Chemopreventive Efficacy of Inositol Hexaphosphate against Prostate Tumor Growth and Progression in TRAMP Mice

Komal Raina,[1] Subapriya Rajamanickam,[1] Rana P. Singh,[1,3] and Rajesh Agarwal[1,2]

Abstract

Purpose: Herein, for the first time, we evaluated the *in vivo* chemopreventive efficacy of inositol hexaphosphate (IP6), a major constituent of high-fiber diets, against prostate tumor growth and progression in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model.

Experimental Design: Beginning at 4 weeks of age, male TRAMP mice were fed 2% (w/v) IP6 in drinking water or only drinking water till 24 weeks of age, and then sacrificed. Prostate tissue was subjected to histopathologic analysis and to immunohistochemical analyses for proliferation and apoptosis.

Results: IP6 feeding did not show any adverse effect on fluid and diet consumption and body weight. There was a significant reduction (40%; $P < 0.01$) in lower urogenital tract weight in IP6-fed mice. IP6 inhibited prostate cancer progression at prostatic intraepithelial neoplasia stage and strongly reduced the incidence of adenocarcinoma (prostatic intraepithelial neoplasia/adenocarcinoma, 75:25% in the IP6 group versus 39:61% in the control group; $P < 0.05$). The incidences of well-differentiated and poorly differentiated adenocarcinomas in the IP6-fed group were reduced by 44% and 62%, respectively. Immunohistochemical analysis of prostate tissue showed a 26% decrease ($P < 0.05$) in proliferation cell nuclear antigen–positive cells and a 3.5-fold increase in apoptotic cells with no effect on Tag expression by IP6.

Conclusions: These findings are both novel and highly significant in establishing for the first time that oral IP6, without any toxicity, suppresses prostate tumor growth and progression at the neoplastic stage, thereby reducing the incidence of adenocarcinoma through its antiproliferative and proapoptotic effects, and thus indicating that IP6 could have potential chemopreventive effects against human prostate cancer.

Inositol hexaphosphate (IP6) or phytic acid is a naturally occurring hexaphosphorylated carbohydrate, ubiquitously present in most plants and mammalian cells (1, 2). The basic carbohydrate moiety "inositol" in IP6 and its other phosphate derivatives (IP1-IP5) are physiologically interconvertible and regulate vital cellular functions (2, 3). It is marketed as a dietary supplement owing to its antioxidant property and known beneficial effects such as prevention against the formation of kidney stone, high cholesterol, and heart and liver diseases (4, 5).

Epidemiologic studies indicating that diets rich in IP6 had a negative correlation with the incidence of colon cancer triggered a series of investigations to determine the anticancer efficacy of IP6 (2). Over the years, several studies pioneered by Shamsuddin et al. (1, 2) and other research groups have shown the promising chemopreventive and anticancer effects of IP6 in various cancer models (3, 6). *In vitro* studies have indicated that IP6 inhibits the growth of human breast (7), colon (8), prostate (9, 10), and liver (11) cancer cells, as well as of rhabdomyosarcoma (12) and erythroleukemia cells (13); inhibits cell transformation in mouse epidermal JB6 cells (14); and reverses the transformed phenotype of HepG2 liver cancer cells (11). Regarding the *in vivo* anticancer efficacy of IP6, it has been shown that exogenous administration of 1% IP6 in drinking water 1 week before or 2 weeks after the administration of azoxymethane inhibits the development of large intestinal cancer in F344 rats (15). In the same model, administration of 2% IP6 in drinking water after 5 months of carcinogen induction was also able to significantly inhibit both tumor number and volume in the large intestine (16). Further more, IP6 has also been shown to suppress dimethyhydrazine-induced large intestinal cancer in CD-1 mice (17); inhibit growth of 7,12-dimethylbenz(*a*)anthracene–induced skin and mammary tumorigenesis (7, 18); regress liver cancer xenotransplant (19); prevent pulmonary adenomas in mice (20); inhibit growth of rhabdomyosarcoma tumor xenograft (12); inhibit

Authors' Affiliations: [1]Department of Pharmaceutical Sciences, School of Pharmacy, and [2]University of Colorado Cancer Center, University of Colorado Denver, Denver, Colorado; and [3]Cancer Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

Received 12/28/07; revised 1/30/08; accepted 3/1/08.

Grant support: National Cancer Institute RO1 grant CA116636.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Rajesh Agarwal, Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Denver, 4200 East Ninth Street, Box C238, Denver, CO 80262. Phone: 303-315-1381; Fax: 303-315-6281; E-mail: Rajesh.Agarwal@uchsc.edu.

©2008 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-07-5275

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024

growth of mouse fibrosarcoma FSA-1 tumor xenografts (21); and inhibit colon carcinogenesis (22).

Following the first report that IP6 causes growth inhibition and induces differentiation in advanced human prostate cancer PC-3 cells (9), successive mechanistic studies conducted by us revealed that IP6 possesses strong anticancer efficacy against both androgen-dependent and androgen-independent prostate cancer, wherein it inhibits cell growth and causes G_1 cell cycle arrest via modulation of cell cycle regulatory molecules in human prostate cancer LNCaP and DU145 cells (10, 23) and also induces their apoptotic death. Further studies by our group revealed that IP6 impairs erbB1 receptor–associated mitogenic signaling (24) and also inhibits constitutive activation of nuclear factor κB in DU145 cells (25). Additionally, the growth inhibitory and proapoptotic effects of IP6 were also observed in mouse tumorigenic TRAMP-C1 cells (26). With regard to the *in vivo* efficacy of IP6 against prostate cancer, we recently reported that 1% and 2% (w/v) IP6 feeding in drinking water inhibits DU145 tumor xenograft growth in athymic nude mice, which was associated with the antiproliferative, proapoptotic, and antiangiogenic effects of IP6 on the tumor (27). However, other than xenograft, the anti–prostate cancer efficacy of IP6 has not been studied in any existing animal models of prostate cancer.

In the present study, for the first time, we evaluated the chemopreventive efficacy of IP6 feeding against prostate cancer growth and progression in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. In TRAMP male mice, hormonally regulated minimal rat probasin promoter (PB) specifically drives the expression of SV40 early genes (T/t; Tag) in prostatic epithelium at sexual maturity, causing the spontaneous induction of neoplastic transformation in the prostate (28, 29). The Tag abrogates p53 and retinoblastoma function, leading to the development of spontaneous progressive stages of prostatic disease with time from initial lesions of prostatic intraepithelial neoplasia (PIN) to late-stage adenocarcinoma (28, 30, 31). This tumorigenesis pattern closely mimics the progressive forms of human prostatic carcinoma (30), and therefore our present findings of chemopreventive efficacy of IP6 in the TRAMP model could have potential clinical significance.

Materials and Methods

Animals and treatment, necropsy, and histopathology. Heterozygous TRAMP females, developed on a pure C57BL/6 background, were crossbred with nontransgenic C57BL/6 breeder males. Tail DNA was subjected to PCR-based screening assay for PB-Tag as previously described (29). The routinely obtained 4-wk-old TRAMP male mice were randomly distributed into positive control and treatment groups. Positive control mice were supplied with regular drinking water and the treatment groups were fed with 2% (w/v) IP6 in regular drinking water for 20 wk. IP6 (sodium salt) was purchased from Sigma, and the freshly prepared solution (as the only source of drinking water) was supplied every Monday, Wednesday, and Friday; the fluid consumption in both groups was also recorded. There were 18 mice in the control group and 16 mice in the IP6-fed group. In parallel, age-matched nontransgenic C57BL/6 male mice (n = 5 per group) were fed with regular drinking water or IP6 for the same duration. During the study, animals were permitted free access to AIN-76A rodent diet. Food consumption and animal body weight were recorded weekly, and the animals were monitored daily for their general health. Animal care and treatments were in accordance with institutional guidelines and approved protocols.

At the time of sacrifice, the animals were anesthetized by ketamine injection and then euthanized by exsanguinations. Each mouse was weighed and lower urogenital tract, including bladder, seminal vesicles, and prostate, was removed en bloc. Animals were also examined for gross pathology, and any evidence of edema, abnormal organ size, or appearance in nontarget organs was also noted. Lower urogenital tract wet weight was recorded, and prostate gland was harvested and microdissected whenever possible (when a tumor obscured the boundaries of the lobes it was taken as such). Tissues were fixed overnight in 10% (v/v) phosphate-buffered formalin and processed conventionally. Briefly, the fixed tissues were dehydrated in ascending grades of ethanol, cleared in toluene, and embedded in paraffin wax. Sections (5 μm) were cut with microtome and mounted on superfrost slides (Fisher Scientific) coated with 0.01% poly-L-lysine (Sigma–Aldrich). Sections (5 μm) of paraffin-embedded tissues were stained with H&E for routine histopathologic evaluation.

Immunohistochemical analysis. Paraffin-embedded sections were deparaffinized and stained with specific primary antibody followed by 3,3-diaminobenzidine (DAB) staining, as previously described (32). Primary antibodies used were anti–proliferation cell nuclear antigen (PCNA; 1:250; DakoCytomation) and anti–SV40 large T antigen (1:400; BD PharMingen). Biotinylated secondary antibody used was rabbit anti-mouse IgG (1:200; DakoCytomation). Apoptotic cells were identified by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining using Dead End Colorometric TUNEL System (Promega Corp.) as per vendor's protocol. PCNA- and TUNEL-positive cells were quantified by counting the brown-stained cells and the total number of cells in five randomly selected fields at ×400 magnification.

Statistical and microscopic analyses. All statistical analyses were carried out with SigmaStat software version 2.03 (Jandel Scientific), and two-sided $P < 0.05$ was considered significant. Fisher's exact test was used to compare the incidence of PIN and adenocarcinoma in positive control versus IP6-fed group. For other data, the difference was analyzed by unpaired two-tailed Student's t test. All microscopic histopathologic and immunohistochemical analyses were done with Zeiss Axioscope 2 microscope (Carl Zeiss, Inc.) and photomicrographs were captured with Carl Zeiss AxioCam MrC5 camera.

Results

IP6 feeding reduces lower urogenital tract weight without any apparent toxicity. In TRAMP mice, IP6 feeding did not show any considerable difference in diet (Fig. 1A) and fluid (Fig. 1B) consumption and body weight gain (Fig. 1C) profiles between positive control and IP6-fed mice during the entire treatment regimen. At the time of necropsy, all animals were examined for gross pathology, and there was no evidence of edema, abnormal organ size, or appearance in nontarget organs. There was, however, a significant difference between the lower urogenital tract weight of positive control and IP6-fed groups. The lower urogenital tract weight of the IP6-fed group was 40% (P<0.01) lower than that of the positive control group (Fig. 2A). When the lower urogenital tract weight was normalized to body weight (Fig. 2B), the difference in weight followed the same trend; the IP6-fed group of mice showed 38% (P<0.01) lower urogenital tract weight compared with the positive control group.

In nontransgenic mice, IP6 feeding did not show any change in diet and fluid consumption and body weight gain profiles (data not shown). In addition, the gross pathology of prostate

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024

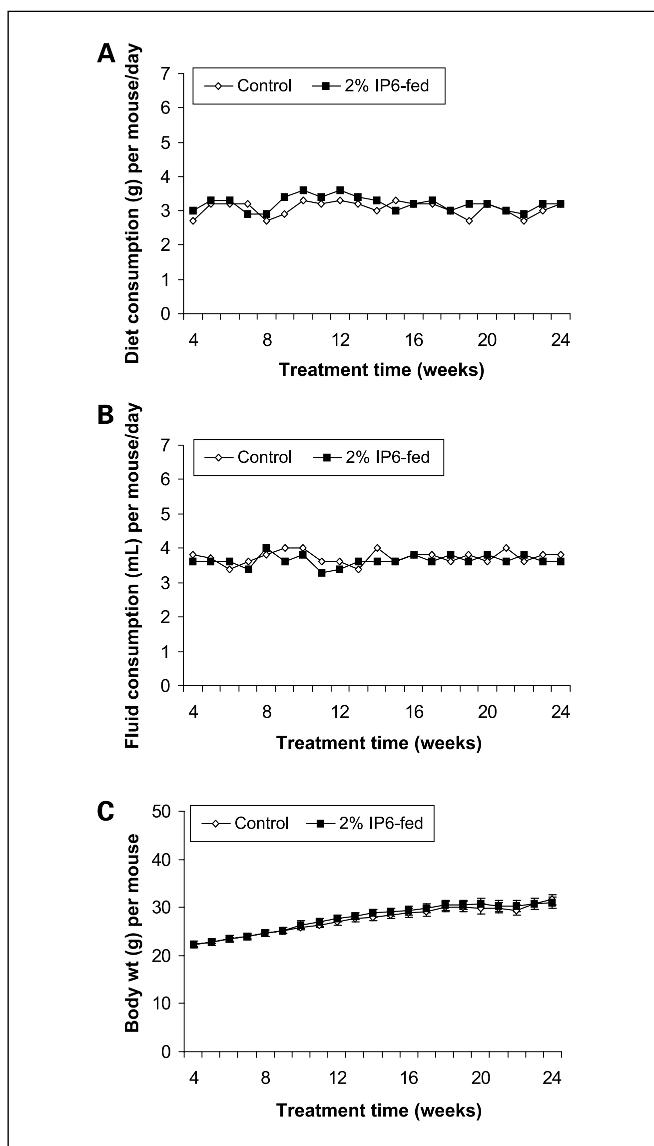

Fig. 1. *A,* effect of oral feeding of IP6 on the daily diet consumption of TRAMP mice. Food consumption by each mouse was recorded weekly throughout the feeding regimen in each group. Diet consumption (in grams) per mouse per day is plotted as a function of time (weeks) for each group. *B,* effect of oral feeding of IP6 on the daily fluid consumption of TRAMP mice. Positive control mice were supplied with regular drinking water and the treatment group was fed with IP6 solution [2% IP6 (w/v) in regular drinking water] for 20 wk. The freshly prepared solutions (as the only source of drinking water) were supplied every Monday, Wednesday, and Friday; and the fluid consumption by different groups was also recorded. Fluid consumption (in milliliters) per mouse per day is plotted as a function of time (weeks) for each group. *C,* effect of oral feeding of IP6 on the body weight of TRAMP mice. Body weight of each mouse was recorded weekly throughout the experiment. Body weights of mice are represented as average of each group and plotted as a function of time (weeks) for each group. Control, positive control (TRAMP) mice.

and other organs in nontransgenic mice was similar in both control and IP6-fed groups, and no considerable change was observed in lower urogenital tract weight (data not shown). These findings clearly indicate that daily IP6 consumption at 2% (w/v) dose for longer duration is nontoxic to animals and inhibits abnormal growth of the prostate in TRAMP mice, which was further examined by histopathologic analysis.

IP6 feeding inhibits prostate cancer progression at PIN and reduces the incidence of adenocarcinoma. Because progressive pathologies of the disease are more evident and aggressive in dorsolateral prostate, studies were conducted with a particular focus on dorsolateral prostate. A detailed histopathologic analysis of neoplastic progression of the dorsolateral prostate in both positive control and IP6-fed groups of TRAMP mice was done. H&E-stained sections were microscopically examined and classified as (*a*) low-grade PIN; (*b*) high-grade PIN; (*c*) well-differentiated adenocarcinoma; (*d*) moderately differentiated adenocarcinoma; and (*e*) poorly differentiated adenocarcinoma, as recently published (33, 34). As shown in Fig. 3A, there was a significant difference in PIN incidence between the IP6-fed and positive control groups ($P < 0.05$). None of the mice showed low-grade PIN in the positive control group; however, 25% of mice in the IP6-fed group had low-grade PIN. In most animals, the tumor progression was arrested at high-grade PIN stage in the IP6-fed group as compared with the positive control group (high-grade PIN incidence, 50% versus 39%, respectively). Further, histopathologic analysis revealed that there was a significant decrease ($P < 0.05$) in the incidence of adenocarcinoma by IP6. As shown in Fig. 3B, there was a 44% reduction in the incidence of well-differentiated

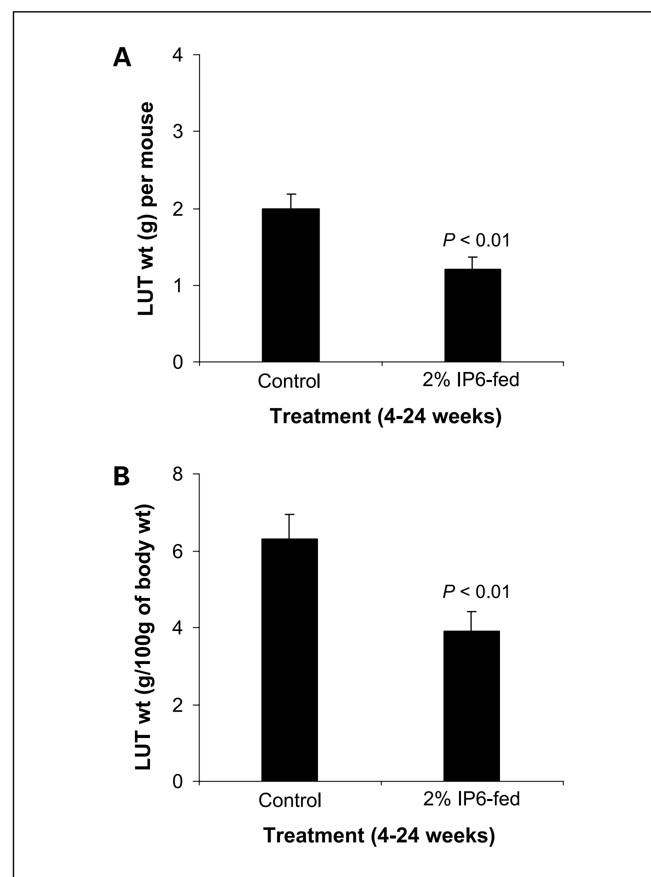

Fig. 2. *A,* effect of IP6 feeding on the weight of the lower urogenital tract (*LUT*) organs. At the time of necropsy after 20 wk of IP6 feeding, starting from 4th wk of age, each mouse was weighed and the lower urogenital tract including the bladder, seminal vesicles, and prostate was removed en bloc and weighed; $n = 18$ (positive control) and $n = 16$ (2% IP6-fed) mice per group. Columns, mean; bars, SE. $P < 0.05$, positive controls versus IP6-fed group (unpaired two-tailed Student's t test). Control, positive control (TRAMP) mice.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024

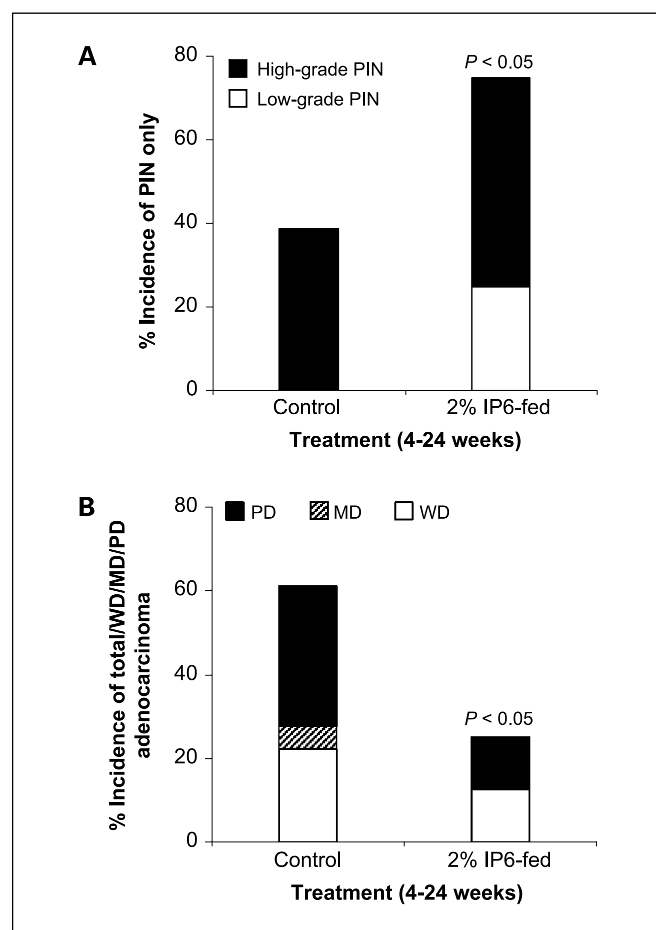

Fig. 3. Inhibitory effect of IP6 feeding on prostate tumor progression in TRAMP mice. The dorsolateral prostate from the study detailed in Fig. 1 was histopathologically analyzed for the different stages of neoplastic progression of the dorsolateral prostate. *A,* effect of IP6 feeding on the incidence of low-grade and high-grade PIN lesions in TRAMP mice. *B,* effect of IP6 on the incidence of prostate adenocarcinoma in TRAMP mice. $P < 0.05$, incidence of PIN and adenocarcinoma in positive control versus IP6-fed group (Fisher's exact test). WD, well differentiated (adenocarcinoma); MD, moderately differentiated (adenocarcinoma); PD, poorly differentiated (adenocarcinoma); Control, positive control (TRAMP) mice.

adenocarcinoma and a 62% reduction in the incidence of poorly differentiated adenocarcinoma in the IP6-fed group when compared with the positive control group (Fig. 3B). These results suggest that IP6 inhibits prostate tumor progression at the neoplastic stage and concomitantly reduces the incidence of adenocarcinoma.

IP6 feeding reduces tumor grade. To assess the severity of prostatic lesions, histologic data of both groups were further analyzed for tumor grade. Tissues were graded as (*a*) normal epithelium, which was assigned a score of 1.0; (*b*) low-grade PIN, 2.0; (*c*) high-grade PIN, 3.0; (*d*) well-differentiated adenocarcinoma, 4.0; (*e*) moderately differentiated adenocarcinoma, 5.0; and (*f*) poorly differentiated adenocarcinoma, 6.0 (33, 34). To generate the mean peak histologic score, the maximum histologic score for individual prostate from each mouse was used to calculate the mean for that treatment group. As shown in Fig. 4A, there was a significant reduction in the severity of lesions in the IP6-fed group. TRAMP mice fed with 2% IP6 had a mean peak score of 3.2, which was significantly lower ($P < 0.05$) than that of the control group (mean peak score, 4.3). Accordingly, the photomicrographs for the tumor grade representative of a treatment group are shown in Fig. 4B. These results suggest that in addition to reducing the incidence of adenocarcinoma, IP6 feeding decreases the severity of prostatic lesions when administered to TRAMP mice.

IP6 feeding reduces proliferation index and increases apoptosis in the prostate of TRAMP mice. To assess the *in vivo* effect of IP6 feeding on proliferation index in the dorsolateral prostate, tissue samples from both positive control and IP6-fed groups were analyzed by PCNA immunostaining. Qualitative microscopic examination of PCNA-stained sections showed a substantial decrease in PCNA-positive cells in the IP6-fed group compared with the positive control group (Fig. 5A). Quantification of PCNA staining showed 39 ± 3% PCNA-positive cells in the IP6-fed group as compared with 53 ± 5% in the positive control group (Fig. 5B), accounting for a 26% ($P < 0.05$) decrease in proliferation index by IP6. These results suggest the *in vivo* antiproliferative effect of IP6 feeding against prostate tumor growth and progression in TRAMP mice.

Further analysis of the prostate tissues was done to assess the *in vivo* apoptotic response to IP6 feeding in TRAMP mice. Microscopic examination of tissue sections showed an increased number of TUNEL-positive cells in the IP6-fed group

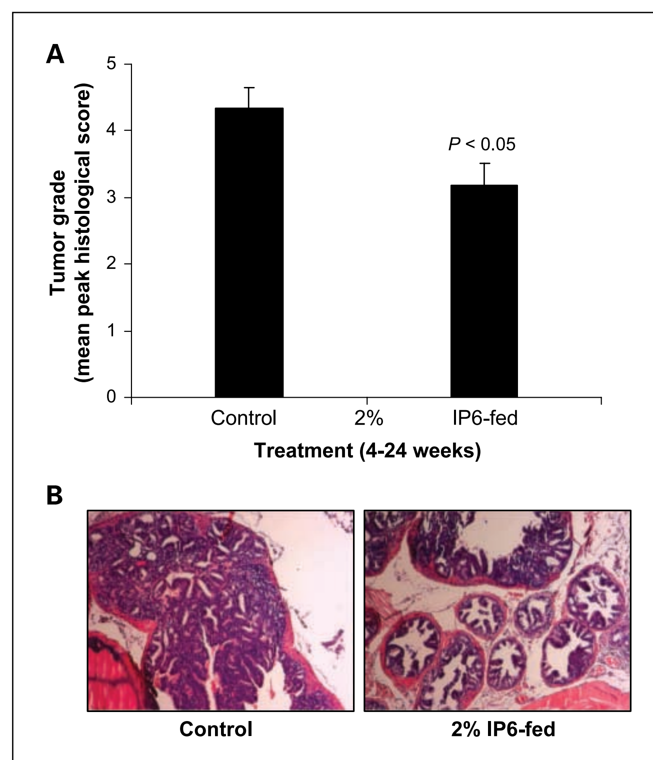

Fig. 4. IP6 feeding reduces the severity of prostatic lesions (tumor grade) of dorsolateral prostate in TRAMP mice. *A,* different stages of prostate tissues were graded as described in Results. The maximum histologic score for the individual prostate from each mouse was used to calculate the mean for the treatment group. Columns, mean peak histologic score of each group; bars, SE. $P < 0.05$, between positive control and IP6-fed groups (unpaired two-tailed Student's t test). *B,* representative photomicrographs (×100 magnification) of a treatment group show the H&E staining of the dorsolateral prostate of positive control and IP6-fed mice sacrificed at 24 wk of age. Control, positive control (TRAMP) mice.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024

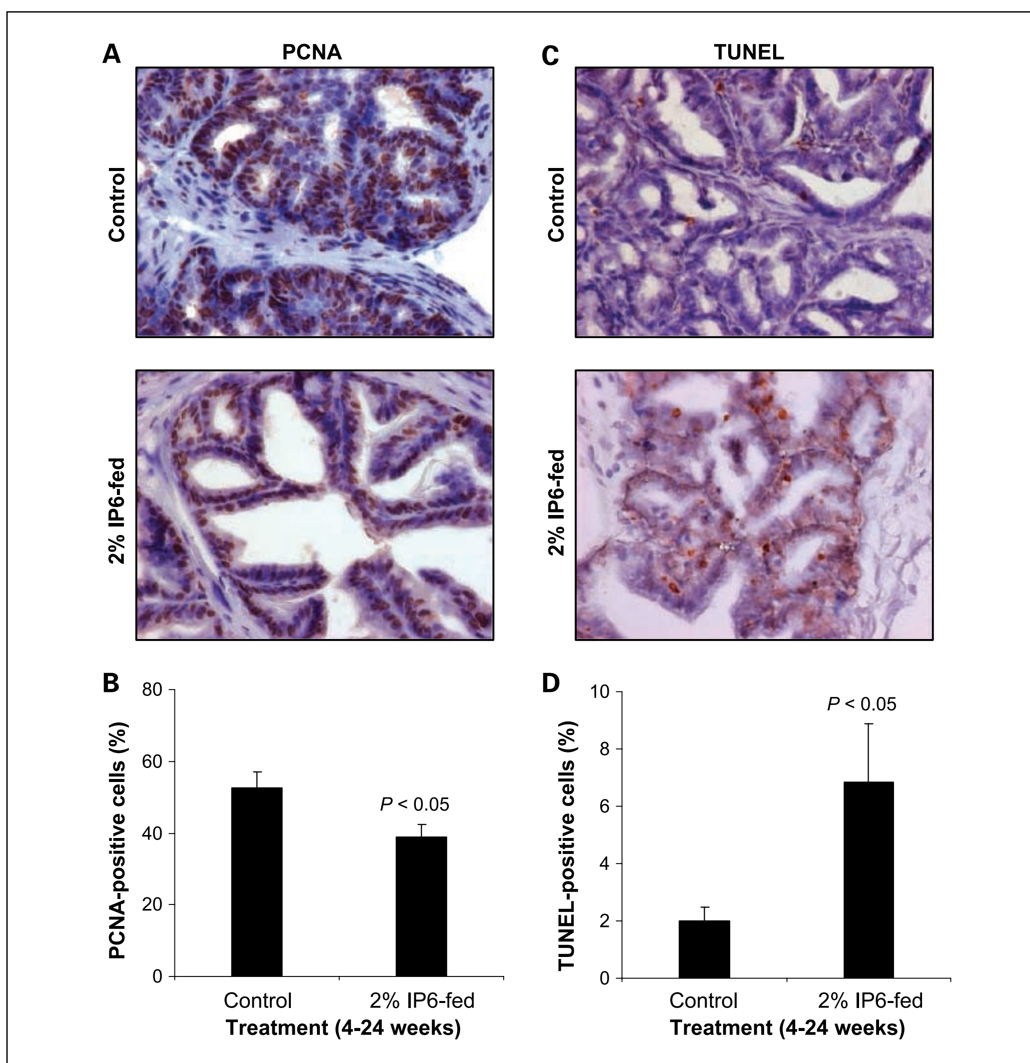

Fig. 5. Antiproliferative and proapoptotic effects of IP6 feeding in TRAMP mice. *A, in vivo* antiproliferative effect of IP6 feeding on dorsolateral prostate of TRAMP mice. Immunohistochemical staining for PCNA in prostate was based on DAB staining as detailed in Materials and Methods. Representative DAB-stained tissue specimens from the positive control and IP6-fed groups showing brown-colored PCNA-positive cells (×400 magnification). *B,* proliferation index was calculated as (number of positive cells / total number of cells counted) × 100, counted under ×400 magnification in five randomly selected areas in each sample. Columns, mean for each group; bars, SE. *C, in vivo* proapoptotic effect of IP6 feeding on dorsolateral prostate in TRAMP mice. Apoptosis was analyzed by TUNEL staining in prostate tissues as detailed in Materials and Methods. Representative DAB-stained tissue specimens from the positive control and IP6-fed groups showing brown-colored TUNEL-positive cells (×400 magnification). *D,* apoptotic index was calculated as (number of TUNEL-positive cells / total number of cells) × 100, counted under ×400 magnification in five randomly selected areas in each sample. Columns, mean for each group; bars, SE. $P < 0.05$, between positive control and IP6-fed groups (unpaired two-tailed Student's t test; *B* and *D*). Control, positive control (TRAMP) mice.

(Fig. 5C). The number of TUNEL-positive apoptotic cells were 7 ± 2% in the IP6-fed group, as compared with 2 ± 0.5% in the positive control group, accounting for a 3.5-fold ($P < 0.05$) increase in apoptotic cells by IP6 (Fig. 5D). These observations suggest that in addition to antiproliferative effect, proapoptotic effect could be another potential mechanism underlying the chemopreventive effect of IP6 against prostate tumorigenesis in the TRAMP model.

IP6 feeding has no effect on SV40 T antigen expression in the prostate of TRAMP mice. Because prostate tumorigenesis in TRAMP mice is driven by the expression of SV40 T antigen specifically in prostate epithelial cells, it is always desirable to find the effect of a given chemopreventive/antitumor agent on SV40 T antigen expression level. In this regard, the IP6-treated group did not show any considerable change in the levels of SV40 T antigen in different stages of prostate tumorigenesis when compared with the positive control group, as observed by the immunohistochemical analysis of the transgene expression (Fig. 6). Therefore, the inhibition of prostate tumor growth and progression by IP6 could most likely be mediated by altering the T antigen–driven neoplastic molecular changes for enhanced cell growth and survival.

Discussion

Prostate cancer is the most frequently diagnosed malignancy in elderly American men (35). Several epidemiologic studies indicate that prostate cancer incidence and associated death rate are lower in Asian countries as compared with Western countries (3, 36). This has been attributed to the difference in

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024

dietary pattern, which is recognized as one of the major etiologic factors responsible for the variation in prostate cancer incidence and mortality between Asian and Western male populations (37). The dietary composition in the industrialized Western countries includes highly processed foods rich in meat, dairy products, and refined carbohydrates; however, in Asian countries, diets are rich in fiber, whole grain cereals, legumes, vegetables, and fruits (36–39). Research groups worldwide have directed considerable efforts toward the identification of dietary or nondietary naturally occurring chemical agents for the prevention and intervention of prostate cancer (40, 41). One such dietary agent is IP6, which has shown anticancer efficacy against various *in vitro* and *in vivo* cancer models, including prostate cancer (3, 42). IP6 is abundantly present in high-fiber diets, mostly cereals, legumes, nuts, and soybean (1, 2). The consumption of these dietary agents has been associated with reduced risk, incidence of, and mortality due to prostate cancer in Asian countries (1, 2, 37).

We have previously studied the *in vivo* anticancer efficacy of oral IP6 against human prostate carcinoma DU145 xenograft, in which IP6 suppressed the tumor growth without any toxicity (27). IP6 has also been found effective in animal tumorigenesis models of other cancer types without any toxicity (3). The effect of IP6 on prostate tumor progression has not been studied till now in any preclinical animal model. In this regard, the most relevant available animal model is TRAMP, which closely mimics the progression of prostate cancer as it occurs in humans (31). Therefore, our present study of the chemopreventive efficacy of IP6 in the TRAMP model could have potential clinical significance. The most important and novel finding in the present study is that oral IP6 feeding for 24 weeks starting from the 4th week of age inhibits prostate tumor growth and progression in TRAMP mice. This antitumor progression effect of IP6 is accompanied by the arrest of tumor progression at PIN stage with a concomitant reduction in the incidence of adenocarcinoma. There were more mice with low-grade PIN and high-grade PIN (75%) and fewer with well-differentiated and poorly differentiated adenocarcinoma (25%) in the IP6-fed group, whereas no mouse was found with low-grade PIN but there were some with high-grade PIN (39%) and more with adenocarcinoma (61%) in the positive control group. IP6 also significantly inhibited the progression through the different stages of adenocarcinoma and overall decreased the severity of the lesions as observed by the mean peak histologic score analysis. Additionally, no apparent toxic or adverse effect was observed in mice having IP6-supplemented drinking water, as monitored by the general health, water and diet

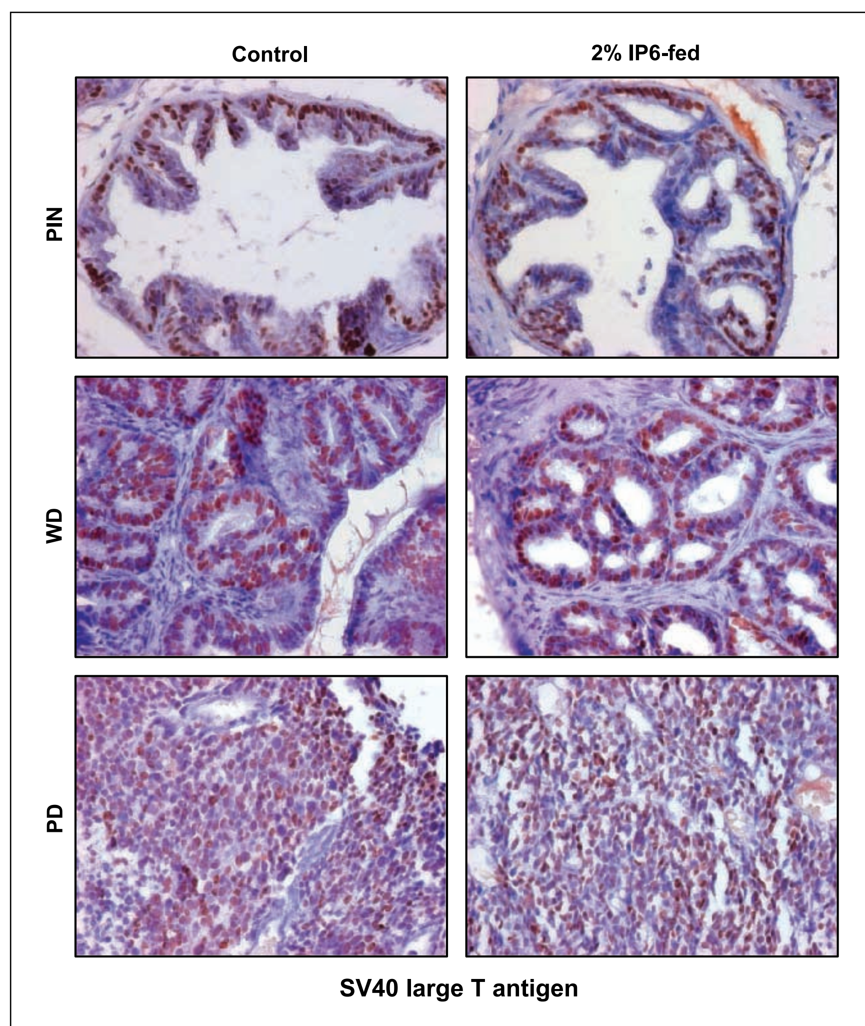

Fig. 6. Effect of oral feeding of IP6 on the expression of SV40 large T antigen in the dorsolateral prostate of TRAMP mice. Immunohistochemical staining was based on DAB staining as detailed in Materials and Methods. Representative DAB-stained tissue specimens are illustrated from positive control and 2% IP6-fed groups (×400 magnification) showing brown staining for the expression of SV40 large T antigen in PIN, well-differentiated adenocarcinoma, and poorly differentiated adenocarcinoma. Control, positive control (TRAMP) mice.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024

consumption, body weight gain, and gross pathologic examination during necropsy. These observations indicate the clinical potential of IP6 in suppressing prostate cancer growth and progression.

Cell proliferation and apoptosis are well-established biomarkers to study the antitumor effect of a given agent (43). Many naturally occurring and synthetic agents have been found to inhibit cell proliferation and induce apoptosis in cancer cells (41). In this regard, IP6 has been found to inhibit cell proliferation as well as induce apoptosis in human and mouse prostate cancer cells in culture and in DU145 tumor xenograft in nude mice (reviewed in ref. 3). In the present study, to examine whether inhibition of prostate tumor growth and progression by IP6 is associated with its effect on cell proliferation and survival, prostate tissues were also immunohistologically analyzed with PCNA and TUNEL staining. IP6 significantly inhibited cell proliferation and induced apoptotic cell population in prostate tissues. These observations suggest the role of the antiproliferative and proapoptotic effects of IP6 in suppressing prostate cancer growth and progression.

Our further concern was to address whether the observed anti–prostate cancer effect of IP6 is due to its effect on Tag expression that drives neoplastic transformation in prostate epithelial cells and, subsequently, prostate tumorigenesis in the TRAMP model or by other mechanisms. The immunohistochemical analysis of prostate tissue did not show any considerable change in Tag expression at different stages of tumor development. This observation suggests that the inhibitory effects of IP6 against prostate cancer growth and progression are not related to suppression of Tag expression but to the direct suppression of tumorigenesis. In this regard, it is likely that IP6 may inhibit cell cycle progression to suppress tumor progression by targeting the cyclin-dependent kinase-cyclin-dependent kinase inhibitor-cyclin axis, retinoblastoma family proteins, and E2F cell cycle regulators, as we have observed in prostate cancer cell culture studies with IP6 (10, 23, 26). Furthermore, other potential mechanisms of IP6 could be the inhibition of epidermal growth factor receptor, phosphatidylinositol 3-kinase/Akt, and nuclear factor-κB signaling and induction of mitochondrial as well as caspase-independent apoptosis, which have been reported in prostate cancer cells (24–26). IP6 may also target the insulin-like growth factor I-insulin-like growth factor binding protein-3 axis for its antiproliferative and proapoptotic effects as has been observed in a DU145 tumor xenograft study (27). However, additional studies are needed in future to examine the molecular mechanisms involved in the anti–prostate cancer efficacy of oral IP6 feeding in this preclinical mouse model.

In humans, prostate tumorigenesis takes considerable time from the onset of the disease to progression to a detectable tumor and then to a hormone-refractory stage. Therefore, a considerable window of time could be available to use various intervention strategies, including dietary chemoprevention (40, 41, 44). In this regard, the findings in the present study are both novel and highly significant in establishing that IP6 feeding suppresses prostate tumor progression at the neoplastic stage thereby reducing the incidence of the advanced forms of the disease, the various progressive stages of adenocarcinoma. Further, this preclinical study advocates for a potential clinical trial of IP6 in prostate cancer patients, which may improve the morbidity and survival time in cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Shamsuddin AM, Vucenik I, Cole KE. IP6: a novel anti-cancer agent. Life Sci 1997;61:343–54.
2. Vucenik I, Shamsuddin AM. Cancer inhibition by inositol hexaphosphate (IP6) and inositol: from laboratory to clinic. J Nutr 2003;133:3778–84S.
3. Singh RP, Agarwal R. Prostate cancer and inositol hexaphosphate: efficacy and mechanisms. Anticancer Res 2005;25:2891–903.
4. Graf E, Eaton JW. Antioxidant functions of phytic acid. Free Radic Biol Med 1990;8:61–9.
5. Jariwalla RJ. Rice-bran products: phytonutrients with potential applications in preventive and clinical medicine. Drugs Exp Clin Res 2001;27:17–26.
6. Fox CH, Eberl M. Phytic acid (IP6), novel broad spectrum anti-neoplastic agent: a systematic review. Complement Ther Med 2002;10:229–34.
7. Shamsuddin AM, Vucenik I. Mammary tumor inhibition by IP6: a review. Anticancer Res 1999;19:3671–4.
8. Saied IT, Shamsuddin AM. Up-regulation of the tumor suppressor gene p53 and WAF1 gene expression by IP6 in HT-29 human colon carcinoma cell line. Anticancer Res 1998;18:1479–84.
9. Shamsuddin AM, Yang GY. Inositol hexaphosphate inhibits growth and induces differentiation of PC-3 human prostate cancer cells. Carcinogenesis 1995;16:1975–9.
10. Singh RP, Agarwal C, Agarwal R. Inositol hexaphosphate inhibits growth, and induces G_1 arrest and apoptotic death of prostate carcinoma DU145 cells: modulation of CDKI-CDK-cyclin and pRb-related protein-E2F complexes. Carcinogenesis 2003;24:555–63.
11. Vucenik I, Tantivejkul K, Zhang ZS, Cole KE, Saied I, Shamsuddin AM. IP6 in treatment of liver cancer. I. IP6 inhibits growth and reverses transformed phenotype in HepG2 human liver cancer cell line. Anticancer Res 1998;18:4083–90.
12. Vucenik I, Kalebic T, Tantivejkul K, Shamsuddin AM. Novel anticancer function of inositol hexaphosphate: inhibition of human rhabdomyosarcoma *in vitro* and *in vivo*. Anticancer Res 1998;18:1377–84.
13. Shamsuddin AM, Baten A, Lalwani ND. Effects of inositol hexaphosphate on growth and differentiation in K-562 erythroleukemia cell line. Cancer Lett 1992;64:195–202.
14. Huang C, Ma WY, Hecht SS, Dong Z. Inositol hexaphosphate inhibits cell transformation and activator protein 1 activation by targeting phosphatidylinositol-3′ kinase. Cancer Res 1997;57:2873–8.
15. Shamsuddin AM, Elsayed AM, Ullah A. Suppression of large intestinal cancer in F344 rats by inositol hexaphosphate. Carcinogenesis 1988;9:577–80.
16. Shamsuddin AM, Ullah A. Inositol hexaphosphate inhibits large intestinal cancer in F344 rats 5 months after induction by azoxymethane. Carcinogenesis 1989;10:625–6.
17. Shamsuddin AM, Ullah A, Chakravarthy AK. Inositol and inositol hexaphosphate suppress cell proliferation and tumor formation in CD-1 mice. Carcinogenesis 1989;10:1461–3.
18. Ishikawa T, Nakatsuru Y, Zarkovic M, Shamsuddin AM. Inhibition of skin cancer by IP6 *in vivo*: initiation-promotion model. Anticancer Res 1999;19:3749–52.
19. Vucenik I, Zhang ZS, Shamsuddin AM. IP6 in treatment of liver cancer. II. Intra-tumoral injection of IP6 regresses pre-existing human liver cancer xenotransplanted in nude mice. Anticancer Res 1998;18:4091–6.
20. Wattenberg LW. Chemoprevention of pulmonary carcinogenesis by myo-inositol. Anticancer Res 1999;19:3659–61.
21. Vucenik I, Tomazic VJ, Fabian D, Shamsuddin AM. Antitumor activity of phytic acid (inositol hexaphosphate) in murine transplanted and metastatic fibrosarcoma, a pilot study. Cancer Lett 1992;65:9–13.
22. Jenab M, Thompson LU. Purified and endogenous phytic acid in wheat bran affects early biomarkers of colon cancer risk. IARC Sci Publ 2002;156:387–9.
23. Agarwal C, Dhanalakshmi S, Singh RP, Agarwal R. Inositol hexaphosphate inhibits growth and induces G_1 arrest and apoptotic death of androgen-dependent human prostate carcinoma LNCaP cells. Neoplasia 2004;6:646–59.
24. Zi X, Singh RP, Agarwal R. Impairment of erbB1 receptor and fluid-phase endocytosis and associated mitogenic signaling by inositol hexaphosphate in human prostate carcinoma DU145 cells. Carcinogenesis 2000;21:2225–35.
25. Agarwal C, Dhanalakshmi S, Singh RP, Agarwal R. Inositol hexaphosphate inhibits constitutive activation of NF-κB in androgen-independent human prostate carcinoma DU145 cells. Anticancer Res 2003;23:3855–61.
26. Sharma G, Singh RP, Agarwal R. Growth inhibitory and apoptotic effects of inositol hexaphosphate in transgenic adenocarcinoma of mouse prostate (TRAMP-C1) cells. Int J Oncol 2003;23:1413–8.
27. Singh RP, Sharma G, Mallikarjuna GU, Dhanalakshmi

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024

S, Agarwal C, Agarwal R. *In vivo* suppression of hormone-refractory prostate cancer growth by inositol hexaphosphate: induction of insulin-like growth factor binding protein-3 and inhibition of vascular endothelial growth factor. Clin Cancer Res 2004;10: 244–50.

28. Greenberg NM, DeMayo F, Finegold MJ, et al. Prostate cancer in a transgenic mouse. Proc Natl Acad Sci U S A 1995;92:3439–43.

29. Greenberg NM, DeMayo FJ, Sheppard PC, et al. The rat probasin gene promoter directs hormonally and developmentally regulated expression of a heterologous gene specifically to the prostate in transgenic mice. Mol Endocrinol 1994;8:230–9.

30. Gingrich JR, Barrios RJ, Foster BA, Greenberg NM. Pathologic progression of autochthonous prostate cancer in the TRAMP model. Prostate Cancer Prostatic Dis 1999;2:70–5.

31. Gingrich JR, Greenberg NM. A transgenic mouse prostate cancer model. Toxicol Pathol 1996;24:502–4.

32. Singh RP, Sharma G, Dhanalakshmi S, Agarwal C, Agarwal R. Suppression of advanced human prostate tumor growth in athymic mice by silibinin feeding is associated with reduced cell proliferation, increased apoptosis, and inhibition of angiogenesis. Cancer Epidemiol Biomarkers Prev 2003;12:933–9.

33. Raina K, Singh RP, Agarwal R, Agarwal C. Oral grape seed extract inhibits prostate tumor growth and progression in TRAMP mice. Cancer Res 2007; 67:5976–82.

34. Raina K, Blouin MJ, Singh RP, et al. Dietary feeding of silibinin inhibits prostate tumor growth and progression in transgenic adenocarcinoma of the mouse prostate model. Cancer Res 2007;67:11083–91.

35. Stewart AB, Lwaleed BA, Douglas DA, Birch BR. Current drug therapy for prostate cancer: an overview. Curr Med Chem Anticancer Agents 2005;5: 603–12.

36. Clinton SK, Giovannucci E. Diet, nutrition, and prostate cancer. Annu Rev Nutr 1998;18:413–40.

37. Boyle P, Severi G, Giles GG. The epidemiology of prostate cancer. Urol Clin North Am 2003;30:209–17.

38. Abdulla M, Gruber P. Role of diet modification in cancer prevention. Biofactors 2000;12:45–51.

39. Bidoli E, Talamini R, Bosetti C, et al. Macronutrients, fatty acids, cholesterol and prostate cancer risk. Ann Oncol 2005;16:152–7.

40. Klein EA. Chemoprevention of prostate cancer. Annu Rev Med 2006;57:49–63.

41. Singh RP, Agarwal R. Mechanisms of action of novel agents for prostate cancer chemoprevention. Endocr Relat Cancer 2006;13:751–78.

42. Vucenik I, Shamsuddin AM. Protection against cancer by dietary IP6 and inositol. Nutr Cancer 2006;55: 109–25.

43. Klein S, McCormick F, Levitzki A. Killing time for cancer cells. Nat Rev Cancer 2005;5:573–80.

44. Agarwal R. Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem Pharmacol 2000;60:1051–9.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/14/10/3177/1975426/3177.pdf by guest on 12 November 2024