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Dietary soy and tea mitigate chronic inflammation and prostate cancer via NFκB pathway in the Noble rat model☆

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Abstract

Chronic inflammation and nuclear factor-kappa B (NFκB) have been implicated in prostate cancer development; thus, dietary factors that inhibit NFκB may serve as effective chemo-preventative agents. Prostate cancer risk is significantly lower in Asian countries compared to the United States, which has prompted interest in the potential chemopreventative action of Asian dietary components such as soy and green tea. This study examined the effects of dietary soy and tea on NFκB activation and inflammation in vivo using a hormone-induced rat model for prostate cancer. Male Noble rats implanted with estradiol and testosterone were divided into 4 dietary groups: control, soy, tea, or soy+tea. NFκB activation and inflammatory cytokines were measured post implantation. The combination of soy and tea suppressed NFκB p50 binding activity and protein levels via induction of IκBα. Soy and tea also decreased prostate inflammatory infiltration, increased Bax/BcL2 ratio and decreased protein expression of tumor necrosis factor-alpha, interleukin (IL)-6 and IL-1β compared to control. Soy and tea attenuated prostate malignancy by decreasing prostate hyperplasia. These effects were not apparent in groups treated with soy or tea alone. The ongoing in vivo studies thus far suggest that combination of foods, such as soy and tea, may inhibit hormone-induced proinflammatory NFκB signals that contribute to prostate cancer development.
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Keywords: Soy; Green tea; Inflammation; Noble rat; Prostate cancer; Nuclear factor kappa B

1. Introduction

Prostate cancer is the second leading cancer-related cause of mortality in American men [1]. Epidemiological studies have shown that the incidence of latent prostatic lesions in men appear to be uniform across Asian and Western countries, but prostate cancer outcome is considerably higher in Western countries [2]. Migration studies indicate that prostate cancer rates increase considerably when Asian migrants move to the United States [3], and the first generation Asian migrants with high soy and tea intake have lower incidences of prostate and mammary cancers [4]. Together, this suggests that the etiology of prostate cancer is highly influenced by environmental factors such as diet. A typical soy-rich Japanese diet consists of 25–100 mg soy isoflavones per day, whereas the typical American diet contains ~1–3 mg soy isoflavones per day [4,5]. In addition, green tea consumption in Asian countries averages 360–480 ml/d only whereas 8% of Americans regularly consume ~180 ml/d [6].

Soy and green tea consumption has been associated with a lower risk of prostate and several other cancers [7–10]. Soybeans and green tea contain bioactive components that have anti-carcinogenic properties, with soy isoflavones and tea catechins as the two major constituents, respectively. The anti-cancer effects of soy isoflavones and green tea catechins may target different stages of the carcinogenesis pathway by inhibiting proliferation [11,12], angiogenesis and metastasis [13,14], activating phase II enzymes or modulating immune functions [4,15,16]. Nonetheless, a gap in knowledge exists as to whether dietary whole soy or green tea modulates chronic inflammation that would otherwise contribute to prostate carcinogenesis *in vivo* [17]. Nuclear factor-kappa B (NFκB), a transcription factor that regulates immune responses and cell proliferation, is constitutively active in prostate cancer, and mediates chronic

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inflammation and carcinogenesis in colitis and hepatitis mouse models [17,18]. Molecular strategies that target NFκB have been shown to suppress prostate cancer progression [19]. Soy isoflavones and green tea catechins have been shown to modulate NFκB in vitro [20–22]. However, the in vivo effects of dietary soy and green tea on hormonal microenvironments, NFκB activation, and inflammation in the prostate require further investigation.

This study sought to examine the effects of dietary soy and green tea on prostate carcinogenesis using the hormone-induced prostate cancer Noble rat model. The outcome of the proposed investigations would enhance our understanding of the significance of NFκB regulation and chronic inflammation in prostate carcinogenesis. We hypothesized that soy or green tea would attenuate prostate cancer development in Noble rats by inhibiting NFκB activation and mitigating inflammatory responses implicated in the development of prostate cancer. The study design also examined the potential for synergism, by investigating the effects of combined soy and green tea treatments on prostate cancer development.

2. Methods and materials

2.1. Animal care and sacrifice

Male Noble rats 5–6 weeks old were housed in pairs, provided laboratory rodent diet (Harlan Teklad, Kent, WA, USA) devoid of soy isoflavones and tea catechins, and acclimated to the temperature and humidity controlled environment with a 12-h dark: light cycle until 10 weeks of age. At 10 weeks of age (1 week prior to hormone implantation), rats were fed ad libitum with the treatment diets. There were 5 different treatment groups, including one sham group and four hormone-treated groups (Fig. 1). The baseline sham without hormone group was fed AIN-93G diet and deionized water. The hormone treated groups included no treatment (AIN-93G+water), green tea (AIN-93G+2% green tea), soy (200 g soy protein/kg AIN-93G based diet+water), and soy+tea combination (200 g soy protein/kg AIN-93G based diet+2% green tea). Diets were formulated commercially (Research Diets, New Brunswick, NJ, USA). Chunmee green tea (Harney & Sons, Salisbury, CT, USA) was brewed for 2 min in boiling water followed by filtration. A final concentration of 0.05% citric acid (Sigma-Aldrich, St. Louis, MO, USA) was added into freshly brewed and cooled green tea [23]. Green tea and soy treatment concentrations were determined based on previous studies, and green tea concentration was calculated by weight (2 g of green tea in 100 ml water) [23,24]. After 4, 8, 20 and 38 weeks of hormone treatment, blood was collected via cardiac puncture while rats were anaesthetized under 2.5% isoflurane. Plasma and serum were obtained by centrifugation from tubes containing EDTA. Spleen and liver were also excised from rats, flash frozen with liquid nitrogen and stored at −80°C. Prostates were divided for tissue extracts, fixed in formalin for histology and flash-frozen for storage in −80°C.

2.2. Hormone treatments

Noble rats exposed to elevated levels of testosterone plus estradiol have NFκB activation and inflammation in the prostate as early as 4 weeks, precancerous lesions at 16 weeks and prostate tumors by 50 weeks [25,26]. At sexual maturity (11 weeks of age), rats in the hormone-treated groups were implanted subcutaneously with two 3-cm silicone tubes (0.62 ID×0.125 OD×0.32 wall; VWR, West Chester, PA, USA) containing ~14 mg of testosterone and one 2 cm tube containing ~14.8 mg of estradiol (Sigma-Aldrich) [27]. Tubes were prepared via vacuum suctioning, and tube endings were sealed with silicone type medical adhesive (Dow Corning, Midland, MI, USA). Tubes were soaked in 1×phosphate-buffered saline (PBS) at 37°C for at least 12 h and wiped with 70% ethanol prior to implantation. Rats were anaesthetized with 2.5% isoflurane in 100% O_2 prior to subcutaneous insertion of the tubes between the shoulder blades, and tubes were replaced every 8 weeks.

2.3. LC-MS analysis of tea

Chromatographic separation was performed on a Shimadzu (Columbia, MD, USA) high-performance liquid chromatography (HPLC) system consisting of two LC-20AD pumps, SIL-20AC HT autosampler maintained at 4°C, SPD-M20A diode array detector, and DGU-20A degasser using a Synergi Hydro-RP column (150×2 mm), 4-μm particle size, and Security Guard Aqua C18 guard column (4×2 mm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of water (A) and acetonitrile (B), both containing 0.1% formic acid (V/V), and separation was performed at a constant flow rate of 200 μl/min. Initial gradient conditions were 10% B, then increased to 20% B over 20 min, to 30% B over 5 min and to 100% B over 5 min. The gradient was held at 100% B for 7 min, then decreased to 10% B over 1 min and held at 10% for 7 min equilibration.

Flow from the HPLC was monitored by UV in a 280 nm channel extracted from the spectrum collected of 220–400 nm. The flow was then directed to the electrospray ionization source of a Shimadzu LCMS-2010A single quadrupole mass spectrometer. Analytes were detected for quantitation using single ion monitoring, with alternating 500 msec periods of negative and positive mode. Catechin and epicatechin (m/z 289.2), gallocatechin and epigallocatechin (m/z 305.2), catechin gallate and epicatechin gallate (m/z 441.3), and gallocatechin gallate and epigallocatechin gallate (m/z 457.3) were detected in negative mode. Caffeine (m/z 195.1) was detected in positive mode. The concentrations of catechins were identified by comparing retention times can relative peak area to standards. Standards included 50 μg/ml of EGCG, EC, catechins, GCG and caffeine (Sigma-Aldrich) in 0.5% citric acid. Instrument control, data acquisition and quantitation were performed with LCMS solution 3.41 software from Shimadzu.

2.4. Plasma soy isoflavone concentrations

Plasma isoflavones (genistein, diadzein and equol) were measured by using HPLC Coularray as described previously [28]. In brief, serum was buffered with ammonium acetate (pH 4.6), mixed with internal standard (4-hydroxybenzophenone; 110 μmol/L), and subjected to enzymatic hydrolysis (overnight, 37°C) using 200 U β-glucuronidase and 15 U sulfatase prepared ammonium acetate (pH 4.6). After incubation, proteins were precipitated by using acetonitrile (1 ml), the sample was delipidated by using hexane (3 ml), and the isoflavones were extracted three times using 3 ml methyl *tert*-

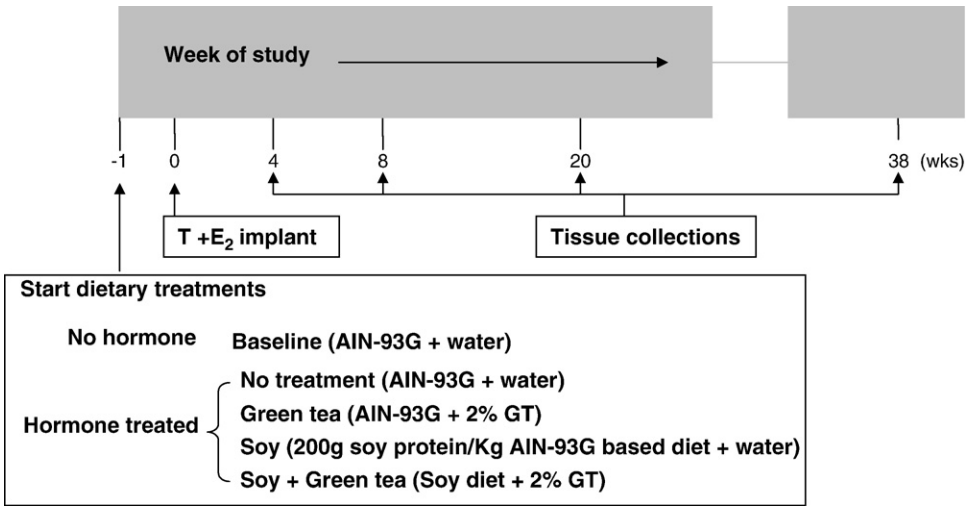

Fig. 1. Dietary treatment plan. Rats were randomly assigned to five dietary treatment groups: baseline without hormone, no treatment (AIN-93G diet), green tea, soy, and soy+tea groups. Testosterone and estradiol were implanted subcutaneously between shoulder blades of hormone-treated rats at sexual maturity (11 weeks of age; time point 0) in the no treatment, green tea, soy and soy+tea groups. Rats were sacrificed and tissue samples collected after 4, 8, 20 and 38 weeks of hormone treatments.

butyl ether. Extracts were combined and dried under nitrogen gas, reconstituted in mobile phase A, and injected on the HPLC system. The sample was separated by binary gradient at 1 ml/min on a C18 Luna, 250×4.6 mm, 5 μm (Phenomenex, Torrance, CA) by using 25 mmol potassium phosphate buffer/L (pH 2.7) as mobile phase A and methanol: acetonitrile:mobile phase A (50:30:20) as mobile phase B. The gradient was delivered as follows: 50% B to 65% B from 0 to 20 min, a linear gradient to 75% B from 20 to 30 min, and a linear gradient to 100% from 30 to 35 min. Initial conditions were restored over 2 min, and the system was equilibrated at 50% B for 12 min before subsequent injection. Analytes were detected by using potential settings of 325, 450, 575 and 700 mV and were quantified on their dominant channel. Plasma isoflavone concentrations were calculated by using area ratios for standards and the internal standard, and the lower limit of quantification was ≈20 nmol/L for each analyte.

2.5. Plasma estradiol concentrations

Plasma estradiol and testosterone concentrations were measured by radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX, USA). Plasma (200 μl) was mixed with estradiol antiserum, followed by addition of radioactive [I-125]-labeled estradiol. The amount of antibody bound [I-125]-estradiol was inversely proportional to the concentrations of estradiol in the samples. Sample counts per minute were taken by Cobra II Auto-Gamma Counter (Packard Instruments, Meriden, CT, USA), and plasma estradiol levels were extrapolated from a standard curve. Testosterone levels were measured under similar procedures.

2.6. NFκB DNA binding activity and cytokine levels

p65 and p50 DNA binding activities were measured by Transcription Factor Assay Kit (ActiveMotif, Carlsbad, CA, USA). Nuclear extract were obtained from prostate tissue using Nuclear Extraction kit (ActiveMotif). Oligonucleotides containing the NFκB consensus site (5′-GGGACTTTCC-3′) were immobilized on a 96-well plate. The active forms of NFκB in the nuclear extracts were bound to the oligonucleotides on the plate, and DNA binding activities were measured colorimetrically by at 450 nm. Tumor necrosis factor-alpha (TNFα), interleukin (IL)-1 beta (IL-1β) and IL-6 enzyme-linked immunosorbent assay (ELISA)-protein levels of TNFα, IL-1β and IL-6 were measured by rat TNFα ELISA and rat IL-1-beta tissue culture ELISA Ready-SET-Go kits (eBiosciences, San Diego, CA, USA) respectively.

2.7. Western blot analysis

Protein levels of pro-apoptotic protein Bax and andti-apoptotic protein Bcl-2, inhibitors of apoptosis proteins (cIAP-1 and cIAP-2) and proteins in the NFκB pathway including p50, p65 (nuclear), IκB kinase (IKK) α, IKKβ, IκBα, and p-IκBα (cytosolic) in treated cells were qualitatively evaluated by the Invitrogen NuPAGE Western blotting system (NuPAGE Novex, Invitrogen, Carlsbad, CA, USA). Nuclear and cytosolic extracts were obtained as described above. All primary antibodies specific against proteins of interest were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), except IKKα and IKKβ were purchased from Cell Signaling Technology (Danvers, MA, USA). Ponceau S red staining and β-actin protein levels were used as protein loading controls. Secondary antibodies were conjugated with horseradish peroxidase (Bio-Rad, Hercules, CA, USA), and proteins were detected by Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, Boston, MA, USA) and imaged by Alpha Innotech photodocumentation system (Alpha Innotech, San Leandro, CA, USA). Densitometry and quantifications were performed using NIH Image J software.

2.8. Prostate histology and scoring

Freshly excised prostate dorsolateral lobes were fixed in 10% paraformaldehyde in PBS over night. One-step sections (3.5 μm) were prepared onto slides and stained with Harris hematoxylin, followed by 1% eosin. Slides were then dehydrated in 95% and 100% ethanol, cleared in xylene and mounted with Permount. The sections were examined by light microscopy in a blinded fashion for morphology and prostate lesions by a board certified veterinary pathologist (C.L.) from the Veterinary Diagnostic Laboratory at Oregon State University. Prostate acini infiltrated by inflammatory cells, prostate stromal volume (10× magnification) and epithelium thickness (40× magnification) were scored using a histomorphometric software (OsteoMeasure system, OsteoMetrics, Atlanta, GA, USA).

2.9. Statistics

Measurements were performed independently for three times with multiple biological samples (n=4–6). Data were analyzed using GraphPad Prism V4.0. One-way analysis of variance and Tukey–Kramer multiple comparison test were used to evaluate the statistical differences. Values not sharing the same superscript letter differ (P<.05).

3. Results

3.1. Estradiol plus testosterone increased prostate mass and circulating estradiol

The average food consumption was approximately 15 g of diet per rat per day, and the green tea group consumed an average of 45 ml green tea per day, which contained 60 mmol of EGCG. There were no significant differences in food intake among groups, but hormone treatments decreased body mass (Table 1) as reported previously. Similar to prior work in this model [27,29], prolonged estradiol and testosterone treatments increased prostate mass and circulating estradiol level without affecting circulating testosterone level. The lack of changes in serum testosterone levels might indicate more stringent regulation, but the specific mechanism is unknown. No significant changes in prostate size, body weight, or plasma hormone levels were observed between groups within the hormone-treated groups.

3.2. Catechin and isoflavone levels

Animals in the tea groups ingested ~86.15 mg/100 ml tea, similar to circulating plasma levels in human consuming green tea [30]. Tea polyphenols were detected by LC-MS. Catechin, epicatechin, epigallocatechin, epigallocatechin gallate, gallocatechin gallate, epicatechin gallate, and caffeine at concentrations of 0.36, 3.6, 11.3, 12.9, 1.1, 5.2 and 16 mg/100 ml tea, respectively. Soy isoflavone concentrations in the soy diet were measured by Covance Laboratories and Research Diets, Inc. Soy isoflavones included glycosides daidzin (542 ppm); glycitin (92.5 ppm); genistin (1160 ppm) and their respective aglycones: dadzein (<50 ppm), glycitein (<50 ppm) and genistein (50.5 ppm). Plasma genistein, daidzein, equol and combined concentrations of all three isoflavones increased with both soy and soy+tea treatment compared to rats fed AIN-93G diets (Table 2). The plasma soy isoflavone concentrations in soy+tea group were significantly higher than the soy alone group, suggesting that consumption of tea might have augmented soy isoflavone uptake or reduced elimination.

3.3. Dietary soy and tea reduced prostate malignancy

Progression to prostate malignancy in the Noble rat model is characterized by morphological changes including thickening of the stroma and epithelial cells, both preceding neoplasia in the prostate [27,31]. Hormone treatment significantly increased prostate epithelial thickness and stromal volume (Table 3). Tea alone and in combination with soy decreased stromal volume, but only the soy +tea group significantly reduced epithelial thickness. Importantly, the incidence of hyperplasia was significantly reduced to baseline levels with combined soy+tea treatment, providing direct evidence for its efficacy in reducing progression to prostate cancer. No apparent decrease in the incidence of hyperplasia was observed in soy or green tea treated groups alone.

3.4. Inflammatory markers

The Noble rat model is characterized by inflammatory response in the prostate at early time points [31–34]. As early as 4 weeks post implantation, hormone treatments increased the infiltration of inflammatory cells including macrophages, B and T cells into the prostate, which appeared to be decreased by combined soy+tea treatment (data not shown). At 20 weeks, the soy and tea group had quantitatively less prostate acini that were infiltrated by inflammatory cells compared to other hormone-treated groups (Fig. 2A), including acini surrounded by inflammatory cells and acini with

Table 1
Animal characteristics

	Food intake (g/rat per day)	Prostate mass	Body mass (g)	Plasma estradiol (log pg/ml)	Plasma testosterone (ng/ml)
Baseline	15.27±0.46	0.71±0.03[a]	398.3±23.9[a]	ND[a]	0.63±0.13
Hormone treated					
No treatment	14.94±0.50	1.29±0.07[b]	305.3±6.8[b]	1.44±0.11[b]	0.54±0.13
Tea	14.33±0.45	1.26±0.04[b]	291.2±6.3[b]	1.46±0.11[b]	0.97±0.35
Soy	14.07±0.39	1.29±0.08[b]	288.5±2.9[b]	1.67±0.23[b]	1.10±0.29
Soy+tea	15.50±0.39	1.32±0.11[b]	315.3±7.5[b]	1.99±0.26[b]	0.92±0.40

Hormone treatments led to enlarged prostates, decreased body weights, and elevated plasma estradiol levels at 20 weeks post implantation. No significant difference in food intake or plasma testosterone levels was observed.
Values represent means±S.E.M., n=4 for Baseline and n=6 for all other groups.
Values in the prostate mass column were normalized to body weight and multiplied by 100.
ND, not detectable.
Values not sharing the same superscript letter (a, b, c) differ (P<.05).

inflammatory cells infiltrated into the epithelium and lumen. In contrast, soy or green tea alone did not show a decrease in infiltrating inflammatory cells compared to hormone-implanted animals fed the control diet (no treatment group).

To profile chronic inflammatory responses, TNFα, IL-6 and IL-1β were assessed (Fig. 2B-D). Hormone implantation had no effect on TNFα and IL-6 but significantly increased the concentration of IL-1β compared to no hormone baseline group. Dietary treatment of soy +tea decreased protein levels of TNFα, IL-6 and IL-1β significantly. At 20 weeks, the transcript levels of IL-1β and IL-6 were increased following hormone treatment, but soy+tea treatment decreased levels of IL-1β and IL-6 mRNA expression in the prostate (data not shown). Notably, the transcript levels of TNFα at 20 weeks were very low, supporting the view that activation of TNFα was likely an early, transient inflammatory response.

3.5. Dietary soy and tea decreased inflammation via NFκB pathway

At 8 weeks post implantation, there were no significant changes in p65 DNA binding activity among treatment groups, but hormone treatments increased p50 DNA binding activity (Fig. 3). Green tea, but not soy, significantly decreased the hormone-induced p50 binding. However, the treatment of soy+tea more effectively decreased NFκB p50 DNA binding activity, suggesting the combination of dietary agents might exert additional inhibitory effects on NFκB DNA binding activity.

Thus, NFκB p65 and p50 protein levels were measured at 8 weeks post hormone implantation in prostate nuclear extracts. Hormone treatments increased nuclear p50 protein levels, which was inhibited by tea, and more so by soy+tea treatment, whereas soy alone had no effect (Fig. 4A). No significant changes in nuclear p65 were detected among groups. NFκB is primarily regulated by a cascade of regulatory proteins [35]. Protein expressions of several major regulator proteins including IKKα, IKKβ, IκBα and p-IκBα in the NFκB pathway were measured. The levels of IKKα and IKKβ were below detection, and there was no significant difference in IκBα expressions among groups, but a trend for decrease in p-IκBα in the soy+tea group (P=.09) (Fig. 4B), suggesting a decrease in degradation of NFκB inhibitory proteins. Interestingly, hormone treatments did not significantly affect NFκB regulatory proteins, implying that there may be other points of deregulation or mechanisms by which the hormones increased NFκB protein and DNA binding ability. Furthermore, inhibition of NFκB might provide higher apoptotic potentials in the soy+tea treated prostates, such that the Bax/BcL2 ratio was increased.

Table 2
Plasma soy isoflavone concentrations

	Genistein	Daidzein	Equol	Total (genistein +daidzein+equol)
	μmol/L			
AIN-93G	ND[a]	0.21±0.03[a]	ND[a]	0.21±0.03[a]
Soy	0.41±0.09[b]	0.24±0.02[b]	0.75±0.05[b]	1.19±0.06[b]
Soy+tea	0.77±0.13[c]	1.23±0.11[c]	1.62±0.20[c]	3.40±0.37[c]

Plasma soy isoflavone concentrations in rats were measured by HPLC; rats in the soy and soy+tea groups had higher plasma concentrations of genistein, daidzein and equol than rats fed the control AIN-93G diet.
Value represent means± S.E.M., n=6.
Values not sharing the same superscript letter (a, b, c) differ (P<.05).

3.6. Dietary soy and tea affected apoptotic pathways

Although no significant differences in prostate mass occurred among all the hormone-treated groups, there was evidence of greater apoptotic signaling in the animals receiving soy+tea in combination. The expression of pro-apoptotic Bax protein and anti-apoptotic Bcl-2 protein were evaluated via Western blotting, and the ratio of Bax/Bcl-2 was determined as a marker for apoptosis. Soy or green tea treatment alone did not significantly change the Bax/Bcl-2 ratio, but the treatments in combination tended (P=.052) to increase Bax and decrease BcL2 levels (Fig. 5A). Inhibitors of apoptosis proteins (cIAP) inhibit caspase activities, positively modulate the NFκB pathways and are over-expressed in prostate cancer cells [36]. There was a nonsignificant trend of increasing protein expressions of cIAP-1 (P=.13) with hormone treatments that was decreased with soy+tea treatment (Fig. 5B). These findings suggest that there was an increase in proapoptotic pathways with combined soy and green tea consumption.

4. Discussion

Prostate cancer is the most common type of cancer found in American men [37]. Low prostate cancer incidence in Asian countries has prompted interest in dietary components in Asian diets, such as soy and tea, as cancer chemoprevention agents. When studying the combinational effects of dietary soy and green tea on hormone-induced chronic inflammation and prostate cancer, we found marked differences in responses between single food and combined dietary strategies. We demonstrated that dietary soy and green tea in combination decreased prostate inflammation and pre-cancerous lesions via attenuation of NFκB and downstream apoptotic pathways. Soy or green tea alone did not exert similar inhibitory actions, suggesting that the interactions between soy and green tea provided additional benefits against prostate cancer. Thus, the concurrent ingestion of soy and green tea may have combined activities that mitigate the risk for developing prostate cancer, particularly in the United States where the consumption of these foods are limited.

There are many concerns about the in vivo bioavailability of soy phytochemicals, but previous studies have shown that soy isoflavonoids accumulate in the prostate gland [38]. The prostate produces active soy isoflavone metabolites and the bioavailabilities of soy

Table 3
Prostate malignancy markers and hyperplasia incidence at 38 weeks post implantation

	Epithelium layer thickness (μm)	Stromal volume Percent (%) total volume	Hyperplasia incidence Percent (%)
Baseline	16.80±1.43[b]	14.10±0.68[b]	16.7
Hormone treated			
No treat	23.64±0.59[a]	31.61±3.74[a]	50
Tea	22.84±0.82[a]	30.50±2.92[b]	66.7
Soy	20.93±0.87[a]	23.91±3.06[a]	50
Soy+tea	18.69±1.73[b]	22.85±2.65[b]	16.7

Epithelium thickness, stromal volume and hyperplasia incidence at 38 weeks post implantation. Tea and soy+tea group decreased stromal volume, but only soy+tea group decreased all three markers of prostate malignancy.
Values represented means± S.E.M., $n=4$ for Baseline and $n=6$ for all other groups.
Values not sharing the same superscript letter (a, b, c) differ ($P<.05$).

isoflavones improve with long-term consumption of soy [38,39]. Our results were consistent with previous observations that soy isoflavones were present at detectable levels in the plasma of soy treated rats [40]. The soy+tea group had higher soy isoflavone concentrations than the soy group (Table 1). It is possible that green tea enhanced the absorption of soy, making soy isoflavones more bioavailable in the prostate. Plasma EGCG was not measured in the present study, but it is possible that soy constituents affected bioavailability of green tea catechins. One previous study reported that co-treatment of genistein increased uptake of EGCG in human colon cancer cells and mice [41]. This study did not explore such mechanisms or examine the effects of other soy constituents on bioavailability of green tea catechins. Green tea may improve absorption of soy constituents by increasing activities of lactase phlorizin hydrolase (LPH) and β-glucosidase that hydrolyze isoflavone glucosides to form aglycones or glucoronidases that metabolize isoflavones for digestion and absorption. Current knowledge of the effects of green tea on β-glucosidase, LPH and glucuronidases responsible for soy metabolism is very limited. Previous studies have indicated that green tea increased hepatic UDP-glucuronosyl

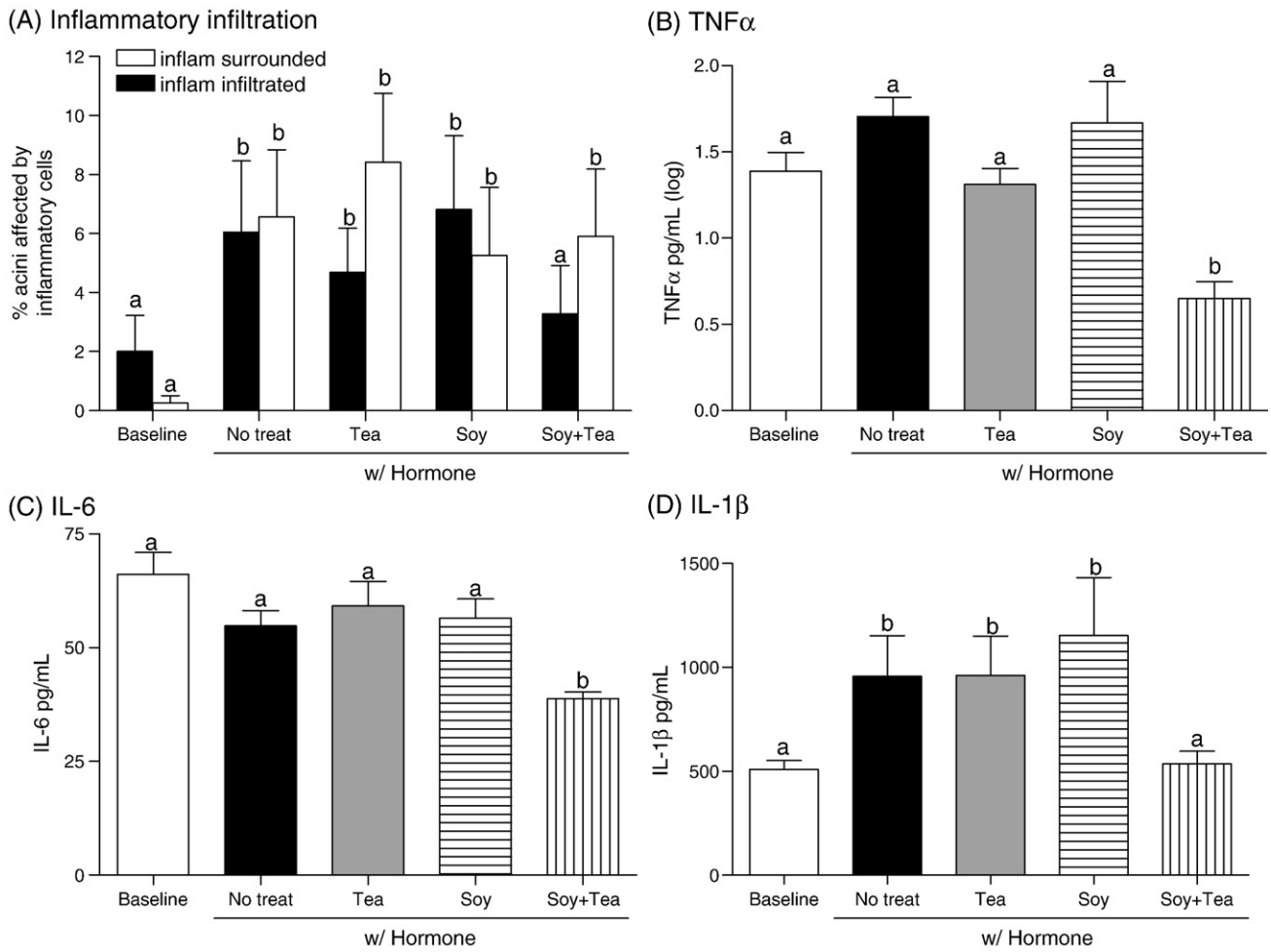

Fig. 2. Dietary soy and tea decreased inflammatory infiltrates, TNF-alpha, IL-6 and IL-1β levels in the prostate of hormone-implanted rats. (A) Scores of inflammatory infiltration in rat prostate at 20 weeks post implantation. At 20 weeks post implantation, soy and tea combination treatments decreased inflammatory infiltration into the rat prostate acini more than other treatments. Protein levels of TNFα (B), IL-6 (C) and IL-1β (D) in prostate cytosolic portions were measured by ELISA. Hormone treatments increased inflammatory cytokines at 4 weeks post implantation, but soy and tea treatment significantly decreased levels of TNFα, IL-6 and IL-1β. Values represented means±S.E.M., $n=4$ for baseline, and $n=6$ for all other groups. Values not sharing the same superscript letter differ ($P<.05$).

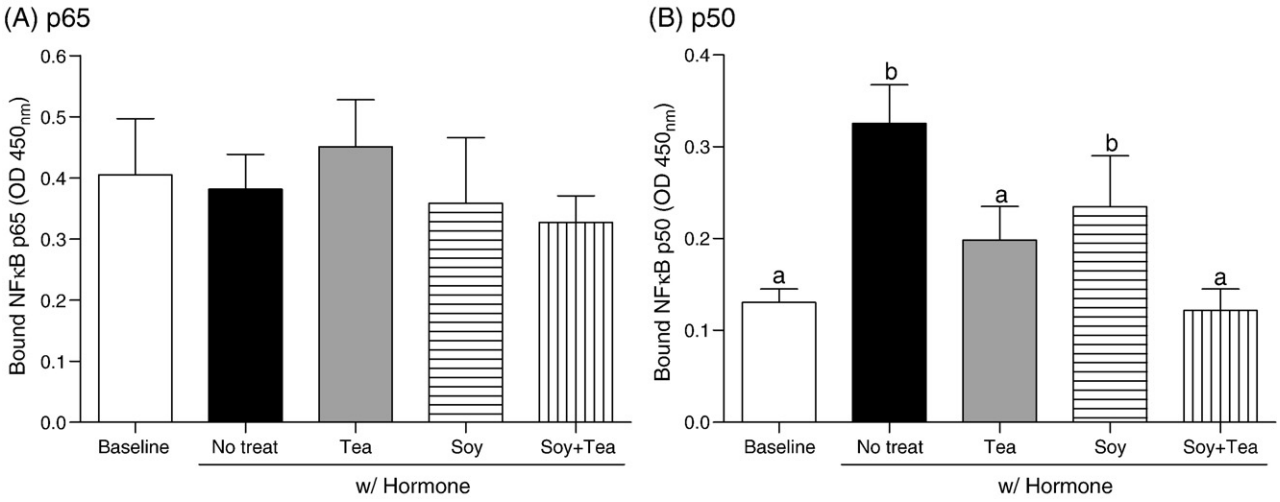

Fig. 3. Dietary tea and soy/tea decreased NFkB p50 binding activity in the prostate. NFκB DNA binding abilities were measured by enzyme immunosorbent assay (ELISA). Dietary treatment of soy+tea significantly decreased NFκB activity in the prostates at 8 weeks post implantation. There were no differences in p65 binding activities (A) among all groups. Hormone implants elevated p50 binding activities (B), but soy and tea significantly decreased p50 activities. Values represent means±S.E.M., $n=4$ for baseline, and $n=6$ for all other groups. Values not sharing the same superscript letter differ ($P<.05$).

transferase activity in rats [42,43], which might contribute to the glucuronidation of soy metabolites for more efficient transport to target tissues [5]. Green tea may also selectively enhance growth of gut bacteria that are crucial for soy isoflavone metabolism and absorption. Studies have showed that green tea had selective bactericidal properties [44], but no studies have investigated the effects of green tea on intestinal flora that are responsible for soy metabolism. Future work on these topics will be essential for understanding the interactions between soy and green tea in vivo.

Previous findings have suggested that blocking inflammation is an effective strategy to prevent prostate cancer progression [45,46]. Inhibition of the COX-2 pathway by non-steroidal anti-inflammatory drugs (NSAIDs) effectively decreased prostate tumor growth [45,46], but long-term NSAIDs usage also elicited adverse gastrointestinal and vascular effects [47,48]. Prostate regions susceptible to carcinoma induction also have lower expressions of anti-oxidative enzymes, including catechol-*O*-methyltransferase, glutathione and quinone reductase [49]. When prostate cells transform to more aggressive

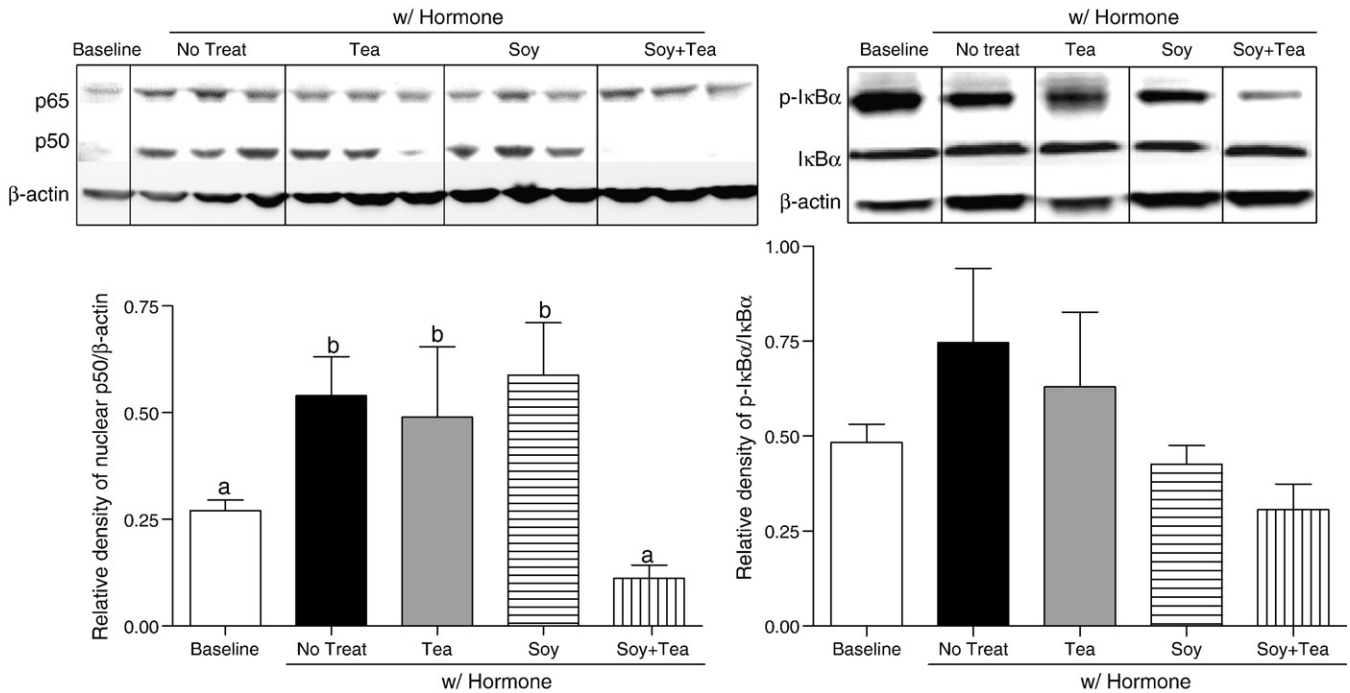

Fig. 4. Dietary soy and tea decreased nuclear NFkB p50 translocation in the prostate. Western blots of NFκB in the prostates at 8 weeks post-implantation. Hormone treatments increased nuclear p50 protein levels, and a trend for increased cytosolic p- IκBα in the prostates. Soy+tea group decreased protein levels of p50, possibly due to decrease p-IκBα. Representative blots are shown. Densitometry values represent means±S.E.M., $n=4$ for baseline, and $n=6$ for all other groups. Values not sharing the same superscript letter differ ($P<.05$).

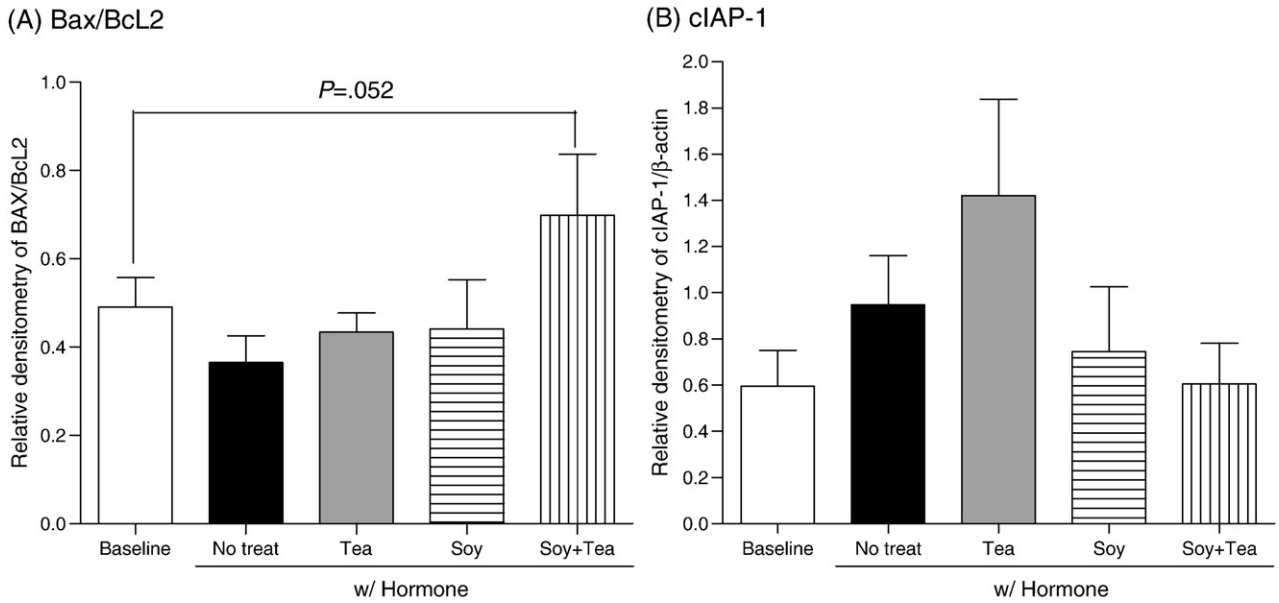

Fig. 5. Dietary soy and tea induced higher BAX/BcL2 ratio and decrease cIAP-1. Protein expression levels of Bax, BcL2 and cIAP-1 of prostate tissues were measured by western blotting. Soy+Tea group increased pro-apoptotic protein Bax and decreased anti-apoptotic protein Bcl2 protein expressions at 4 weeks post implantation. Soy+tea group also showed a nonsignificant trend of decreasing protein expressions of inhibitor of apoptosis, cIAP-1 at 8 weeks post implantation. The densitometry values represented means±S.E.M., $n=4$ for baseline, and $n=6$ for all other groups. $P=.052$ compared to no treatment group.

cancerous cells, the redox balance in estrogen/testosterone metabolism shifts towards production of estradiol and activation of testosterone and dihydroxytestosterone (DHT), leading to proliferative pressure on cells and unregulated prostatic growth [50]. In addition, carcinogenic estrogen metabolites such as 4-hydroxyestradiol can serve as a co-oxidants and strongly stimulate production of pro-inflammatory prostaglandins [51,52]. The NFκB pathway influences many cellular responses that attribute to carcinogenesis, such as regulation of cell cycle, apoptosis and inflammation, and also intimately interacts with hormonal homeostasis. With the loss of redox balance and androgen dependency, deregulation of NFκB becomes a major promoting factor for transformation to malignancy and poor prognosis [53]. Targeting NFκB may have important prevention or therapeutic values against prostate inflammation and cancer.

Results from the present study suggested that hormone treatments increased NFκB p50 DNA binding activities and protein expressions without affecting p65 (Figs. 3 and 4). NFκB p50 has lower affinity for the IκBα regulatory element compared to p65 [54]; therefore, a selective increase in p50 subunits may lack adequate feedback mechanisms and contribute to a chronic inflammatory response. Dietary soy+tea appeared to mitigate NFκB at several levels. The combined soy+tea treatment significantly decreased protein levels of p-IκBα, NFκB p50 protein expressions and DNA binding activity. Thus, the combined treatment of soy+tea may inhibit NFκB activation via decreasing phosphorylation and subsequent degradation of its inhibitory unit, IκBα. Further examination of possible candidate upstream kinases that control IκB phosphorylation are an important area of future interest. Interestingly, soy or green tea alone did not exhibit similar protective effects as the combination treatment. Under the present conditions, neither soy nor green tea alone significantly affected inflammatory infiltration and inflammatory cytokine levels in the prostate. However, green tea decreased p50 DNA binding activity, and soy showed a trend for restoring levels of p-IκBα to that of the baseline group. Green tea also reduced hormone- induced prostate stroma enlargement, but neither soy nor green tea alone changed prostate hyperplasia outcome. Thus, soy or green tea alone might target different molecular endpoints further upstream in the NFκB pathway. Previous studies have shown that EGCG inhibits NFκB inducing kinase (NIK) and subsequent IKK/NIK signaling complex in human lung cancer cells [21], and genistein inhibits proteasome activity responsible for degradation of IκBα [55]. It is possible that dietary levels of soy or green tea alone were not sufficient, but given in combination, they work targeted different pathways, and the anti-inflammatory and anti-cancer effects were amplified.

The current study shows a lack of chemopreventive effects with dietary soy or tea alone, which is in contrast to prior studies that showed high concentrations of soy isoflavone or green tea extracts induced protective effects in other models [56–59]. However, similar to our findings, Cohen et al. utilized lower concentrations of soy protein isolate (≤20% by weight) in the diet and showed a lack of preventative effects against hormone refractory prostate tumor growth in rats [60]. Previous studies utilizing green tea polyphenol extracts containing high concentrations of catechins showed efficacy in inhibiting inflammation and prostate tumorigenicity [15,57,61], but concentrations utilized do not reflect normal human consumption. Studies looking specifically at the combined effects of soy and green tea are limited. One report indicated that genistein increased bioavailability of EGCG, but also increased intestinal tumorigenesis in $APC^{min/+}$ mice [41]. Utilizing high levels of individual bioactive agents, rather than lower levels of agents in combination, may induce adverse effects or ignore cooperative interactions among several components [62,63]. Zhou et al. reported that combination of soy phytochemical concentrate and brewed green tea synergistically inhibited prostate tumorigenicity and metastasis possibly through modulating serum testosterone and DHT in a mouse prostate cancer model [64,65]. Nevertheless, they also found that green tea alone was not sufficient in inducing beneficial effects. Moreover, this study utilized a xenograft model to introduce prostate tumors, which limits the ability to examine chemoprevention at early stages of carcinogenesis. In contrast to these previous studies, the Noble rat model permitted investigation of preventative effects of dietary compounds at the early initiation and promotion stages of prostate cancer, and allowed the examination of chronic inflammation and NFκB activation. Further studies are needed to pinpoint the precise mechanism leading to the synergy between soy and green tea.

In summary, dietary soy and green tea treatments worked together, but not alone, in inhibiting inflammatory cytokine production and inducing apoptosis, possibly through NFκB-dependent pathways. Mitigation of NFκB resulted in attenuation of inflammation in the prostates and inhibited prostate carcinogenesis. Combination of soy and green tea may have provided additional beneficial effects by targeting multiple points along the NFκB pathway, and/or by targeting other mechanisms, such as improving bioavailability of active compounds in the prostate. Overall, we conclude that dietary modifications incorporating soy and green tea, which together target inflammatory and apoptotic pathways, have preventative and therapeutic values against prostate cancer development. The precise mechanism warrant further study.

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