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

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ABSTRACT

Purpose: Diet composition is an important etiologic factor in prostate cancer (PCA) growth and has significant impact on clinical PCA appearance. Because inositol hexaphosphate (IP6) is a dietary phytochemical present in cereals, soy, legumes, and fiber-rich foods, we evaluated efficacy of IP6 against PCA growth and associated molecular events.

Experimental Design: DU145 cells were injected into nude mice, and animals were fed normal drinking water or 1 or 2% IP6 in drinking water for 12 weeks. Body weight, diet, water consumption, and tumor sizes were monitored. Tumors were immunohistochemically analyzed for proliferating cell nuclear antigen, terminal deoxynucleotidyl transferase-mediated nick end labeling, and CD31. Tumor-secreted insulin-like growth factor binding protein (IGFBP)-3 and vascular endothelial growth factor (VEGF) were quantified in plasma by ELISA.

Results: IP6 feeding resulted in suppression of hormone-refractory prostate tumor growth without any adverse effect on body weight gain, diet, and water consumption during entire study. At the end of study, tumor growth inhibition by 1 and 2% IP6 feeding was 47 and 66% (P = 0.049–0.012) in terms of tumor volume/mouse and 40 and 66% (P = 0.08–0.003) in terms of tumor weight/mouse, respectively. Tumor xenografts from IP6-fed mice showed significantly (P < 0.001) decreased proliferating cell nuclear antigen-positive cells but increased apoptotic cells. Tumor-secreted IGFBP-3 levels were also increased up to 1.7-fold in IP6-fed groups. Additionally, IP6 strongly decreased tumor microvessel density and inhibited tumor-secreted VEGF levels.

Conclusions: IP6 suppresses hormone-refractory PCA growth accompanied by inhibition of tumor cell proliferation and angiogenesis and increased apoptosis. IP6-caused increase in IGFBP-3 and decrease in VEGF might have a role in PCA growth control.

INTRODUCTION

Prostate cancer (PCA) is the major nonskin cancer in elderly men and the second most common male malignancy in the United States and European countries (1). It is estimated that 220,900 new cases and 28,900 associated deaths will occur from PCA in 2003 (1). Similar to other epithelial malignancies, the autocrine and paracrine growth factor-receptor interactions and associated mitogenic signaling are the major contributors to the deregulated PCA growth (2, 3). An advanced and hormone-refractory stage of PCA does not respond to androgen-deprivation therapy and is also resistant to apoptosis by many cancer chemotherapeutic drugs (4, 5). Accordingly, intervention of hormone-refractory advanced stage of PCA by nontoxic phytochemicals that target proliferation and survival could be an alternative strategy.

Insulin-like growth factor (IGF) signaling is known to play a crucial role in both mitogenic and antiapoptotic events in cancer cells, including PCA (3, 6). Epidemiological studies show that high levels of circulating IGF-I and low levels of IGF binding protein (IGFBP)-3 are associated with an increased risk of PCA (7–10). There is ample evidence that IGF-I not only stimulates cell proliferation but also inhibits apoptosis, and this combination has an enormous favorable effect on tumor growth (6). Conversely, IGFBP-3 sequesters IGF-I and antagonizes both mitogenic and antiapoptotic functions of IGF-I (3, 6, 10). Furthermore, it has been shown that IGFBP-3 could cause apoptotic cell death independent of IGF-I (11). An uncontrolled growth of tumors also depends upon the proper blood supply, which involves recruiting new blood vessels from the surrounding tissues (12). Angiogenesis is absolutely required for the growth of normal and drug-resistant tumors, as well as for the regrowth of dormant tumors (13). Therefore, it has been hypothesized that targeting tumor angiogenesis could be another promising strategy to control and treat various malignancies, including PCA (12, 13). Taken together, based on these facts, we rationalized that dietary [diets containing nontoxic anticancer agent(s)] intervention of IGF-I signaling, as well as angiogenesis could be a practical and translational approach to control PCA. This assumption is also supported by several epidemiological and laboratory studies showing that consumption of yellow-green vegetables, fruits, and certain grains, which con-
tain phytochemicals with known antitumor activity, affords significant protection against various cancers (14–16).

Most cereals, legumes, nuts, oil seeds, and soybean contain up to 6.4% (w/w) or even higher amounts of hexaphosphate (IP6; Ref. 17), which is also marketed as a dietary supplement and is consumed by many cancer patients for its several health benefits. The beneficial effects of inositol hexaphosphate (IP6) include the prevention of kidney stone, high cholesterol, and heart and liver diseases (17). Consumption of IP6-rich cereals and legumes has been shown to be associated with a reduction in breast, colon, and prostate cancers (18). The laboratory investigation on the antitumor efficacy of IP6 started in mid 1980s by Shamsuddin et al. (17), and since then, several studies have shown the anticancer effects of IP6 in various in vitro as well as in vivo cancer models, including skin, mammary, intestine, lung, and liver (17–22). To the best of our knowledge, there is no study showing in vivo efficacy of IP6 against PCA. There are only a few studies showing in vitro activity of IP6 in human PCA cell lines (23–25). Therefore, the findings in this study are both novel and highly significant in addressing for the first time the oral efficacy of IP6 in controlling the in vivo growth of hormone-refractory human PCA xenografts and its in vivo association with a decrease in proliferation and angiogenesis together with increased apoptosis.

MATERIALS AND METHODS

Cell Lines and Animals. DU145 human prostate carcinoma cells were obtained from the American Type Culture Collection (Manassas, VA) and grown in RPMI 1640 with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin at 37°C in a 5% CO2 atmosphere. Athymic male nu/nu mice were purchased from the National Cancer Institute-Frederick (Frederick, MD) and housed in nude mice care facility at the University of Colorado Health Sciences Center. Animal care was in accordance with current regulations and standards of the NIH, as well as our institutional guidelines for animal care.

In Vivo Tumor Xenograft Model. DU145 cells were collected from the culture dishes by trypsinization and then washed, and resuspended in serum-free RPMI 1640. To establish DU145 tumor xenografts in mice, 6-week-old athymic nu/nu male mice were injected s.c. with 2 × 106 DU145 cells mixed with Matrigel (Collaborative Biomedical Products, Bedford, MA) in the right flank of each mouse. Next day, the mice were randomly divided into three groups: group I (n = 9), normal drinking water (control); group II (n = 9), 1% IP6 (w/v) in drinking water; and group III (n = 8), 2% IP6 (w/v) in drinking water for 12 weeks. The selection of these IP6 doses and their feeding protocol in drinking water was based on several published studies showing anticancer efficacy of IP6 in different animal tumor models (Refs. 17, 20, 21 and references therein). Body weight, diet, and water consumption were recorded two to three times/week throughout the study. Once xenografts started growing, their sizes were measured twice weekly. The tumor volume was calculated by the formula “0.5236 L1(L2)2”, where L1 is the long axis and L2 is the short axis of the tumor.” At the end of experiment, tumors were excised, weighed, and stored at −80°C until additional analysis.

Immunohistochemical Detection of Proliferating Cell Nuclear Antigen (PCNA) in Tumor Xenografts. Tumor samples were fixed in 10% buffered formalin for 12 h and processed conventionally. The paraffin-embedded tumor sections (5-µm thick) were heat immobilized, deparaffinized using xylene, and then rehydrated in a graded series of ethanol. Antigen retrieval was done by incubating the sections in 10 mM citrate buffer (pH 6.0) in microwave for 20 min. Endogenous peroxidase activity was blocked by immersing the sections in 3% (v/v) H2O2 in methanol. Sections were then incubated with mouse monoclonal anti-PCNA antibody IgG2a (Dako, Carpinteria, CA) and finally stained with 3,3’-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) as detailed recently (26). Proliferating cells were quantified by counting the PCNA-positive (brown) cells and the total number of cells at 10 arbitrarily selected fields at ×400 magnification by an independent observer. The proliferation index (per ×400 microscope field) was determined as number of PCNA-positive cells × 100/total number of cells.

In Situ Apoptosis Detection by Terminal Deoxynucleotidyl Transferase-Mediated Nick End Labeling (TUNEL) Staining. The formalin-fixed and paraffin-embedded 5-µm thick sections of all tumor samples (those used for PCNA staining) were also used for TUNEL staining. DNA fragmentation in individual apoptotic cells was visualized by detection of biotinylated nucleotides incorporated onto the free 3’-hydroxyl residues of the DNA fragments using Tumor TACS in Situ Apoptosis Detection kit (R&D Systems, Inc., Minneapolis, MN) as published recently (26). The apoptosis was evaluated by counting the positive cells (brown-stained), as well as the total number of cells in 10 arbitrarily selected fields at ×400 magnification by an independent observer. The apoptotic index (per ×400 microscope field) was calculated as number of apoptotic cells × 100/total number of cells.

Immunohistochemical Staining for CD31 Expression in Tumor Xenografts. Staining procedure for CD31 (an endothelial cell-specific antigen also known as platelet endothelial cell adhesion molecule 1) was similar to that of PCNA staining. Briefly, sections from paraffin-embedded tumors were incubated overnight with goat antismouse CD31 polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in PBS containing 10% rabbit serum. Sections were then incubated with biotinylated rabbit antigen secondary antibody (Santa Cruz Biotechnology, Inc.) followed by streptavidin-conjugated horseradish peroxidase (Dako). Antigen-antibody complex was visualized by incubation with 3,3’-diaminobenzidine substrate and counterstaining with diluted Harris hematoxylin. CD31-stained (brown) microvessels were quantified in 10 random microscopic (×400) fields/tumor by an independent observer.

ELISA for Human IGFBP-3 and Vascular Endothelial Growth Factor (VEGF). At the termination of tumor xenograft experiment, blood was collected intracardially from mice in heparinized tubes, and plasma was separated for IGFBP-3 and VEGF analyses. Quantikine human IGFBP-3 and VEGF immunoassay kits from R&D Systems, Inc., were used to determine the IGFBP-3 and VEGF concentrations secreted from DU145 tumors in mouse plasma. The principle of the assays is based on quantitative sandwich enzyme immunoassay using precoated monoclonal antibody specific for human IGFBP-3 or...
human VEGF onto a microplate for solid-phase ELISA. Briefly, 100 μl of plasma were used in each assay, and after following step-by-step protocol provided by the vendor, final absorbance of the developed color was determined using a microplate reader set to 450 nm with correction wavelength at 540 nm. IGFBP-3 or VEGF concentrations were extrapolated from the standard curves generated using recombinant human IGFBP-3 or recombinant human VEGF in the assay. We observed that known concentrations of recombinant human IGFBP-3 or recombinant human VEGF added in mouse plasma yield linear standard curve similar to that generated in assay diluents.

**Immunohistochemical and Statistical Analyses.** All of the microscopic examinations were done by Zeiss Axioscop 2 microscope (Carl Zeiss, Jena, Germany). Microscopic images were taken by Kodak DC290 zoom digital camera and processed by Windows Millennium DC290 Kodak microscopy documentation system (Eastman Kodak Co., New Haven, CT). Quantitative data are presented as mean and SE. The statistical significance of difference between control and IP6-fed groups was determined by ANOVA followed by Tukey test for multiple comparisons. The level of statistical significance was additionally confirmed by Student’s two-tailed t test, and P was considered significant at \( P < 0.05 \).

**RESULTS**

**Oral Feeding of IP6 Inhibits Hormone-Refractory DU145 Tumor Xenograft Growth in Athymic Nude Mice.** To study the anticancer effects of any compound in preclinical model, *in vivo* tumor xenograft growth in athymic mouse is a preferred animal model. Here, we used s.c. hormone-refractory human DU145 prostate tumor xenograft growth in athymic nude mouse as a model to assess the *in vivo* anticancer efficacy of IP6 against PCA growth. IP6 was given in drinking water at 1 and 2% (w/v) doses for 12 weeks. As shown in Fig. 1A, the control group (normal drinking water) of mice showed a progressive tumor xenograft growth during the entire study. The IP6 feeding, however, resulted in a clear separation in tumor growth with control group throughout the study; the effect of IP6 oral feeding was more prominent starting sixth to seventh week of the study (Fig. 1A). This observation suggested that a consistent level of IP6 might be necessary for its observable *in vivo* antitumor effects in PCA. The inhibitory effect of IP6 on DU145 tumor xenograft growth was more evident and significant at the end of the study where compared with control group of mice with 741.0 ± 145.9 mm³ tumor volume/mouse; IP6 feeding at 1 and 2% dose levels resulted in 389.7 ± 89.9 mm³ (\( P = 0.049 \)) and 247.6 ± 70.3 mm³ (\( P = 0.012 \)) tumor volume/mouse accounting for 47 and 66% inhibition in tumor growth, respectively (Fig. 1A). Consistent with the tumor volume results, the wet tumor weight measurements, at the end of the study, also showed a significant reduction in IP6-fed groups of mice (Fig. 1B). In this case, compared with control groups of mice with 0.483 ± 0.069 g/mouse tumor weight, IP6 feeding at 1 and 2% dose levels showed 0.291 ± 0.073 and 0.163 ± 0.050 g/mouse tumor weight accounting for 40% (\( P = 0.08 \)) and 66% (\( P = 0.003 \)) inhibition, respectively (Fig. 1B).

We would like to emphasize here that to validate and check the reproducibility of the findings of the above experiment, the study was repeated once where we followed with identical 12 weeks of feeding regimen, including normal (control) and 2% IP6 dose in drinking water. As shown in Fig. 1, C and D, the results of this second experiment were similar to that of the first experiment in terms of both time kinetics of growth inhibitory
Effect of IP6, as well as amount of tumor growth inhibition in terms of tumor volume and tumor weight (401.2 ± 52.2 mm³ in control group versus 246.3 ± 36.8 mm³ in 2% IP6 dose, P = 0.025). However, we noticed that tumor growth in control group was a little slower, as well as the inhibitory effect of IP6 was slightly lower when compared with the results of the first experiment (Fig. 1, C and D). We also observed that inhibitory effect of IP6 on tumor growth was significantly (P < 0.05) evident from eighth week onward, whereas in the first experiment, it was evident from tenth week onwards (Fig. 1).

The primary parameters used in the assessment of gross toxicity of a test compound in cancer chemoprevention/therapy studies include body weight gain profiles, as well as diet and water consumption (14, 26). Accordingly, we also assessed whether IP6 feeding to mice during 12 weeks study period causes any adverse health effects in terms of changes in animal body weight and diet and water consumption. In IP6-fed mice, body weight gain and diet, as well as water consumption profiles, were similar to control group of mice and did not show any significant alterations (Fig. 2, A–C). Furthermore, IP6 feeding did not show any significant changes in the prostate plus seminal vesicle weight/mouse, although the values in IP6-fed groups were slightly lower than the control group (Fig. 2D). Similar findings were also observed in the repeated experiment during 12 weeks of feeding regimen with control and 2% (w/v) IP6-fed groups of mice (data not shown). Overall, the reproducible inhibitory effect of IP6 on DU145 tumor xenograft growth without any gross toxicity in two independent studies were encouraging and suggested that a consistent high level of physiological IP6 might have potential to suppress the in vivo growth of prostate tumor. In additional studies, we analyzed biomarkers associated with the antitumor efficacy of IP6 in prostate tumors (from the first experiment) by immunohistochemical staining.

**Antiproliferative and Proapoptotic Effects of IP6 in Prostate Tumor Xenografts.** On the basis of the data showing inhibition of prostate tumor xenograft growth by IP6, we next assessed whether this effect of IP6 is associated with an in vivo alteration in cell proliferation and/or apoptosis. Microscopic examination of tumor sections stained for PCNA clearly showed a lesser number of PCNA-positive cells in IP6-fed groups as compared with control group (Fig. 3, A–C). Quantification of this data showed that IP6 decreases proliferation index (percent PCNA-positive cells) from 26.4 ± 0.6 (control) to 19.2 ± 0.5 (1% IP6; P < 0.001) and 12.4 ± 0.3 (2% IP6; P < 0.001), which accounted for 27 and 53% inhibition as compared with control, respectively (Fig. 3D). In TUNEL analysis of tumor sections, we observed increased staining for TUNEL-positive cells in the tumor sections from IP6-fed groups (Fig. 3, E–G). Furthermore, quantification of TUNEL-positive cells showed a significant dose-dependent increase in apoptotic index by IP6, which was 1.6- and 3.6-fold (P < 0.001) in 1 and 2% IP6-fed groups compared with control, respectively (Fig. 3H). Taken together, these results convincingly suggest the possible involvement of both antiproliferative as well as apoptotic effect of IP6 in the in vivo inhibition of prostate tumor growth.

**Oral Feeding of IP6 Increases IGFBP-3 Secretion from Prostate Tumors.** Tumor-secreted IGFBP-3 levels in mouse plasma were determined at the end of the experiment. A standard curve was first generated using recombinant human IGFBP-3 in the assay to extrapolate the plasma level of IGFBP-3 (data not shown). Oral feeding of IP6 in drinking
water at 1 and 2% doses for 12 weeks resulted in 16.70 ± 2.23 and 14.51 ± 0.77 ng IGFBP-3/ml plasma as compared with 9.82 ± 1.39 (ng/ml) in control group, respectively (Table 1). Overall, the increase in IGFBP-3 level by 1 and 2% IP6 feeding was 1.7- (P = 0.019) and 1.5-fold (P = 0.009) over that of control, respectively (Table 1).

**Antiangiogenic Effect of IP6 in Prostate Tumors.** Tumor sections were additionally analyzed for platelet endothelial cell adhesion molecule 1 (CD31) staining to assess the effect of IP6 feeding on tumor vasculature. Microscopic analysis of the tumor sections clearly showed that IP6 strongly inhibits microvessel density in prostate tumor xenografts (Fig. 3, I–K). Quantification of microvessels showed 15.3 ± 0.3 (P = 0.003) and 9.2 ± 0.2 (P < 0.001) microvessels/×400 field in the tumor sections from 1 and 2% of IP6-fed groups of mice as compared with control group showing 20.1 ± 1.4 microvessels/×400 field, which accounted for 24 and 53% decrease in microvessel density in the tumors from 1 and 2% IP6-fed groups, respectively (Fig. 3L). On the basis of this finding, we rationalized that IP6 might have an inhibitory effect on VEGF secretion from tumor cells (as one of the possible mechanisms) in exerting its in vivo antiangiogenic effect. To support this possibility, we next analyzed the mouse plasma samples for tumor-secreted VEGF using an ELISA kit specific for human VEGF. The quantification of the data showed 75.53 ± 10.17, 26.12 ± 7.58, and 35.53 ± 16.24 pg VEGF/ml plasma in the control (normal drinking water) and 1% IP6-fed and 2% IP6-fed groups of mice, respectively (Table 1).

### Table 1: Effect of inositol hexaphosphate (IP6) feeding on insulin-like growth factor binding protein 3 (IGFBP-3) and vascular endothelial growth factor (VEGF) secretion from DU145 tumor xenografts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IGFBP-3 (ng/ml plasma)</th>
<th>VEGF (pg/ml plasma)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>9.82 ± 1.39</td>
<td>75.53 ± 10.17</td>
</tr>
<tr>
<td>1% IP6</td>
<td>16.70 ± 2.23 (P = 0.019)</td>
<td>26.12 ± 7.58 (P = 0.002)</td>
</tr>
<tr>
<td>2% IP6</td>
<td>14.51 ± 0.77 (P = 0.009)</td>
<td>35.51 ± 16.24 (P = 0.05)</td>
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which accounted 65% ($P = 0.002$) and 53% ($P = 0.05$) decrease in VEGF levels by these two doses of IP6 feeding in drinking water, respectively (Table 1). VEGF is the most potent angiogenic growth factor, and targeting VEGF is one of the widely hypothesized antiangiogenic strategies. Therefore, these results suggest that inhibition of VEGF secretion from tumors could be one of the possible antiangiogenic mechanisms of IP6-caused decrease in microvessel density in prostate tumors.

DISCUSSION

The data presented here demonstrate that IP6 feeding inhibits the growth of hormone-refractory DU145 human prostate tumor xenograft, which is accompanied by an inhibition of cell proliferation, enhanced apoptosis, and suppression of tumor angiogenesis. In addition, IP6 up-regulates IGFBP-3 secretion, which could be one of the possible mechanisms of its antiproliferative and apoptotic efficacy in tumor. In other studies, an inhibition in VEGF secretion from tumor cells is identified as one of the in vivo antiangiogenic mechanisms of IP6 that might have a significant role in the inhibition of tumor microvessel density. Taken together, these findings substantiate in vivo antitumor efficacy of IP6 against hormone-refractory PCA. Furthermore, these findings could be useful in describing the global incidence of PCA, as well as deciding the dietary intervention of PCA patients as an adjunct to conventional cancer therapy.

The major significance of our findings related to inhibitory effect of IP6 on PCA xenograft growth is its relevance to diet, nutrition, and global PCA incidence. For example, several epidemiological studies have reported that the incidence of PCA development and associated death rate are lower in Asian countries as compared with Western countries (reviewed in Ref. 27). In this regard, the difference in dietary pattern has been identified as one of the major etiologic factors responsible for a variation in PCA incidence and mortality between Asian and Western male populations (27, 28). It is important to emphasize here that the dietary composition in Western countries includes highly processed foods rich in meat, diary products, and refined carbohydrates; however, in Asian countries, the major portions of the diets are fiber-rich food, whole grain cereals, legumes, vegetables, and fruits (27, 28). Consistent with these studies, the data of the present study showing that a dietary agent IP6 suppresses in vivo growth of PCA might support the role of IP6 in reduced risk, incidence, and mortality associated with PCA in Asian countries. Additional studies, however, are needed to evaluate IP6 efficacy in other in vivo PCA models to support this assumption.

The role of Asian dietary pattern has been convincingly demonstrated in the reduced risk and incidence of colorectal cancer (29); however, translational studies on dietary intervention in PCA are very limited. Other limitations are the lack of appropriate and less expensive animal models and relevant biomarkers of PCA development and progression, which could be targeted via dietary intervention. In the present study, we studied three surrogate biomarkers, namely cell proliferation, apoptosis, and angiogenesis, which are widely used and are linked to the growth and progression of cancer, including PCA (30–32). Deregulated cell proliferation with predominating survival mechanisms are hallmarks for almost every cancer (Ref. 33 and references therein). Hormone-refractory PCA is also characterized by constitutive mitogenic and cell survival signaling leading to uncontrolled growth and apoptosis resistance in PCA (2, 5, 34). In such cases, patients do not respond to radiotherapy or chemotherapy, and their life span is reduced to <1 year (28). In the present study, we observed that IP6 causes up to 53% decrease in proliferation and up to ~4-fold increase in apoptosis in prostate tumors, which correlated with the decrease in tumor size. These findings suggest that proliferation and apoptosis could be relevant surrogate biomarkers in dietary intervention of PCA by IP6.

Recent epidemiological as well as laboratory studies have demonstrated that lower IGFBP-3 levels and/or higher circulating IGF-I levels are positively correlated with increased risk of PCA in humans (7–10). The importance of IGF-I signaling and IGFBPs in deregulated cellular growth has also been established in prostate carcinoma cells and transgenic mice (3, 6, 34, 35). It has also been suggested that mitogenic, as well as cell survival signaling via IGF-I/IGF-I receptor pathway, are constitutively activated in human PCA cells and are, in part, responsible for the growth and metastatic potential of PCA (Refs. 3, 34, 36 and references therein). In the present study, we observed that in vivo PCA xenograft growth inhibition by IP6 is associated with an increase in IGFBP-3 level, suggesting a possible inhibition of IGF-I receptor signaling as one of the in vivo antitumor mechanisms of IP6 in PCA xenograft growth inhibition. Additionally, IGFBP-3 has been shown to induce apoptosis independent of its IGF-sequestering action (11). Therefore, in vivo apoptotic effect of IP6 on tumor growth might also be via an up-regulation of IGFBP-3 by IP6 feeding as observed in the present study. Although in vivo involvement of such mechanisms remains to be established, based on our findings, we suggest that in dietary intervention studies with IP6 in human PCA patients, plasma levels of IGFBP-3 may serve as a prognostic biomarker and could be correlated with a decreased incidence of PCA.

Tumor angiogenesis is a reliable prognostic biomarker as well as attractive therapeutic target for prevention and therapy of various cancers, including PCA (12, 13, 31). Angiogenesis is required at every stage of tumor growth and progression after tumor initiation and becomes obligatory for the sustained growth of the tumor beyond a certain size (12, 13, 37). Tumor angiogenesis is regulated by the balance between angiogenic and antiangiogenic factors secreted by tumor cells or present in the circulation (12, 13, 31, 37). Tumors usually remain dormant in the absence of angiogenesis, which may be prolonged up to two decades or more (12, 13, 31, 37). Therefore, it could be hypothesized that dietary factors might have an important role in the regulation of angiogenesis, and the suppression of tumor angiogenesis by these factors could be one of the major underlying causes in the increased latency of tumor appearance (Ref. 38 and references therein). This hypothesis could be supported by our present findings, as well as other studies, showing several phytochemicals of dietary origin modulating the equilibrium between pro- and antiangiogenic growth factors/cytokines and altering the course of pathological angiogenesis (38). In the present study, the results of CD31 immunohistochemical staining showed the decrease in tumor angiogenesis by IP6 feeding, which was also accompanied by the decrease in VEGF secretion by prostate tumors. The observed antiangiogenic efficacy of IP6 might be, in part, due to a shift in the balance between pro- and antiangiogenic factors in prostate tumors.

In summary, the results of the present study suggest that
IP6 could have pleiotropic in vivo antitumor effects targeting cell proliferation, survival, as well as angiogenesis, leading to suppression of prostate tumor growth and that these effects of IP6 might be mediated via up-regulation of IGFBP-3 and down-regulation of VEGF, and associated molecular events.

REFERENCES

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Clin Cancer Res 2004;10:244-250.

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