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Effects of Soy Protein Isolate Consumption on Prostate Cancer Biomarkers in Men With HGPIN, ASAP, and Low-Grade Prostate Cancer

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Fifty-eight men at high risk of prostate cancer or with low-grade prostate cancer were randomly assigned to consume 1 of 3 protein isolates containing 40 g protein: 1) soy protein (SPI+, 107 mg isoflavones/d); 2) alcohol-washed soy protein (SPI–, <6 mg isoflavones/d); or 3) milk protein (MPI). Proliferating cell nuclear antigen (PCNA), epidermal growth factor receptor, B-cell non-Hodgkin lymphoma-2 (Bcl-2), and Bcl-2-associated X protein (Bax) were assessed in baseline and ending prostate biopsy cores. Serum collected at 0, 3, and 6 mo was analyzed for total and free prostate specific antigen (PSA). Consumption of SPI+ did not alter any of the prostate cancer tumor markers. Bax expression decreased from baseline in the SPI– group, resulting in lower Bax expression than the MPI group. PCNA expression also decreased from baseline in the SPI– group, but this was not different from the other 2 groups. PSA did not differ among the groups at 3 or 6 mo. Interestingly, a lower rate of prostate cancer developed in the soy groups compared to the milk group ($P = 0.01$). These data suggest that 6-mo SPI+ consumption does not alter prostate tissue biomarkers, SPI– consumption exerts mixed effects, and less prostate cancer is detected after 6 mo of soy consumption regardless of isoflavone content.

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INTRODUCTION

Men with biopsy-proven, low-grade cancer, or preneoplastic lesions such as atypical small acini suspicious for prostatic adenocarcinoma (ASAP) or high-grade prostatic intraepithelial neoplasia (HGPIN) would be ideal candidates for a nontoxic dietary supplement with proven efficacy for reversing or retarding these early prostate tissue lesions. Phytoestrogenic soy isoflavones have been shown to exert prostate cancer preventive effects, and soy consumption has been associated with decreased prostate cancer risk in epidemiological studies (1). Isoflavone supplementation has been shown to suppress serum prostate specific antigen (PSA), a biomarker associated with prostate cancer progression. In men with prostate cancer, soy food interventions have significantly decreased mean total serum PSA compared to controls (2,3), although several studies have not shown statistically significant effects of soy or isoflavone consumption on total PSA (4–15). Because total PSA is a nonspecific biomarker for prostate cancer, clinicians often evaluate the free to total PSA percent to differentiate between cancer and benign conditions (16). The lower the value of free PSA percent, the greater the probability that elevated PSA represents cancer and not benign prostatic hyperplasia. In men with PSA concentrations between 4 and 10 ng/ml and a free PSA percent below 10%, risk of cancer is 56% compared to men with a free PSA percent above 25% whose risk of cancer is only 8% (17). Only two studies to date have evaluated the effects of soy or isoflavone consumption on free PSA percent. Dalais et al. (3) reported that soy grits increased free PSA percent, but Kranse et al. (10) did not observe a change in free PSA percent with isoflavone supplementation.

It has been suggested that intraprostatic expression of antigens related to carcinogenesis may be useful molecular biomarkers in dietary intervention studies (18). Soy isoflavone interventions in various models have decreased cell proliferation, downregulated the epidermal growth factor receptor (EGFr), and increased programmed cell death or apoptosis. Soy has suppressed cell proliferation as detected by proliferating cell nuclear antigen (PCNA) staining in rodents dosed with either soy protein concentrate (19) or physiologic concentrations of the isoflavone genistein (20). Physiological doses of dietary genistein have downregulated EGFr messenger RNA (mRNA) expression during the early phase of prostate cancer development in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model (20,21). Increased prostate tissue apoptosis has been shown in prostatectomy specimens obtained from patients treated with isoflavones derived from red clover when compared to historically matched controls (8), and soy protein concentrate has increased apoptotic index in the immune compromised mouse model (19). Genistein has increased apoptosis in vitro as detected by the proapoptotic signaling protein, B-cell non-Hodgkin lymphoma-2 (Bcl-2)-associated X protein (Bax) (22). Comparing Bax to the antiapoptotic signaling protein, Bcl-2 may indicate apoptosis status in prostate biopsy specimens (23,24).

The aim of this study was to assess the effects of soy protein isolate consumption on prostate cancer biomarkers in men at high risk of prostate cancer. A randomized placebo-controlled trial was performed in 58 men who consumed either isoflavone-rich soy protein isolate, isoflavone-poor soy protein isolate, or milk protein isolate for 6 mo. The purpose of this study was to evaluate the efficacy of a soy intervention at the beginning of prostate carcinogenesis and to determine whether isoflavones are the responsible bioactive components of soy. The underlying hypothesis was that isoflavone-rich soy protein isolate consumption would increase Bax and decrease Bcl-2, EGFr, PCNA, and serum PSA.

MATERIALS AND METHODS

The 6-mo randomized controlled trial was conducted at the Minneapolis Veteran's Administration Medical Center and was approved by the University of Minnesota Institutional Review Board: Human Subjects Committee, the Minneapolis Veterans Affairs Institutional Review Board, and the U.S. Army Medical Research and Materiel Command's Human Subjects Research Review Board. Subjects were recruited from a pool of patients who, due to their high-risk status, had already undergone a transrectal ultrasound and biopsy, and the biopsy results showed either preneoplastic lesions ($n = 53$) or low-grade prostate cancer with Gleason scores of 6 or below ($n = 5$). Subjects were considered high risk if they had high-grade prostatic intraepithelial neoplasia (PIN) ($n = 40$) or ASAP (atypical small acinar proliferation) ($n = 13$). The 5 patients with low-grade prostate cancer had elected to undergo active surveillance. Urologists invited patients to participate in the study at their postbiopsy clinic visit, and the patients' medical records were reviewed by a research nurse to determine eligibility. Patients were not allowed to participate if they were morbidly obese [body mass index (BMI) $>$ 40 kg/m^2], had prostate cancer that required medical treatment, had chronic prostatitis, consumed more than 14 alcoholic drinks per week, were allergic to soy or milk, used antibiotics frequently, or were on medically prescribed protein-restricted diets.

All 58 subjects supplemented their habitual diets twice daily with 1 of 3 study protein isolates: 1) soy protein isolate (SPI+); 2) alcohol-extracted soy protein isolate (SPI–); or 3) milk protein isolate (MPI; The Solae Company, St. Louis, MO). The protein isolates provided 40 g protein/day and 200–400 kcal/day. The isoflavone content (analyzed by Dr Pat Murphy, Department of Food Science and Human Nutrition, Iowa State University) was 107 $\pm$ 5.0 mg/day for the SPI+; $<6 \pm 0.7$ mg/day for the SPI–; and 0 mg/day for the MPI (mean $\pm$ SD) expressed as aglycone equivalents. The mean distribution of isoflavones was 53% genistein, 35% daidzein, and 11% glycitein in SPI+ and 57% genistein, 20% daidzein, and 23% glycitein in SPI–. Compliance was assessed by self-report as detailed previously (25). To minimize isoflavone consumption from other sources, subjects were given a detailed list of soy-containing products to avoid.

Subject retention has been previously described in detail (25). Data from 58 participants were included in the serum PSA analysis ($n = 58$), and data from 44 subjects were included in the antigen expression analysis. Fewer participants were eligible for antigen expression analysis because 7 subjects did not undergo the final prostate biopsy [liver cancer ($n = 1$), heart complication ($n = 1$), not clinically indicated ($n = 1$), opted out of procedure ($n = 1$), and early withdrawal from study ($n = 3$)], and 7 subjects had insufficient biopsy tissue at either baseline or study end for the analyses.

Serum Collection and Analysis

Participants reported for clinic visits at 0, 3, and 6 mo. Fasting blood was drawn in the morning. Serum was separated and frozen at –70°C until analysis. Serum PSA was measured in one batch at the Minneapolis Veteran's Administration Hospital by the Architect total PSA chemiluminescence microparticle immunoassay (Architect ci8200, Abbott Laboratories, Chicago, IL). Intraassay variability was 2.5%. Free PSA was measured in one batch at Associated Regional and University Pathologists Laboratories by the Roche Modular E170 free PSA electrochemiluminescent immunoassay. Intraassay variability was 7.1%.

Tissue Collection and Analysis

Prostate cores were obtained before the initial screening and obtained again after the 6-mo dietary intervention. Biopsy cores were fixed in formalin and paraffin embedded. The paraffin-embedded blocks were sectioned onto slides, and the slides were

evaluated and diagnosed by the pathologist at the Minneapolis Veteran's Administration Hospital. After diagnosis, slides were obtained from pathology to perform immunohistochemistry for PCNA, EGFr, Bax, and Bcl-2 expression. The tissue sections were deparaffinized, rehydrated in graded alcohol, and transferred to phosphate-buffered solution (pH 7.3). Epitope retrieval was induced by pressure cooking at 103 kPa in citrate buffer with a pH of 6.0 for 10 min and submerged in quenching solution (3% H_2O_2 in 100% MeOH) for 5 min. After blocking (10% milk, 5% serum, and 1% bovine serum albumin), the samples were incubated overnight at 4°C with mouse monoclonal anti-PCNA antibody (555566; BD Biosciences, San Diego, CA; 1:500), mouse monoclonal anti-Bcl-2 antibody (551107; BD Biosciences, San Diego, CA; 1:500), or rabbit polyclonal anti-Bax antibody (554104; BD Biosciences, San Diego, CA; 1:1000). The samples for the EGFr assay were incubated at room temperature for 30 min with the mouse monoclonal anti-EGFr antibody (08-1205; Zymed, Invitrogen Corporation, Carlsbad, CA; ready to use). After rinsing, the samples were incubated with the corresponding biotinylated secondary antibody. Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine (DAB) were utilized to stain the expressed antigens brown. The slides were rinsed with water and counterstained with Harris' Modified Hematoxylin (Fisher Scientific, Pittsburgh, PA). The slides were photographed, and the digital images were optimized for scoring as described in our previous report (25). Positive and negative controls were run in each batch, and the images were scored using the immunohistochemical histological score (HSCORE) semiquantative method (26). The HSCORE is a sum of the percentage of counted epithelial cells weighted by their staining intensity above control. HSCORE = Σ percentage of cells (PC) (i + 1); where i is the intensity of staining with a value of 0 (absent), 1 (weak), 2 (moderate), or 3 (strong) and PC is the percentage of stained epithelial cells for each intensity varying from 0–100%. The range of the HSCORE is a minimum of 1 and a maximum of 4. The vast majority of the glands scored represented normal, hyperplasic, or premalignant acini. The immunostained slides were evaluated independently by 2 technicians blinded to each patient's medical history. There was good agreement between the 2 observers; the Spearman correlation between them was 0.8. On average, 5 intact glands (range = 3–8) were scored per subject slide. Due to poor staining or incomplete glands on tissue sections, some slides were not scored; thus, a few patients were excluded from analysis: PCNA ($n = 3$) and EGFr ($n = 2$).

Statistical Analysis

Baseline comparisons between groups were performed with 1-factor analysis of variance for continuous endpoints and χ^2 for categories of prostate cancer markers. Analysis of covariance was used to compare groups adjusted for the baseline value of the final endpoint. Overall F tests comparing groups are reported, with preplanned pairwise comparisons of all groups for each endpoint as dictated by the study hypotheses. Paired t-tests were used to examine within-group changes. Prostate cancer incidence rates were compared between groups using Fisher's exact test and logistic regression. Statistical significance was defined as $P < 0.05$. All analyses were performed using SAS version 9.1 (27).

TABLE 1
Baseline pathological diagnosis[a]

Diagnosis	SPI+ ($n = 20$)	SPI– ($n = 20$)	MPI ($n = 18$)
PIN (n (%))	15 (75)	12 (60)	13 (72)
ASAP (n (%))	3 (15)	7 (35)	3 (17)
CaP (n (%))	2 (10)	1 (5)	2 (11)

[a] All values are n (%). Abbreviations are as follows: SPI+, isoflavone-rich soy protein isolate (40 g soy protein, 107 mg isoflavones/day); SPI–, alcohol-extracted soy protein isolate (40 g soy protein, <6 mg isoflavones/day); MPI, milk protein isolate (40 g milk protein); PIN, prostatic intraepithelial neoplasia; ASAP, atypical small acini suspicious for prostatic adenocarcinoma; CaP, prostate cancer. Subjects were categorized by most advanced pathological diagnosis.

RESULTS

Baseline

Anthropometrics and dietary intake did not differ between treatment groups as described previously (25). The average age for all men was 68 yr; the average BMI was 30 kg/m^2. At baseline, cancer status and aggregate antigen expression HSCORES did not differ among the groups (Tables 1 and 2). Similarly, there were no differences in baseline total or free PSA concentrations, prostate volume, or PSA density among the groups (Tables 3 and 4).

Antigen Expression

After 6 mo, Bax expression was lower in prostate biopsies in the SPI– group compared to the MPI group (pairwise comparison, $P = 0.03$) and approached a significant difference compared to the SPI+ group (pairwise comparison, $P = 0.10$; Table 2 and Fig. 1). PCNA expression was decreased from baseline in the SPI– group, but baseline-adjusted PCNA expression was not significantly different from the other two groups (Table 2 and Fig. 1). There were no effects of treatment or differences among the groups in Bcl-2, EGFr, Bax:Bcl-2 ratio, or Bax:PCNA ratio.

PSA and Prostate Volume

There were no effects of treatment or differences among the groups in total PSA, free PSA, or PSA percent (Table 3). Prostate volume at 6 mo was increased in the SPI– group relative to the MPI group (pairwise comparison, $P = 0.04$), but PSA density (serum total PSA/prostate volume) was not different among the groups (Table 4).

TABLE 2
Antigen expression (HSCORE) in benign prostate tissue[a]

	SPI+	SPI–	MPI
Bax[b]			
Baseline	1.38 ± 0.08	1.45 ± 0.07	1.35 ± 0.06
6 mo	1.41 ± 0.06[ab]	1.27 ± 0.05[a*]	1.44 ± 0.06[b]
PCNA[c]			
Baseline	1.61 ± 0.1	1.93 ± 0.1	1.86 ± 0.1
6 mo	1.69 ± 0.1	1.57 ± 0.1*	1.81 ± 0.1
Bcl-2[d]			
Baseline	1.11 ± 0.03	1.17 ± 0.07	1.09 ± 0.03
6 mo	1.15 ± 0.04	1.15 ± 0.04	1.19 ± 0.04
EGFr[e]			
Baseline	1.34 ± 0.08	1.42 ± 0.10	1.39 ± 0.11
6 mo	1.36 ± 0.06	1.37 ± 0.06	1.33 ± 0.06
Bax:Bcl-2 ratio[f]			
Baseline	1.25 ± 0.07	1.30 ± 0.10	1.23 ± 0.06
6 mo	1.20 ± 0.05	1.14 ± 0.05	1.22 ± 0.05
Bax: PCNA ratio[g]			
Baseline	0.88 ± 0.05	0.76 ± 0.05	0.76 ± 0.05
6 mo	0.89 ± 0.05	0.82 ± 0.05	0.84 ± 0.05

[a]Baseline data are unadjusted means + SE. All other data are least-squares means adjusted for baseline measurement ± SE. Abbreviations are as follows: HSCORE, immunohistochemical histological score; SPI+, isoflavone-rich soy protein isolate (40 g soy protein, 107 mg isoflavones/day); SPI–, alcohol-extracted soy protein isolate (40 g soy protein, <6 mg isoflavones/day); MPI, milk protein isolate (40 g milk protein); Bax, B-cell non-Hodgkin lymphoma-2 (Bcl-2)-associated X protein; PCNA, proliferating cell nuclear antigen; EGFr, epidermal growth factor receptor. Means in a row without a common subscript letter differ ($P < 0.05$). *, significant within-group change from baseline ($P < 0.05$).

[b]$n = 14$ for SPI+, n = 16 for SPI–, and $n = 14$ for MPI.

[c]$n = 14$ for SPI+, $n = 13$ for SPI–, and $n = 14$ for MPI.

[d]$n = 15$ for SPI+, $n = 14$ for SPI–, and $n = 16$ for MPI.

[e]$n = 15$ for SPI+, $n = 14$ for SPI–, and $n = 13$ for MPI.

[f]$n = 14$ for SPI+, $n = 14$ for SPI–, and $n = 13$ for MPI.

[g]$n = 13$ for SPI+, $n = 13$ for SPI–, and $n = 12$ for MPI.

Cancer Incidence

Of the 53 men without evidence of cancer at baseline biopsy, 49 completed the final prostate biopsy [elected not to undergo final biopsy procedure ($n = 2$), withdrew after 3 mo and elected not to follow up ($n = 1$), and advanced liver cancer ($n = 1$)]. Prostate cancer incidence was more than 6 times higher in the MPI group than in the combined soy groups ($P = 0.013$). Prostate cancer incidence was 38% ($n = 6/16$) in the MPI group vs. 6% ($n = 1/16$) in the SPI+ group and 6% ($n = 1/17$) in the SPI– group.

DISCUSSION

This study evaluated the effects of SPI on prostate tissue antigen expression levels, serum total and free PSA, prostate volume, and PSA density. Consumption of isoflavone-rich SPI had no effects on any of the prostate cancer tumor markers analyzed. However, in the postintervention biopsy tissue from the men consuming alcohol-extracted SPI, we observed lower Bax expression levels (reflecting decreased apoptosis) compared to those consuming MPI and decreased PCNA expression levels (reflecting decreased proliferation) compared to baseline values. Despite these seemingly contradictory effects, there was a trend toward decreased risk of cancer in the soy groups compared to the MPI group.

The lack of effect of SPI+ consumption on total PSA concentrations is consistent with several soy or isoflavone intervention studies in which no change in total PSA was observed (4–13) but inconsistent with a few reports of significant reductions (2) or trends toward reductions (14,15) in total PSA concentrations. Because a nearly significant difference in prostate volume was observed, we also evaluated total PSA standardized to prostate size (PSA density). Neither PSA density nor free PSA concentrations were different among the groups, which is consistent with all studies to date (3,4,10,12,14). We also found no effect of treatment on free PSA percent, which is consistent with Kranse et al. (10) but inconsistent with Dalais et al. (3), who reported increased free PSA percent. A limitation of this study was the lack of data available to calculate PSA doubling time or PSA velocity before and after the intervention.

TABLE 3
Serum PSA differences from baseline[a]

	SPI+ ($n = 20$)[b]	SPI– ($n = 20$)[b]	MPI ($n = 18$)[b]
Total PSA (ng/mL)			
Baseline	5.4 ± 1	5.0 ± 1	5.1 ± 1
3-mo change	−0.8 ± 0.5	−0.8 ± 0.5	−0.6 ± 0.6
6-mo change	−0.5 ± 0.6	−0.8 ± 0.6	−0.2 ± 0.6
Free PSA (ng/ml)			
Baseline	0.9 ± 0.09	0.8 ± 0.1	0.9 ± 0.2
3-mo change	−0.09 ± 0.09	0.04 ± 0.09	−0.10 ± 0.1
6-mo change	−0.07 ± 0.07	−0.02 ± 0.07	−0.06 ± 0.07
PSA %			
Baseline	22 ± 2	19 ± 2	22 ± 2
3-mo change	−0.21 ± 1	0.67 ± 1	−0.74 ± 1
6-mo change	1.03 ± 1	1.18 ± 1	−0.22 ± 1

[a]Baseline data are unadjusted means ± SE. Differences are post-intervention minus baseline and are least-squares means adjusted for baseline measurement + SE. Abbreviations are as follows: PSA, prostate specific antigen; PSA %, free PSA/total PSA; SPI+, isoflavone-rich soy protein isolate (40 g soy protein, 107 mg isoflavones/day); SPI–, alcohol-extracted soy protein isolate (40 g soy protein, <6 mg isoflavones/day); MPI = milk protein isolate (40 g milk protein).

[b]Sample sizes listed at column headings are for all time points except 3 mo MPI ($n = 17$), 6 mo SPI+ ($n = 18$), and 6 mo SPI– ($n = 19$).

TABLE 4
Prostate volume and PSA density differences from baseline[a]

	SPI+ ($n = 10$)	SPI– ($n = 13$)	MPI ($n = 15$)
Prostate volume (cm^3)			
Baseline	52 ± 5	47 ± 5	54 ± 6
6-mo change	-4.3 ± 3^{ab}	$1.6 \pm 2_a$	-5.5 ± 2^b
PSA density (ng/ml/cc)			
Baseline	0.1 ± 0.03	0.09 ± 0.02	0.1 ± 0.02
6-mo change	0.0001 ± 0.01	-0.003 ± 0.01	-0.005 ± 0.01

[a]Baseline data are unadjusted means ± SE. Differences are postintervention minus baseline and are least squares means adjusted for baseline measurement ± SE. Abbreviations are as follows: PSA, prostate specific antigen; PSA density, total PSA/prostate volume; SPI+, isoflavone-rich soy protein isolate (40 g soy protein, 107 mg isoflavones/day); SPI–, alcohol-extracted soy protein isolate (40 g soy protein, <6 mg isoflavones/day); MPI, milk protein isolate (40 g milk protein). Means in a row without a common subscript letter differ ($P < 0.05$).

Consumption of SPI+ did not affect the expression of the apoptotic cancer biomarkers, Bax and Bcl-2, analyzed in baseline and ending prostate biopsy cores. The lack of effect on apoptotic markers is in contrast to in vitro data showing increased Bax when LNCaP cells were exposed to 100 μM genistein (22), and one study that reported higher apoptotic index in prostate specimens obtained from men who consumed isoflavone extract compared to historically matched controls (8). The disparity between the results of this last study (8) and our results could be explained by different treatment regimens (red clover vs SPI), control groups (historically matched vs. placebo controlled), and analytical methods for apoptosis (apoptotic index vs specific signaling proteins, i.e., Bax and Bcl-2). Although comparing Bax to the antiapoptotic signaling protein Bcl-2 may indicate apoptosis status in prostate cancer biopsy specimens (23,24), most of our subjects did not have prostate cancer. Bcl-2 was scored only in the luminal layer, and consistent with the literature, we found that benign glands had minimal to absent staining of Bcl-2 in these cells (28). Given the small range in Bcl-2 HSCORE in our study, larger tissue sections and more subjects would need to be evaluated for improved reliability of the Bax to Bcl-2 ratio within precancerous lesions. Thus, utilizing biopsy cores from preneoplastic prostate glands for this endpoint was a limitation of our study design.

Although consumption of SPI+ did not influence Bax, consumption of SPI– significantly decreased Bax from baseline such that at 6 mo, it was lower than the other 2 groups. These findings are consistent with the hypothesis that isoflavones increase Bax and suggest that a different constituent of SPI+ decreases Bax, resulting in a neutral effect when they are present together and a reduction of Bax when isoflavones are removed (29–31). Given that this study is the first to evaluate these markers and reproducibility problems have been documented, this finding may also be explained by chance.

Consumption of SPI+ did not alter PCNA, whereas consumption of SPI– decreased PCNA from baseline, although there were no differences among the groups at 6 mo. These results are inconsistent with rodent studies that have shown that soy protein concentrate (19) or physiologic concentrations of genistein (20) suppressed PCNA staining. PCNA is an auxiliary protein of DNA polymerase that reaches maximal expression during the DNA replication phase (S phase) of the cell cycle. Therefore, abundant PCNA in the cell reflects DNA replication,

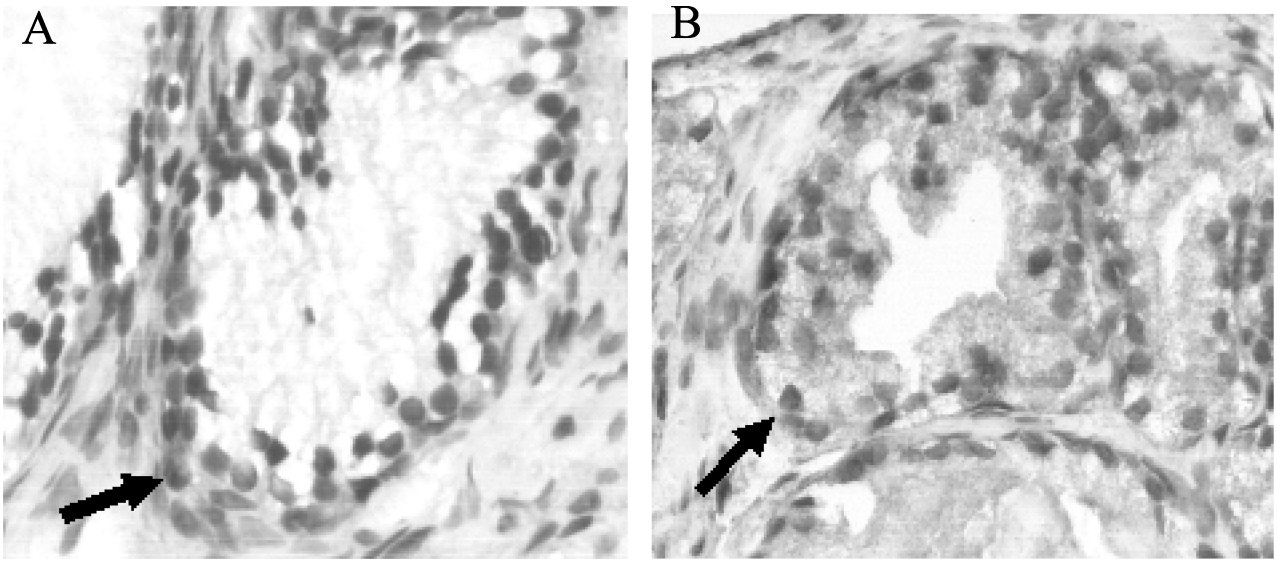

FIG. 1. Representative immunohistochemical staining of human prostate core biopsies for HSCORE (A) PCNA and (B) BAX. Arrow indicates stained acinar cell.

and several studies have confirmed that the PCNA index is directly correlated with prostate cancer progression (32–35).

We found no change in EGFr by either SPI+ or SPI– consumption, which is in contrast to animal studies in which dietary genistein downregulated EGFr mRNA expression during the early phase of prostate cancer development in the TRAMP model at physiologically plausible doses (20,21). EGFr activates transcription through either the EGFr-Shc-SOS-Ras-Raf-ERK1/2 or the phosphatidylinositol-3′kinase-AKT pathways leading to cellular proliferation, angiogenesis, and apoptosis evasion. A limitation of our study is that we measured EGFr expressed and not phosphorylated or activated. Thus, further studies are needed to evaluate the effects of SPI consumption on the activation of the EGFr pathways.

Last, we observed different rates at which patients progressed to a malignant diagnosis at study end. Malignancy was diagnosed 6 times more often in the MPI group than in the combined soy groups (SPI+ and SPI–). Though interesting and relevant, this finding should be interpreted with caution given that this short-term study was not designed to investigate progression to cancer. In light of data associating soy consumption with decreased prostate cancer risk in epidemiological studies (1) and mechanistic evidence of hormonal changes in this population (25,36), further soy interventions designed with prostate cancer onset or progression as endpoints are warranted.

To our knowledge, this is the first randomized controlled study on the effects of SPI intervention on prostate tissue biomarkers in men at high risk of developing prostate cancer. Consumption of isoflavone-rich SPI had no effects on any of the prostate cancer tumor markers analyzed. However, consumption of alcohol-washed (isoflavone-poor) SPI had mixed effects, decreasing proapoptotic Bax expression levels and decreasing proliferation as reflected in PCNA expression levels. These data suggest that there may be multiple constituents of SPI that exert varied effects on prostate cancer biomarkers. Importantly, we observed a lower rate of prostate cancer development in men in the soy groups compared to the milk group. Further research should be conducted to determine whether soy delays the onset and progression of clinically significant prostate cancer and to identify the responsible soy components.

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