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

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Abstract

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

Experimental Design—Beginning 4 weeks of age, male TRAMP mice were fed with 2% (w/v) IP6 in drinking water or only drinking water till 24 weeks of age, and then sacrificed. Prostate tissue was subjected to histopathology, and 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 the weight of lower urogenital tract organs in the IP6-fed mice. IP6 inhibited prostate cancer (PCa) progression at prostatic intraepithelial neoplasia (PIN) stage and strongly reduced the incidence of adenocarcinoma (PIN:adenocarcinoma, 75:25% mice in IP6 group versus 39:61% in control group, $P < 0.05$). The incidences of well differentiated and poorly differentiated adenocarcinomas in IP6-fed group were reduced by 44% and 62%, respectively. Immunohistochemical analysis of prostate tissue showed 26% ($P < 0.05$) decrease in proliferation cell nuclear antigen (PCNA)-positive cells, and 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 feeding, without any toxicity, suppresses prostate tumor growth and progression at the neoplastic stage thereby reducing the incidence of adenocarcinoma involving anti-proliferative and pro-apoptotic effects, and thus could have potential against human PCa.

Keywords

chemoprevention; TRAMP; prostate cancer; inositol hexaphosphate; phytic acid

Introduction

Inositol hexaphosphate (IP6) or phytic acid is a naturally occurring hexaphosphorylated carbohydrate, ubiquitously present in most of the plants and mammalian cells (1,2). The

basic carbohydrate moiety 'inositol' in IP6 and its other phosphate derivatives (IP1-5) are physiologically inter-convertible and regulate vital cellular functions (2,3). It is marketed as a dietary supplement owing to its anti-oxidant property and known beneficial effects such as prevention against the formation of kidney stone, high cholesterol, and heart and liver diseases (4,5).

Epidemiological studies indicating that diets rich in IP6 content 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 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 cancer cells (11), and 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 prior 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 been also shown to suppress dimethylhydrazine induced large intestinal cancer in CD-1 mice (17); inhibit growth of DMBA 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 the 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 (PCa) PC-3 cells (9), successive mechanistic studies conducted by us revealed that IP6 possesses strong anticancer efficacy against both androgen-dependent and -independent PCa, wherein, it inhibits cell growth and causes G1 cell cycle arrest via modulation of cell cycle regulatory molecules in human PCa 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 NF- κ B in DU145 cells (25). Additionally, growth inhibitory and pro-apoptotic effects of IP6 were also observed in mouse tumorigenic TRAMP-C1 cells (26). With regard to the *in vivo* efficacy of IP6 against PCa, 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 anti-proliferative, pro-apoptotic and anti-angiogenic effects of IP6 on the tumor (27). However, other than xenograft, the anti-PCa efficacy of IP6 has not been studied in any existing animal models of PCa.

In the present study, for the first time, we evaluated the chemopreventive efficacy of IP6 feeding against PCa growth and progression in transgenic adenocarcinoma of the mouse prostate (TRAMP) model. In TRAMP male mice, hormonally regulated minimal rat probasin promoter (PB) specifically drives the expression of SV-40 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 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 cross-bred with non-transgenic C57BL/6 breeder males. Tail DNA was subjected to PCR-based screening assay for PB-Tag as previously described (29). The routinely obtained four week-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 weeks. IP6 (sodium salt) was purchased from Sigma (St. Louis, MO), and the freshly prepared solution (as the only source of drinking water) was supplied every Monday, Wednesday and Friday; and the fluid consumption in both groups was also recorded. There were 18 mice in control and 16 mice in IP6-fed group. In parallel, age-matched non-transgenic C57BL/6 male mice (n=5 mice/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 protocol.

At the time of sacrifice, the animals were anaesthetized by ketamine injection and then euthanized by exsanguinations. Each mouse was weighed and lower urogenital tract (LUT) 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 non-target organs was also noted. LUT 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, Houston, TX) coated with 0.01% poly-L-Lysine (Sigma-Aldrich, St. Louis, MO). Sections (5 µm) of paraffin-embedded tissues were stained with H&E for routine histopathological evaluation.

Immunohistochemical Analysis

Paraffin-embedded sections were deparaffinized and stained using specific primary antibody followed by 3, 3'-diaminobenzidine (DAB) staining, as previously described (32). Primary antibodies used were anti-PCNA (1:250; DakoCytomation, Carpinteria, CA) and anti-SV40 large T antigen (1:400; BD Pharmingen, San Diego, CA). Biotinylated secondary antibody used was rabbit anti-mouse IgG (1:200; DakoCytomation, Carpinteria, CA). Apoptotic cells were identified by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) staining using Dead End Colorimetric TUNEL System (Promega Corp., Madison, WI) as per vendor's protocol. PCNA- and TUNEL-positive cells were quantified by counting brown-stained cells within total number of cells at 5 randomly selected fields at ×400 magnification.

Statistical and Microscopic Analyses

All statistical analyses were carried out with Sigma Stat software version 2.03 (Jandel Scientific, San Rafael, CA), and two-sided *P* values <0.05 were considered significant. Fishers Exact test was used to compare 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 histopathological and immunohistochemical analyses

were done by Zeiss Axioscope 2 microscope (Carl Zeiss, Inc., Jena, Germany) and photomicrographs were captured by Carl Zeiss AxioCam MrC5 camera.

Results

IP6 Feeding Reduces LUT 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 the 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 non-target organs. There was however a significant difference between the LUT weight of positive control and IP6-fed groups. The LUT weight of the IP6-fed group was 40% ($P<0.01$) lower than that of the positive control group (Fig. 2A). When the LUT 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 LUT weight compared to the positive control group.

In non-transgenic mice, IP6 feeding did not show any change in diet and fluid consumption, and body weight gain profiles (data not shown). Also, the gross pathology of prostate and other organs in non-transgenic mice were similar in both control and IP6-fed groups, as well as no considerable change was observed in LUT weight (data not shown). These findings clearly indicate that daily IP6 consumption at 2% (w/v) dose for longer duration is non-toxic to animals and inhibits abnormal growth of the prostate in TRAMP mice, which was further examined by histopathological analysis.

IP6 Feeding Inhibits PCa Progression at PIN and Reduces the Incidence of Adenocarcinoma

As 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 histopathological analysis of the 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 prostatic intraepithelial neoplasia (LGPIN); (b) high grade prostatic intraepithelial neoplasia (HGPIN); (c) well differentiated adenocarcinoma; (d) moderately differentiated adenocarcinoma; and (e) poorly differentiated adenocarcinoma, as published recently (33,34). As shown in Figure 3A, there was a significant difference in PIN incidence between IP6-fed and positive control group ($P<0.05$). None of the mice showed LGPIN in the positive control group; however, 25% of mice in IP6-fed group had LGPIN. In most animals, the tumor progression was arrested at HGPIN stage in IP6-fed group as compared to the positive control group (HGPIN incidence, 50% versus 39%, respectively). Further, histopathological analysis revealed that there was a significant decrease ($P<0.05$) in the incidence of adenocarcinoma by IP6. As shown in Figure 3B, there was a 44% reduction in the incidence of well differentiated adenocarcinoma and 62% reduction in the incidence of poorly differentiated adenocarcinoma in IP6-fed group when compared to 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 severity of prostatic lesions, histological data of both groups were further analyzed for tumor grade. Tissues were graded as (a) normal epithelium was assigned a score of 1.0; (b) LGPIN as 2.0; (c) HGPIN as 3.0; (d) well differentiated adenocarcinoma as 4.0; (e) moderately differentiated adenocarcinoma as 5.0; and (f) poorly differentiated

adenocarcinoma as 6.0 (33,34). To generate a mean peak histological score, the maximum histological score for individual prostate from each mouse was used to calculate a mean for that treatment group. As shown in Figure 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 the control group (mean peak score, 4.3). Accordingly, the photomicrographs for the tumor grade representative of a treatment group are shown in Figure 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 IP6-fed group compared to the positive control group (Fig. 5A). Quantification of PCNA staining showed $39 \pm 3\%$ PCNA-positive cells in IP6-fed group as compared to $53 \pm 5\%$ 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* anti-proliferative effect of IP6 feeding during prostate tumor growth and progression in TRAMP mice.

Further analysis of the prostate tissues was done to assess the *in vivo* apoptotic response of IP6 feeding in TRAMP mice. Microscopic examination of tissue sections showed an increased number of TUNEL-positive cells in IP6-fed group (Fig. 5C). The number of TUNEL-positive apoptotic cells were $7 \pm 2\%$ in IP6-fed group, as compared to $2 \pm 0.5\%$ 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 anti-proliferative effect, pro-apoptotic effect could be another potential mechanism underlying the chemopreventive effect of IP6 on prostate tumorigenesis in TRAMP model.

IP6 Feeding has no Effect on SV40T Transgene Expression in the Prostate of TRAMP Mice

Since, prostate tumorigenesis in TRAMP mice is driven by the expression of SV40T antigen specifically in prostate epithelial cells, it is always desired to find the effect of a given chemopreventive/antitumor agent on its expression level. In this regard, IP6 treated group did not show any considerable change in the levels of SV40 T antigen in different stages of prostate tumorigenesis when compared to 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

PCa is the most frequently diagnosed malignancy in elderly American men (35). Several epidemiological studies indicate that the PCa incidence and associated death rate are lower in Asian countries as compared to Western countries (3,36). This has been attributed to the difference in dietary pattern which is recognized as one of the major etiologic factors responsible for a variation in PCa incidence and mortality between Asian and Western male population (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 content, whole grain cereals, legumes, vegetables, and fruits (36-39). Research groups, worldwide, have directed considerable efforts towards the

identification of dietary or non-dietary naturally occurring chemical agents for both prevention and intervention of PCa (40,41). One such dietary agent is IP6, which has shown anticancer efficacy against various *in vitro* and *in vivo* cancer models, including PCa (3,42). IP6 is abundantly present in high fiber content diets, most cereals, legumes, nuts and soybean (1,2). The consumption of these dietary agents have been associated with reduced risk, incidence of, and mortality due to PCa in Asian countries (1,2,37).

We have previously studied the *in vivo* anticancer efficacy of oral IP6 against human prostate carcinoma DU145 xenograft growth, 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 chemopreventive efficacy of IP6 in TRAMP model could have potential clinical significance. The most important and novel findings in the present study are that oral IP6 feeding for 24 weeks starting from the 4th week of age inhibits prostate tumor growth and progression in TRAMP mice. This anti-tumor 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 LGPIN and HGPIN stages (75%) and fewer with well differentiated and poorly differentiated adenocarcinoma stages (25%) in IP6-fed group. Whereas in the positive control group, no mouse was found with LGPIN but there were some with HGPIN (39%) and more with adenocarcinoma stages (61%). 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 histological 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 consumption, body weight gain, and gross pathological examination during necropsy. These observations indicate for the clinical potential of IP6 in suppressing the PCa growth and progression.

Cell proliferation and apoptosis are well established biomarkers to study the anti-tumor 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 PCa 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 for PCNA and TUNEL staining. IP6 significantly inhibited cell proliferation and induced apoptotic cell population in prostate tissues. These observations suggest the role of the anti-proliferative and pro-apoptotic effects of IP6 in suppression of PCa growth and progression.

Our further concern was to address whether the observed anti-PCa effect of IP6 is due to its effect of Tag expression that drives neoplastic transformation in prostate epithelial cells and subsequently prostate tumorigenesis in 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 anti-PCa growth and progression effects of IP6 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 CDK (cyclin-dependent kinase)-CDKI (CDK inhibitor)-cyclin axis and RB (retinoblastoma) family proteins and E2F cell cycle regulators as we have observed in PCa cell culture studies with

IP6 (10,23,26). Furthermore, other potential mechanisms of IP6 could be the inhibition of EGFR (epidermal growth factor receptor), PI-3K (phosphatidylinositol-3 kinase)-Akt and NF- κ B (nuclear factor-kappaB) signaling and induction of mitochondrial as well as caspase-independent apoptosis which have been reported in PCa cells (24-26). IP6 may also target IGF-1 (insulin-like growth factor-1)-IGFBP-3 (IGF binding protein-3) axis for its antiproliferative and proapoptotic effects as has been observed in DU145 tumor xenograft study (27). However, additional studies are needed in future to examine the molecular mechanisms involved in the anti-PCa efficacy by oral IP6 feeding in this pre-clinical mouse model.

In humans, prostate tumorigenesis takes considerable time from the onset of the disease and progression to a detectable tumor and then to a hormone-refractory stage. Therefore, a considerable window of time could be available to employ 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 causes suppression of 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 pre-clinical study advocates for a potential clinical trial of IP6 in PCa patients which may improve the morbidity and survival time in cancer patients.

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Abbreviations

PCa	prostate cancer
IP6	inositol hexaphosphate
TRAMP	transgenic adenocarcinoma of the mouse prostate
LUT	lower urogenital tract
PIN	prostatic intraepithelial neoplasia
PCNA	proliferation cell nuclear antigen
TUNEL	terminal deoxynucleotidyl transferase dUTP nick-end labeling.

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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 (g) per mouse/ 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 weeks. 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 (mL) per mouse/ 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; IP6, inositol hexaphosphate.

Fig. 2.

(A) Effect of IP6 feeding on the weight of the lower urogenital tract (LUT) organs. At the time of necropsy after 20 weeks of IP6 feeding, starting from 4th week of age, each mouse was weighed and the LUT 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. Error bars indicate $\pm$ SEM. The statistical significance of difference between positive controls versus IP6-fed group was analyzed by unpaired two-tailed Student's *t*-test. *P* values <0.05 were considered significant. LUT, lower urogenital tract; Control, positive control (TRAMP) mice; IP6, inositol hexaphosphate.

Fig. 3.

Inhibitory effect of IP6 feeding on prostate tumor progression in TRAMP mice. The dorsolateral prostate from the study detailed in Fig. 1, were histopathologically analyzed for the different stages of the neoplastic progression of the dorsolateral prostate. **(A)** Effect of IP6 feeding on the incidence of low and high grade PIN lesions in TRAMP mice. **(B)** Effect of IP6 on the incidence of adenocarcinoma of prostate in TRAMP mice. Fishers Exact test was used to compare incidence of PIN and adenocarcinoma in positive control versus IP6-fed group. P values < 0.05 were considered significant. PIN, prostate intraepithelial neoplasia; WD, well differentiated (adenocarcinoma); MD, moderately differentiated (adenocarcinoma); PD, poorly differentiated (adenocarcinoma); Control, positive control (TRAMP) mice; IP6, inositol hexaphosphate.

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 histological score for the individual prostate from each mouse was used to calculate a mean for the treatment group. Data is presented as mean peak histological score $\pm$ SEM (error bars) of each group. The statistical significance of difference between positive control versus IP6-fed group was analyzed by unpaired two-tailed Student’s *t*-test. *P* values <0.05 were considered significant. **(B)** The photomicrographs ($\times 100$ magnification) representative of a treatment group show the H&E staining of the dorsolateral prostate of positive control and IP6-fed mice sacrificed after 24 weeks of age. Control, positive control (TRAMP) mice; IP6, inositol hexaphosphate.

Fig. 5.

Anti-proliferative and pro-apoptotic effects of IP6 feeding in TRAMP mice. **(A)** *In vivo* anti-proliferative 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 positive control and IP6-fed groups showing brown-colored PCNA-positive cells are depicted at $\times 400$ magnifications. **(B)** Proliferation index was calculated as the number of positive cells $\times 100$ / total number of cells counted under $\times 400$ magnifications in 5 randomly selected areas in each sample, and shown as mean $\pm$ SEM (error bars) for each group. **(C)** *In vivo* pro-apoptotic 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 positive control and IP6-fed groups showing brown-colored TUNEL-positive cells are depicted at $\times 400$ magnifications. **(D)** Apoptotic index was calculated as the number of TUNEL-positive cells $\times 100$ / total number of cells counted under $\times 400$ magnifications in 5 randomly selected areas in each sample, and shown as mean $\pm$ SEM (error bars) for each group. For both (B) & (D), the statistical significance of difference between positive control versus IP6-fed group was analyzed by unpaired two-tailed Student’s *t*-test. *P* values < 0.05 were considered significant. PCNA, proliferation cell nuclear antigen; Control, positive control (TRAMP) mice; IP6, inositol hexaphosphate.

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 ($\times 400$ magnifications) showing brown staining for the expression of SV40 large T antigen in prostatic intraepithelial neoplasia (PIN), well differentiated (WD) adenocarcinoma, and poorly differentiated (PD) adenocarcinoma. Control, positive control (TRAMP) mice; IP6, inositol hexaphosphate.