Published in final edited form as:
J Nutr. 2007 April ; 137(4): 964–972.

Soy Isoflavones Exert Differential Effects on Androgen Responsive Genes in LNCaP Human Prostate Cancer Cells[1]

Lori Rice[2,*], **Renita Handayani**[3], **Yuehua Cui**[8], **Theresa Medrano**[3], **Von Samedi**[3], **Henry Baker**[4], **Nancy J. Szabo**[5], **Charles J. Rosser**[6], **Steve Goodison**[7], and **Kathleen T. Shiverick**[3]

2*Department of Radiation Oncology, College of Medicine, University of Florida, Gainesville, FL 32610*

3*Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, FL 32610*

4*Department of Statistics, College of Liberal Arts and Sciences, University of Florida, Gainesville, FL 32610*

5*Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL 32610*

6*Department of Urology, College of Medicine, University of Florida, Gainesville, FL 32610*

7*Department of Analytical Toxicology Core Laboratory, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610*

8*Department of Pathology, University of Florida, Jacksonville, FL 32209*

Abstract

The high consumption of soy isoflavones in Asian diets has been correlated to a lower incidence of clinically important cases of prostate cancer. This study characterized the effects of a soy-derived isoflavone concentrate (ISF) on growth and gene expression profiles in the LNCaP, an androgen-sensitive human prostate cancer cell line. ISF caused a dose-dependent decrease in viability ($P<0.05$) and DNA synthesis ($P<0.01$), as well as an accumulation of cells in G_2/M, and G_0/G_1 phases of the cell cycle compared with controls. Using Affymetrix oligonucleotide DNA microarrays (U133A), we determined that ISF upregulated 80 genes and downregulated 33 genes ($P<0.05$) involving androgen-regulated genes and pathways controlling cell cycle, metabolism, and intracellular trafficking. Changes in the expression of the genes of interest, identified by microarrays, were validated by Western immunoblot, Northern blot, and luciferase reporter assays. Prostate-specific antigen, homeobox protein NKX3, and cyclin B mRNA were significantly reduced, whereas mRNA was significantly upregulated for $p21^{CIP1}$, a major cell cycle inhibitory protein, and fatty acid and cholesterol synthesis pathway genes. ISF also significantly increased cyclin-dependent kinase inhibitor $p27^{KIP1}$ and FOXO3A/FKHRL1, a forkhead transcription factor. A differential pattern of androgen-regulated genes was apparent with genes involved in prostate cancer progression being downregulated by ISF, whereas metabolism genes were upregulated. In summary, we found that ISF inhibits the growth of LNCaP cells through the modulation of cell cycle progression and the differential expression of androgen-regulated genes. Thus, ISF treatment serves to identify new therapeutic targets designed to prevent proliferation of malignant prostate cells.

[1]This work was supported by grant CA91231 from the National Cancer Institute, NIH, and P42 ES07375 from the National Institute of Environmental Health Sciences.

[*]To whom correspondence should be addressed. E-mail: lrice@ufl.edu.

Introduction

An estimated 234,000 men will be diagnosed with prostate cancer in the United States in 2006, and ~27,000 will result in death (1). Epidemiological studies have correlated a relatively low incidence of prostate and other cancers with populations having a high dietary intake of soy products (2-3). These findings have generated an interest in the chemotherapeutic effects of isoflavones, a class of phytoestrogens found in high concentrations in soy and other legumes (4).

The antiproliferative effects of genistein, the predominant isoflavone in soy, on various types of cancers, including prostate cancer, have been well documented (5). Genistein can induce apoptosis and inhibit the activation of the antiapoptotic protection factor, NF-κβ, in prostate cancer cells (6). In addition, genistein inhibits growth in prostate and other cancer cells by the upregulation of $p21^{cip1/waf1}$ and a concomitant decrease in cyclin B, resulting in G_2/M arrest (7). Studies have shown that higher levels of p21 expression are associated with a more favorable prognosis for patients with recurrent prostate cancer after radiation therapy (8). Thus, identifying molecular targets may further the development of new therapeutic strategies.

The advent of cDNA microarray technology allows researchers to profile virtually the entire expressed genome of specific cell types as well as to investigate the effects of potentially useful antiproliferative agents on thousands of genes simultaneously. Using prostate-specific filter arrays, we showed that biochanin A, a red clover-derived isoflavone, inhibits the growth of the prostate cancer cell line, LNCaP, in vitro and in mouse xenografts (9). In this study, because a growing number of cancer patients self-medicate with nutritional supplements (10), with >15% of prostate cancer patients surveyed taking a soy product after diagnosis (11), we used microarray chips to investigate the effects of a commercially available dietary soy-derived isoflavone (ISF)[9] supplement (NovaSoy) on LNCaP cells.

Materials and Methods

Cell culture and treatment conditions

The human prostate carcinoma cell line, LNCaP, was obtained from American Type Cell Culture Collection, cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum, 100 kU/L penicillin-streptomycin, and 2 mmol/L L-glutamine, and maintained in a humidified 5% CO_2 incubator at 37°C. A tissue culture-compatible form of NovaSoy, containing 49% ISF by weight, was obtained from Archer Daniels Midland Company (12) and dissolved in 0.1% dimethyl sulfoxide (DMSO). NovaSoy approximates the natural composition of ISF in soybeans and is low in protein (8.5%) and fat (0.42%). LNCaP cells were treated with NovaSoy at indicated concentrations, or with 0.1% DMSO vehicle alone, for 48 h.

[9]Abbreviations used:

AR androgen receptor

DMSO dimethyl sulfoxide

ISF soy isoflavone extract

PSA prostate specific antigen

Isoflavone analysis

The amounts of free and conjugated glucosides of genistein and daidzein in NovaSoy-treated medium were determined using reversed-phase HPLC with UV and mass spectral detection in series (13-14), as described in detail previously (15). This allowed us to determine the extent to which LNCaP cells hydrolyze the glucoside forms of ISF in NovaSoy to the metabolically active aglycone forms.

Cell viability

LNCaP cell viability was determined by the ability of ISF-treated and control cells to exclude 0.1% trypan blue (Sigma-Aldrich).

[^{3}H]-Thymidine incorporation assay

Cells were treated with 0.1-200 mg/L ISF or DMSO vehicle for 48 h. [^{3}H]-Thymidine (4 μCi/well) was added to the medium and pulsed for 16 h. Cells were collected on glass fiber filters using a Brandel cell harvester and the amount of incorporated [^{3}H]-thymidine was determined by liquid scintillation counting.

Flow cytometry

Isolation and staining of cell nuclei were performed using the Cycle Test Plus DNA reagent kit (Becton Dickinson) according to the manufacturer's protocol. The cells were then subjected to flow cytometric analysis on FACSort (Becton Dickinson) and analyzed by CELL Fit software program. MODFit software was used to quantify nucleic DNA content and extrapolate cell cycle phase distribution based on ratios of mean fluorescence.

Western immunoblots

Western immunoblotting was performed as described previously (11,15), using primary antibodies to human p21$^{CIP1/WAF1}$, cyclin B (BD Transduction Laboratories), p27^{KIP1}, prostate specific antigen (PSA) (DakoCytomation), human GST-A1 (Oxford Biomedical Research), or FKHRL1 (United Biochemicals), and secondary horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG secondary antibodies (Bio-Rad Laboratories). Subsequently, membranes were incubated with anti-human actin mouse monoclonal antibody (Oncogene Research Products) to verify equal loading and transfer efficiency. Specific proteins were detected using enhanced chemiluminescent detection system (Amersham Pharmacia Biotech).

Northern blots

Northern blotting was performed, as described previously, on samples of total RNA isolated by the guanidine thiocyanate method (16). Membranes were hybridized with ^{32}P-labeled probe prepared from a p21 cDNA plasmid, generously provided by Dr. Bert Vogelstein, Johns Hopkins Oncology center, Baltimore, MD (17) and exposed to radiographic film for detection and quantification of mRNA signals using Scion Image software (Scion). The signals were normalized for the total RNA loading and transfer efficiency with β-actin mRNA.

Transfection

LNCaP cells were transfected with a plasmid containing wild-type p21 promoter (p21p)/luciferase reporter obtained from Dr. Vogelstein (17) and cotransfected with pRL-TK vector (Promega) using Lipofectamine 2000 (Invitrogen Life Technologies) according to supplier instructions. Cells were then exposed to 150 mg/L ISF or DMSO vehicle for 48 h and subsequently assayed with the Dual Luciferase Assay System (Promega). Luciferase activity was measured using a MonoLight Illuminator-3010 (Pharmingen). The activity of each assay

was normalized to the activity of the internal control reporter (pRL-TK) to correct for differences in transfection efficiency.

Statistical analysis of biological assays

Experiments were repeated 3 times and all treatments were expressed relative to the control, which was set at 100%. Values from 3 experiments were presented as means ± SEM. Data were analyzed by ANOVA and Tukey's post hoc test for pairwise comparisons. Statistical analyses were performed using Microsoft Excel with Analyze-It add-in software. Differences were considered significant at $P \leq 0.05$.

Gene expression profiling using oligonucleotide microarray chips

The expressed genomes of ISF-treated and control cells were analyzed using Affymetrix Human Genome U133A GeneChip microarrays, according to manufacturer's protocol. Fluorescence intensity of GeneChips transcripts and present or absent calls were calculated using Affymetrix Microarray Suite 5.0 (MAS 5.0). Hierarchical clustering was performed using DNA-Chip Analyzer (dChip) software (18), as described previously (15). Normalized expression intensities were then visualized using colorimetric matrices with red colors indicating relative overexpression and green colors indicating relative underexpression for a given probe set. For genes of interest, average-linkage hierarchical clustering of the data were applied using Cluster and the results displayed using TreeView (19).

Genes were determined to have altered expression levels in ISF-treated samples compared with DMSO-treated samples based on the following criteria: *1*) $P \leq 0.05$ and *2*) 1.4-fold or greater difference between the means of the 2 groups, using the lower bound of the 90% CI (20). The reliability of the comparison criteria was assessed by checking the false discovery rate via permuting the samples. Differentially expressed genes were then categorized based on their cellular component, biological process, and molecular function using Onto-Express (21).

Associations with gene ontology biological process, molecular function, cellular component groups, and GenMAPP biological pathways were obtained with MAPPFinder, a freely available software tool that colors biological pathways with gene expression data (22). MAPPFinder Z-scores, a statistical measure of significance for gene expression in a given group, were calculated by subtracting the number of genes expected to be randomly changed in a gene ontology term from the observed number of changed genes in that term.

Results

Effect of ISF on proliferation and cell cycle progression

Based on ^{3}H-thymidine incorporation and trypan blue dye exclusion assays, ISF dose-dependently inhibited both cell viability ($P < 0.05$) and DNA synthesis ($P < 0.01$) (Fig. 1). A concentration of 42 mg/L ISF produced a 50% inhibition (IC_{50}) of DNA synthesis. A cytostatic concentration of 150 mg/L, resulting in >90% inhibition, was used in subsequent assays.

Flow cytometry was used to determine whether the antiproliferative effects of ISF were associated with alterations in cell cycle phase distribution. ISF treatment for 48 h decreased the percentage of cells in S phase by 74.6% ($P < 0.01$). Concomitantly, ISF increased the accumulation of cells in G_0/G_1 and G_2/M phases by 8.9 and 82.5%, respectively ($P < 0.05$). Interestingly, based on a lack of a sub-G_0 peak and absence of PARP cleavage (data not shown), there was no indication of apoptosis. These data indicate that ISF has potent effects on LNCaP cell proliferation and cell cycle progression.

Availability of free isoflavones in culture medium

Analysis by HPLC-MS showed that the ISF stock solution contained 11% total aglycone equivalents of genistein and 9% daidzein total aglygone equivalents by weight. These predominant isoflavones were present in the bound glucoside form (97%) with only 3% as free aglycones.

Following the exposure of LNCaP cells to 150 mg/L ISF for 48 h, virtually all of the daidzein and genistein was in the metabolically active aglycone form at concentrations of 5.6 mg/L (21.8 μmol/L) and 7.7 mg/L (28.5 μmol/L), respectively, indicating there was complete hydrolysis of the glucoside conjugate (Table 1). In addition, the total amount of genistein and daidzein in the culture medium was reduced by 40%, providing evidence that these cells were able to metabolize the ISF in this soy extract.

Effect of ISF on gene expression profiles, including changes in androgen-regulated genes

Gene expression profiles were evaluated using DNA microarray analyses to identify pathways involved in growth inhibition. Samples were 1st analyzed by hierarchical clustering using a filtered subset of 348 variably expressed genes. A total of 113 known genes were significantly altered, with 80 genes upregulated and 33 genes downregulated (Fig. 2*A*). When permuting samples 50 times, the median false discovery rate was 1.1%, indicating that the comparison criteria were reasonable.

A subset of ISF-regulated genes was identified by a cross-comparison with previously published datasets of androgen-regulated genes in prostate (23-30). Androgen-regulated genes of interest that were altered by ISF treatment are represented by a hierarchical clustering image (Fig. 2*A*). Table 2 lists the fold-changes and putative functions of 27 ISF-regulated genes that have been directly or indirectly related to androgen regulation.

LNCaP is an androgen-sensitive cell line, so it is likely that phytoestrogenic agents, such as ISF, could affect expression of androgen-regulated genes. Of particular interest in this group were the highly downregulated genes kallikrein-2 (KLK2) and kallikrein-3 (KLK3) that encode PSA, a major marker for prostate cancer proliferation (23). In addition, ISF markedly decrease NKX3.1, a homeobox protein, and MAF, a v-maf oncogene homolog (31). An unexpected finding was that ISF upregulated a number of genes involved in metabolic pathways reported to be androgen regulated. This group included malic enzyme 1 (ME1), stearoyl-CoA desaturase (SCD), isopentenyl-diphosphate delta isomerase (IDI1), and 3-hydroxy-3-methyl-glutaryl-CoA synthase (HMGCS1). These data are consistent with reports of androgen regulation of several lipogenic enzymes (29-32). In this regard, however, a divergent pattern of androgen-regulated genes apparent with the primary genes involved in prostate cancer progression were downregulated by ISF, whereas the metabolism genes were upregulated (Table 2). However, several important androgen-related genes were not altered by ISF, including the androgen receptor, prostate androgen-related transcript-1 (Part-1), and alpha-methyl CoA racemase (AMACR).

Table 3 lists a number of genes involved in metabolism, apoptosis/stress responses, molecular function, and cell cycle regulation. Among key upregulated genes were FOXO3A, a forkhead transcription factor involved in the regulation of pro-apoptotic genes (31) and CDKN1A, the gene that encodes $p21^{cip1}$ protein, a major cyclin-dependent kinase inhibitor. Similarly, cyclin B, which is essential for G_2/M cell cycle progression, was downregulated. Overall, changes induced by ISF in cell cycle regulatory genes support our cell viability and flow cytometry data.

An unexpected finding was that ISF upregulated a number of genes involved in lipid metabolism pathways, including fatty acid desaturase-2 (FADS2), lanosterol synthase (LSS),

and fatty acid-CoA synthetase long-chain family member 1 (FACL2/ACSL1). Figure 2*A* includes a hierarchical clustering image showing coordinated regulation of a subset of genes in fatty acid and cholesterol synthesis pathways that includes the androgen-regulated genes from Figure 2*B*. It is striking that they were nearly all upregulated by ISF, suggesting that ISF may be acting to alter expression of sterol-regulatory binding proteins (SREBP), lipogenic transcription factors that regulate cellular lipid homeostasis. However, SREBPs-1c and 2 were not present on our array list. MAPPFinder analysis identified several biological processes influenced by ISF, including cell cycle, G-protein signaling, and apoptosis. Of particular interest was the validation of microarray data showing the upregulation of cholesterol synthesis genes by ISF (Fig. 2B). Seven of 14 genes in the cholesterol synthesis MAPP were significantly increased.

ISF upregulates $p21^{cip1}$ mRNA and protein via transcriptional and translational activation

Microarray data indicated that ISF upregulated CDKN1A, the gene that encodes the $p21^{cip1}$ protein. Northern blot analysis also showed that ISF increased the expression of CDKN1A mRNA by 400% (Fig. 3*A*) and protein by 60% (Fig. 3*B*), ($P < 0.05$), indicating regulation at both the transcriptional and translational levels.

It was of further interest to determine whether the ISF effect on $p21^{cip1}$ mRNA level was due to increased transcriptional regulation of p21 or other mechanisms such as message stability. LNCaP cells, transfected with a $p21^{cip1}$ promoter/luciferase reporter construct and then analyzed by dual luciferase assay following treatment with ISF or DMSO vehicle alone, showed that ISF enhanced transcription of the $p21^{cip1}$luciferase reporter by 175% ($P < 0.003$) (Fig. 3*C*), which suggests direct transcriptional regulation through the gene promoter. To our knowledge, this is the first reported evidence of direct transcriptional regulation of the p21 promoter by an isoflavone mixture.

Soy ISF downregulate PSA mRNA and protein expression

Differential expression of selected genes identified by microarray analyses was validated by independent methods. Microarray data indicated that ISF significantly downregulated the level of PSA mRNA by 86%. PSA is an androgen-regulated biological marker positively associated with prostate cell number. Western immunoblots confirmed that protein levels were also decreased by 96% ($P < 0.01$) (Fig. 4*A*).

ISF affects expression of cyclin B, p27, and FOXO3A, but not glutathione S-transferases

Microarray data indicated that ISF significantly decreased the abundance of cyclin B2 mRNA, a regulator of G_2/M progression, by 60% compared with control values. Western immunoblot analysis confirmed that ISF decreased cyclin B protein by 54% ($P = 0.007$) (Fig. 4*B*). In contrast, ISF increased protein levels of $p27^{KIP1}$, a 2nd major cyclin-dependent kinase inhibitor ($P = 0.019$) (Fig. 4*C*). An unexpected finding of a novel gene upregulated by ISF, as validated by Western immunoblotting (Fig. 4*D*), was FOXO3A/FKHRL1, a forkhead transcription factor involved in the regulation of proapoptotic genes, including p27 (31).

Decreased expression of glutathione *S*-transferases (GST), a family of proteins with antioxidant free-radical scavenging properties has been associated with disease progression in prostate cancer patients (32). Microarray data showed that GST-A1, but not GST-M1 or GST-P1, mRNA was expressed in LNCaP cells and that the level of GST-A1 transcripts was not affected by ISF treatment. Western immunoblot analysis confirmed that GST-M1 and GST-P1 were below the assay level of detection, and that ISF did not alter GST-A1 expression at the protein level (data not shown).

Discussion

This study shows that treatment of LNCaP cells with cytostatic doses of a soy isoflavone concentrate alters global gene expression. The development of microarray technology to analyze multiple genes simultaneously allows the evaluation of changes in differentially regulated genes along functional pathways as well as the identification potential therapeutic targets. The present study utilized Affymetrix GeneChips with the capacity to interrogate over 17,000 human genes to determine the effect of a soy extract on the gene expression profiles of LNCaP cells.

The isoflavones in NovaSoy are predominantly in the bound glucoside form. However, extensive hydrolysis by LNCaP cells during the 48-h incubation period resulted in virtually all of the major ISF, daidzein and genistein, being converted to their more metabolically active "free" aglycone forms, with a 40% reduction in the total amount of these ISF.

During these studies, LNCaP cells were exposed to an ISF supplement containing 59 μmol/L genistein. As little as 15 μmol/L genistein has been shown to affect a 40-60% decrease in PC-3 DNA synthesis, cell viability, and colony formation (33). This agrees with other studies showing that 2.6-79 μmol/L genistein was required to produce a 50% growth inhibition in most cancer cell lines (34). Although 13 μmol/L is considered the upper limit of genistein in the serum of people consuming a high soy diet (34-35), prostate cancer patients given high doses (300-600 mg) of NovaSoy had circulating genistein concentrations of up to 27 μmol/L, with no evidence of genotoxicity (36). Mice with orthotopically implanted LNCaP xenografts that were fed 4 mg/d total genistein equivalents, either as genistein or as a component of an ISF concentrate similar to the one used in these studies, had similar serum genistein levels of 1.6 μmol/L and 1.8 μmol/L, respectively (37). This was sufficient to cause a 70% reduction in tumor growth compared with controls. However, the isoflavone diet resulted in significantly greater induction of apopotosis, enhancement of p53 expression, and inhibition of metastatic activity, compared with genistein alone. Although genistein, the predominant isoflavone in soy, is by itself a potent antiproliferative agent against prostate cancer cells, the authors conclude that the concentrate was even more effective. These studies indicate that isoflavones can be consumed in adequate quantities to exert biological effects. At the same time, it is recognized that higher doses of isoflavones are required in cell culture models to attain the same degree of growth inhibition of prostate cancer cells as seen with xenograft tumors in mice fed an ISF-supplemented diet (12,34,37). Therefore, the multiple biological processes influenced by isoflavones may have a greater impact in the microenvironment of solid tumors.

Our data demonstrate that ISF caused a dose-dependent inhibition of cell viability and DNA synthesis in LNCaP cells. Furthermore, ISF at 150 mg/L was found to inhibit DNA synthesis by 91% and induce accumulation of cells in G_0/G_1 and G_2/M phases of the cell cycle. Microarray analysis identified 113 genes were significantly altered by ISF treatment, with 80 genes upregulated and 33 downregulated. The changes in expression of genes such as p21 (upregulated) and cyclin B2 and kallikrein/PSA (downregulated) suggest a role in ISF-induced cytostasis. Genes involved in fatty acid metabolism also appeared prominently in the list of ISF-regulated genes.

LNCaP cells are androgen sensitive with a mutated but functional androgen receptor (AR). Antiandrogens that suppress androgen production, or competitively inhibit ligand binding, are used clinically to slow the growth of malignant prostate cells that continue to retain expression of the androgen receptor. Therefore, downregulation of the AR or AR-regulated genes also may be useful in reducing androgen-stimulated proliferation of tumor cells. Our dataset agrees with those of other investigators who found that, although isoflavones regulate androgen-responsive genes, the AR itself may or may not have altered expression. These agents can work

to affect activity of the AR via transacting factors. Several datasets comparing the transcriptomes of androgen-supplemented or deprived LNCaP cells, generated using techniques such as SAGE (24-25), oligonucleotide/cDNA arrays (23-24,26), tissue arrays (38), or proteomics (39-40) have been published. Therefore, we were able to identify 46 differentially expressed genes from our data that have been determined to be directly or indirectly regulated by androgens.

Interestingly, although several of the genes listed in Table 2 were also reportedly altered in LNCaP cells by PC-SPES, an herbal supplement known to downregulate the AR (41), some AR-regulated genes responded differently to these 2 extracts. ISF and PC-SPES both inhibited genes with proliferative functions such as KLK/PSA, a serine protease marker for prostate growth (23); beta tubulin, often a target of anticancer drugs such as paclitaxel and vincristine (42); and NKX3.1, a prostate-specific transcription factor. However, there are conflicting reports in the literature regarding changes in NKX3.1 expression as related to tumor progression (38). MAF, a member of a family of differentiation response proteins, is sometimes overexpressed in cancer (23). MAF was downregulated by ISF but not by PC-SPES.

Both ISF and PC-SPES upregulated the cdk-inhibitor CDKN1A/p21 in LNCaP cells. However, ISF upregulated DnaJ, whereas there was no change in PC-SPES-treated cells. Selenium-binding protein 1 (SELENBP1) was upregulated by ISF but decreased by PC-SPES. The ongoing Selenium and Vitamin E Cancer Prevention Trial (SELECT) is based on the theory that selenium may have potent anticancer effects by protecting healthy cells from oxidative damage (43).

Many androgen-regulated genes are involved in metabolism, which may explain the growth-promoting effect of steroids on prostate cell lines with a functioning AR. Most of the genes involved in the biosynthesis of fatty acids and cholesterol are upregulated, such as malic enzyme (ME), fatty acid CoA ligase, long chain 2 (FACL2), stearoyl-CoA desaturase (SCD), and 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS1) (Table 3). Many are known to be under transcriptional control of the sterol regulatory element-binding proteins SREBP-1 and SREBP-2. SREBP are major activators and regulators of fatty acid and cholesterol biosynthesis and link these pathways to nutritional status (44). Furthermore, ISF have significantly upregulated the expression of isopentenyl-diphosphate isomerase (IDI1) whose protein encodes an enzyme involved in sterol synthesis (45). In contrast, UDP-N-acetylglucosamine pyrophosphorylase 1 (UAP1), an enzyme involved in the synthesis of complex carbohydrates, was downregulated. Lipid metabolism genes such as acyl CoA synthase, HMG CoA synthase, and reductase are known to be transcriptionally regulated through androgen response elements in the promoter regions (21,38,41). Cytostatic doses of ISF, which can have weak estrogenic effects, may trigger a damage or stress response that mimics the metabolic effects of androgens.

In agreement with our microarray data, Western immunoblot analysis of the expression of GST in LNCaP cells revealed the presence of GST-A1, which was not altered by ISF treatment. GST-M1 and GST-P1 expressions were below the level of detection. Although GST-P1 expression is abundant in most tumors, it is often lost in prostate cancer and has been implicated in disease progression (46).

This study demonstrated that ISF treatment significantly altered genes involved in multiple cellular processes, including proliferation, cell cycle regulation, cholesterol synthesis, and lipid metabolism. More than 40 androgen-regulated genes were affected. Although the effects of isoflavones on reducing the risk of prostate cancer, or providing benefits to those already diagnosed with this disease, have yet to be unequivocally determined, reports of the low incidence of prostate cancer in populations with high soy diets have resulted in many men taking soy-based supplements (47). It is not yet known how high levels of circulating

isoflavones may affect treatments such as androgen ablation or brachytherapy or the incidence of recurrence. Thus, further studies investigating isoflavone-targeted genes may open new avenues for chemoprevention or therapy for prostate cancer.

Acknowledgments

The authors thank Dr. Burt Vogelstein (Johns Hopkins Oncology Center, Baltimore, MD) for plasmids containing $p21^{CIP1}$ cDNA.

Literature Cited

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer Statistics, 2006. CA Cancer J Clin 2006;56:106–30. [PubMed: 16514137]
2. Adlercreutz CH, Goldin BR, Gorbach SL, Hockerstedt KA, Watanabe S, Hamalainen EK, Markkanen MH, Makel TH, Wahala KT, Adlercreutz T. Soybean phytoestrogen intake and cancer risk. J Nutr 1995;125(Suppl):757S–70S. [PubMed: 7884562]
3. Setchell KD. Phytoestrogens: The biochemistry, physiology, and implications for human health of soy isoflavones. Am J Clin Nutr 1998;68:1333S–46S. [PubMed: 9848496]
4. Agarwal R. Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem Pharmacol 2000;60:1051–9. [PubMed: 11007941]
5. Sarkar FH, Li Y. Mechanisms of cancer chemoprevention by soy isoflavone genistein. Cancer Metastasis Rev 2002;21:265–80. [PubMed: 12549765]
6. Davis JN, Kucu O, Sarkar FH. Genistein inhibits NF-kappa β-activation in prostate cancer cells. Nutr Cancer 1999;35:167–74. [PubMed: 10693171]
7. Davis JN, Singh B, Bhuiyan M, Sarkar FH. Genistein-induced up-regulation of p21WAF1, downregulation of cyclin B, and induction of apoptosis in prostate cancer cells. Nutr Cancer 1998;32:123–31. [PubMed: 10050261]
8. Cheng L, Lloyd RV, Weaver AL, Pisansky TM, Cheville JC, Ramnani DM, Leibovich BC, Blute ML, Zincke H, Bostwick DG. The cell cycle inhibitors, p21WAF1 and p27KIP1, are associated with survival in patients treated by salvage prostatectomy after radiation therapy. Clin Cancer Res 2000;6:1896–9. [PubMed: 10815913]
9. Rice L, Samedi VG, Medrano TA, Sweeney CA, Baker HV, Stenstrom A, Furman J, Shiverick KT. Mechanisms of the growth inhibitory effects of the isoflavonoid biochanin A on LNCaP cells and xenografts. Prostate 2002;52:201–12. [PubMed: 12111696]
10. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. JAMA 1998;280:1569–75. [PubMed: 9820257]
11. Wilkinson S, Gomella LG, Smith JA, Brawer MK, Dawson NA, Wajsman Z, Lanting D, Chodak GW. Attitudes and use of complementary medicine in men with prostate cancer. J Urol 2002;168:2505–9. [PubMed: 12441950]
12. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. J Nutr 1999;129:1628–35. [PubMed: 10460196]
13. Coward L, Kirk M, Albin N, Barnes S. Analysis of plasma isoflavones by reversed-phase HPLC multiple reaction ion monitoring-mass spectrometry. Clin Chim Acta 1996;247:121–42. [PubMed: 8920232]
14. Griffith AP, Collison MW. Improved methods for the extraction and analysis of isoflavones from soy-containing foods and nutritional supplements by reversed-phase liquid chromatography and liquid chromatography-mass spectrometry. J Chromatogr A 2001;913:397–413. [PubMed: 11355838]
15. Handayani R, Rice L, Cui Y, Medrano TA, Samedi VG, Baker HV, Szabo NJ, Shiverick KT. Soy isoflavones alter expression of genes associated with cancer progession, including interleukin-8, in androgen-indpendent PC-3 human prostate cancer cells. J Nutr 2006;136:75–82. [PubMed: 16365062]

16. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidine thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9. [PubMed: 2440339]
17. El Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. Cell 1993;75:817–25. [PubMed: 8242752]
18. Li C, Wong WH. 2001. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc Natl Acad Sci USA 2001;98:31–36. [PubMed: 11134512]
19. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998;95:14863–8. [PubMed: 9843981]
20. Li C, Wong WH. Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. Genome Biology 2001;2research 0032.1-.11.
21. Khatri P, Draghici S, Ostermeier GC, Krawetz SA. Profiling gene expression using onto-express. Genomics 2002;79:266–70. [PubMed: 11829497]
22. Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using gene ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol 2003;4:R7. [PubMed: 12540299]
23. Nelson PS, Clegg N, Arnold H, Ferguson C, Bonham M, White J, Hood L, Lin B. The program of androgen-responsive genes in neoplastic prostate epithelium. Proc Natl Acad Sci USA 2002;99:11890–5. [PubMed: 12185249]
24. Velasco AM, Gillis KA, Li Y, Brown EL, Sadler TM, Achilleos M, Greenberger LM, Frost P, Bai W, Zhang Y. Identification and validation of novel androgen-regulated genes in prostate cancer. Endocrinology 2004;145:3913–24. [PubMed: 15131019]
25. Xu LL, Su YP, Labiche R, Segawa T, Shanmugam N, McLeod DG, Moul JW, Srivastava S. Quantitative expression profile of androgen-regulated genes in prostate cancer cells and identification of prostate-specific genes. Int J Cancer 2001;92:322–8. [PubMed: 11291065]
26. Shi X, Ma AH, Tepper CG, Xia L, Gregg JP, Gandour-Edwards R, Mack PC, Kung HJ, deVere White RW. Molecular alterations associated with LNCaP cell progression to androgen independence. Prostate 2004;60:257–71. [PubMed: 15176055]
27. Takahashi Y, Lavigne JA, Hursting SD, Chandramouli GV, Perkins SN, Barrett JC, Wang TT. Using DNA microarray analyses to elucidate the effects of genistein in androgen-responsive prostate cancer cells: identification of novel targets. Mol Carcinog 2004;41:108–19. [PubMed: 15378649]
28. McPherson R, Gauthier A. Molecular regulation of SREBP function: the Insig-SCAP connection and isoform-specific modulation of lipid synthesis. Biochem Cell Biol 2004;82:201–11. [PubMed: 15052338]
29. Swinnen JV, Heemers H, van de Sande T, de Schrijver E, Brusselmans K, Heyns W, Verhoeven G. Androgens, lipogenesis and prostate cancer. J Steroid Biochem Mol Biol 2004;92:273–9. [PubMed: 15663990]
30. Heemers H, Verrijdt G, Organe S, Claessens F, Heyns W, Verhoeven G, Swinnen JV. Identification of an androgen response element in intron 8 of the sterol regulatory element-binding protein cleavage-activating protein gene allowing direct regulation by the androgen receptor. J Biol Chem 2004;279:30880–7. [PubMed: 15133039]
31. Korkmaz CG, Korkmaz K, Manola J, Xi Z, Risberg B, Danielsen H, Kung J, Sellers WR, Loda M, Saatcioglu F. Analysis of androgen regulated homeobox gene NKX3.1 during prostate carcinogenesis. J Urol 2004;172:1134–9. [PubMed: 15311057]
32. Lynch RL, Konicek BW, McNulty AM, Hanna KR, Lewis JE, Neubauer BL, Graff JR. The progression of LNCaP human prostate cancer cells to androgen independence involves decreased FOXO3a expression and reduced p27KIP1 promoter transactivation. Mol Cancer Res 2005;3:163–9. [PubMed: 15798096]
33. Nelson WG, De Marzo AM, Isaacs WB. Mechanism of disease prostate cancer. N Engl J Med 2003;349:366–81. [PubMed: 12878745]
34. Hillman GG, Forman JD, Kucuk O, Yudelev M, Maughan RL, Rubio J, Layer A, Tekyi-Mensah S, Abrams J, Sarkar FH. Genistein potentiates the radiation effect on prostate carcinoma cells. Clin Cancer Res 2001;7:382–90. [PubMed: 11234894]

35. Barnes S, Peterson TG, Coward L. Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. J Cell Biochem Suppl 1995;22:181–7. [PubMed: 8538197]
36. Adlercreutz H, Markkanen H, Watanabe S. Plasma concentrations of phyto-oestrogens in Japanese men. Lancet 1993;342:1209–10. [PubMed: 7901532]
37. Miltyk W, Craciunescu CN, Fischer L, Jeffcoat RA, Koch MA, Lopaczynski W, Mahoney C, Jeffcoat RA, Crowell J, et al. Lack of significant genotoxicity of purified soy isoflavones (genistein, daidzein, and glycitein) in 20 patients with prostate cancer. Am J Clin Nutr 2003;77:875–82. [PubMed: 12663286]
38. Zhou JR, Yu L, Zhong Y, Nassr RL, Franke AA, Gaston SM, Blackburn GL. Inhibition of orthotopic growth and metastasis of androgen-sensitive human prostate tumors in mice by bioactive soybean components. Prostate 2002;53:143–53. [PubMed: 12242729]
39. Martin DB, Gifford DR, Wright ME, Keller A, Yi E, Goodlett DR, Aebersold R, Nelson PS. Quantitative proteomic analysis of proteins released by neoplastic prostate epithelium. Cancer Res 2004;64:347–55. [PubMed: 14729644]
40. Wright ME, Eng J, Sherman J, Hockenbery DM, Nelson PS, Galitski T, Aebersold R. Identification of androgen-coregulated protein networks from the microsomes of human prostate cancer cells. Genome Biol 2003;5:R4. [PubMed: 14709176]
41. Oh WK, Kantoff PW, Weinberg V, Jones G, Rini BI, Derynck MK, Bok R, Smith MR, Bubley GJ, Rosen RT, DiPaola RS, Small EJ. Prospective, multicenter, randomized phase II trial of the herbal supplement, PC-SPES, and diethylstilbestrol in patients with androgen-independent prostate cancer. J Clin Oncol 2004;22:3705–12. [PubMed: 15289492] Epub 2004 Aug 2.
42. Bonham M, Arnold H, Montgomery B, Nelson PS. Molecular effects of the herbal compound PC-SPES: identity of activity pathways in prostate carcinoma. Cancer Res 2002;62:3920–4. [PubMed: 12124319]
43. Klein EA. Clinical models for testing chemopreventative agents in prostate cancer and overview of SELECT: the Selenium and Vitamin E Cancer Prevention Trial. Recent Results Cancer Res 2003;163:212–25. [PubMed: 12903856]
44. Shimano H. Sterol regulatory element-binding protein family as global regulators of lipid synthetic genes in energy metabolism. Vitam Horm 2002;65:167–94. [PubMed: 12481547]
45. Breitling R, Laubner D, Clizbe D, Adamski J, Krisans SK. Isopentenyl-diphosphate isomerases in human and mouse: evolutionary analysis of a mammalian gene duplication. J Mol Evol 2003;57:282–91. [PubMed: 14629038]
46. Aliya S, Reddamma P, Thyagaraju K. Does glutathione S-transferase Pi (GST-Pi) a marker protein for cancer? Mol Cell Biochem 2003;253:319–27. [PubMed: 14619983]
47. Greenlee H, White E, Patterson RE, Kristal AR. Vitamins and lifestyle (VITAL) study cohort. Supplement use among cancer survivors in the vitamins and lifestyle (VITAL) study cohort. J Altern Complement Med 2004;10:660–6. [PubMed: 15353022]

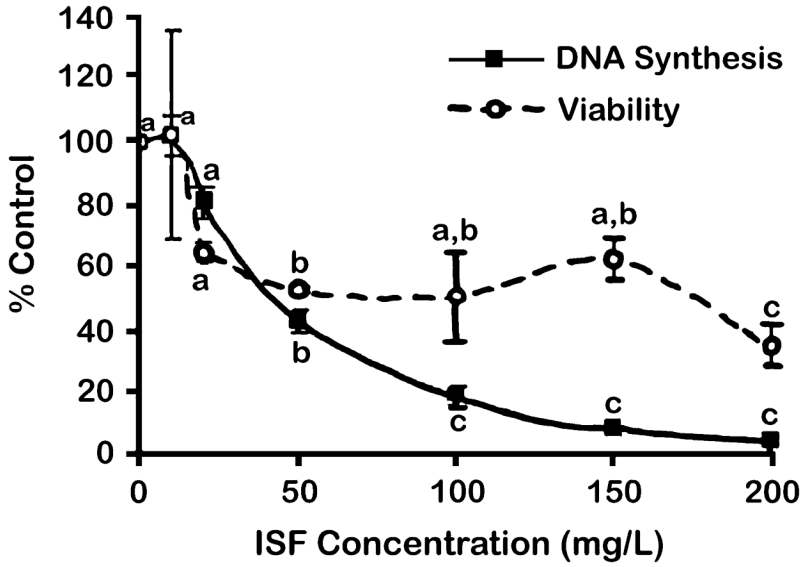

Figure 1.
Effects of ISF on DNA synthesis and viability of LNCaP cells treated with 0-150 mg/L for 48 h. Data are means ± SEM, $n = 3$. Superscripts without a common letter differ from treatment groups: $^{*}P < 0.05$.

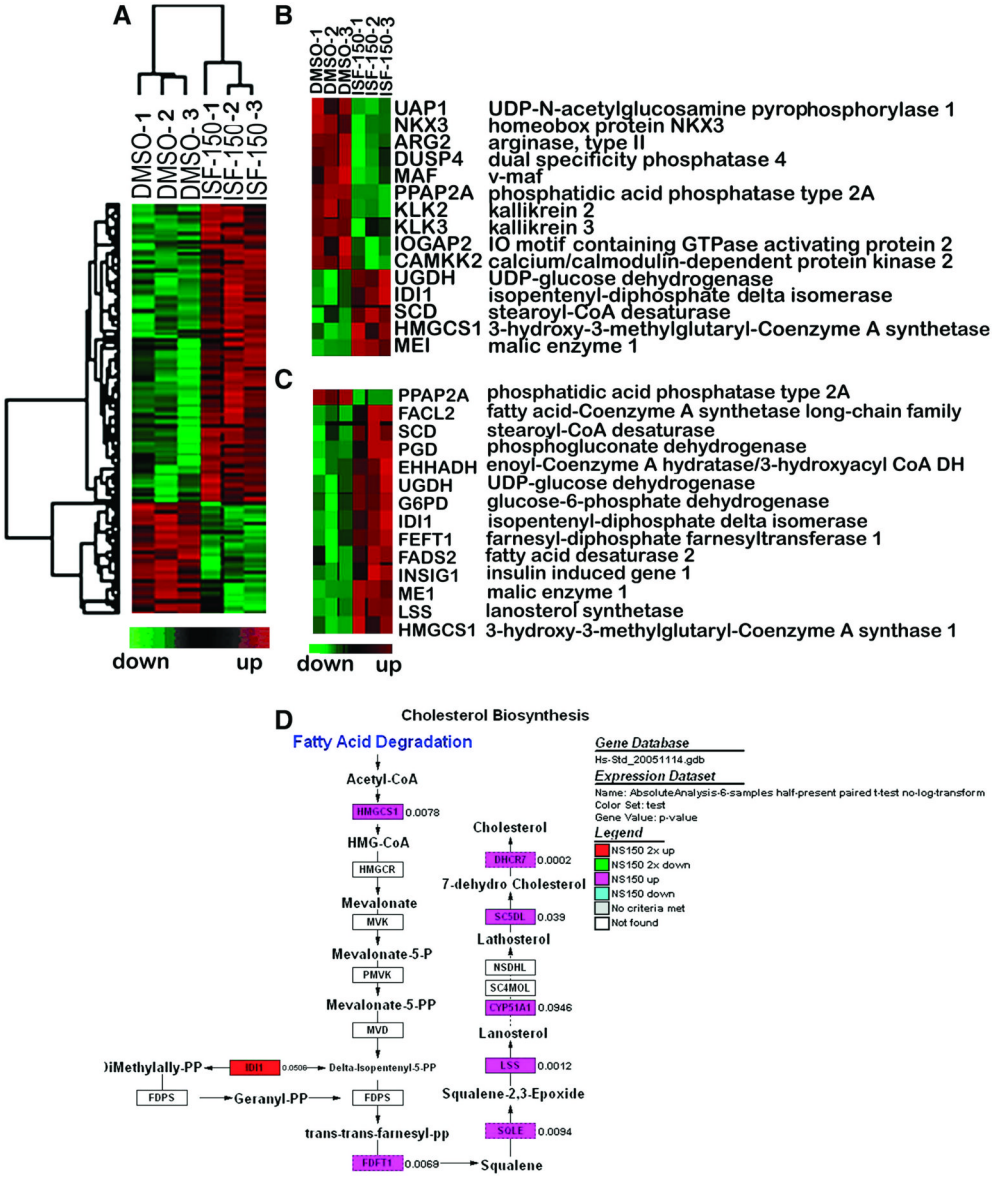

Figure 2.

Hierarchical cluster images of LNCaP genes altered by 150 g/L ISF. The treatment groups are DMSO, cells treated with 0.1% DMSO vehicle alone, and ISF-150, with 150 mg/L soy ISF extract. Column labels represent chip replicates. Cluster image of 113 differentially expressed genes (*A*). Of these, 80 were upregulated and 33 were downregulated. Cluster image, generated by Cluster and TreeView, of a subset of the differentially expressed genes that are directly or indirectly regulated by androgens (*B*). Cluster image showing coordinated regulation of a subset of genes in fatty acid and cholesterol synthesis pathways that includes the androgen-regulated genes from panel B (*C*). Expression changes in genes of the cholesterol biosynthesis pathway (*D*). Using gene-association files from the Gene Ontology Consortium, MAPPFinder assigns genes in the dataset to numerous GO terms and creates functional MAPP if significant (i.e., Z-score > 2.0).

The color scale is obtained by normalization so that the magnitude (sum of the squares of the values) of a row vector = 1; red indicates relative overexpression and green relative underexpression for a given probe set (*A-C*). Genes that increased 2 times more than control

are shown in red, those that increased less than 2 times are shown in purple (*D*). Figures reproduced with permission from (22). Lieberman, M. and Mantei, N. Gladstone Institutes.

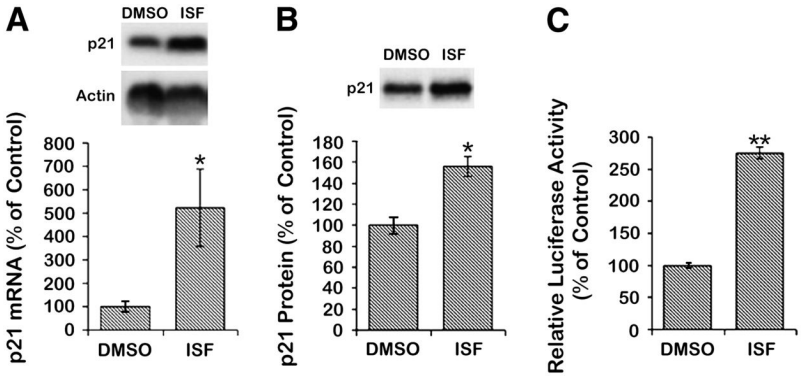

Figure 3.

Validation of microarray data for $p21^{CIP1}$ protein and mRNA expression in LNCaP cells treated with 150 mg/L ISF. Northern blot of $p21^{CIP1}$ mRNA (*A*). Western immunoblot of $p21^{CIP1}$ protein expression (*B*). Representative blots are shown above histograms. Data from Northern and Western blots were normalized to β-actin signals and to control cells treated with DMSO vehicle alone. Activation of a p21 promoter-luciferase construct by ISF treatment in cells transfected with a wild-type p21 promoter/luciferase reporter (*C*). Values are means ± SEM, $n = 3$. Asterisks indicate different from DMSO control: $*P < 0.05$, $**P < 0.003$.

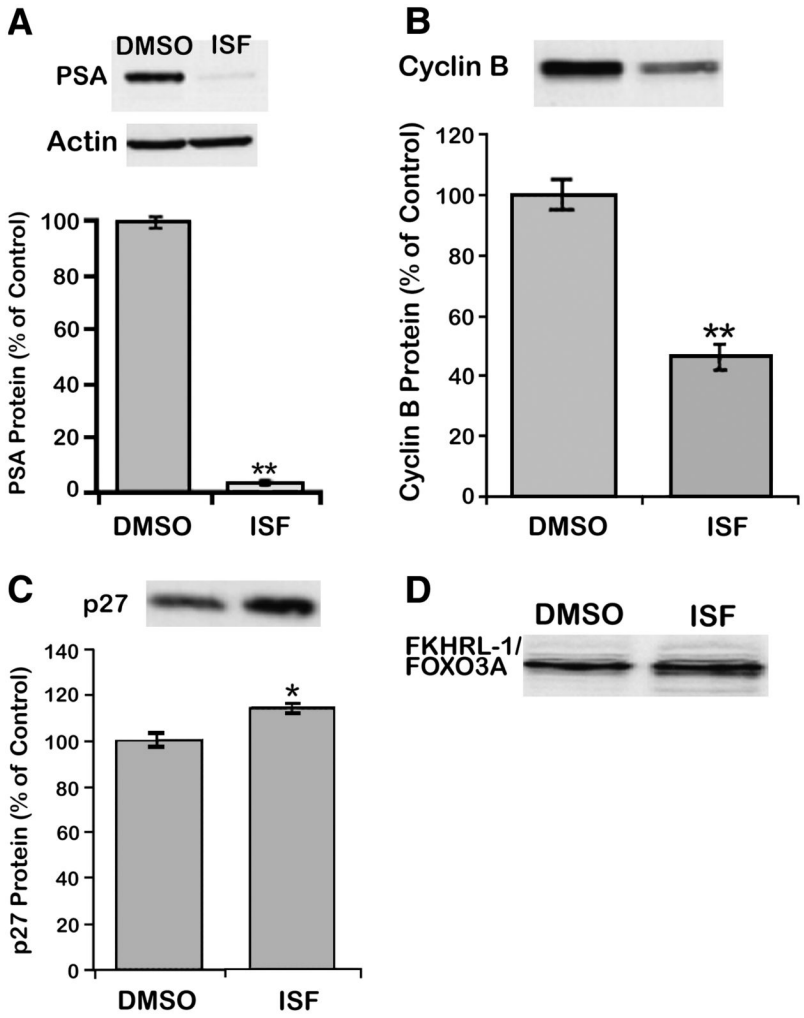

Figure 4.

Validation of microarray data for PSA (*A*), cyclin b (*B*), p27 (*C*), and FOXO3A (*D*) expression by Western immunoblotting in LNCaP cells treated with 150 mg/L soy isoflavone extract. β-Actin expression was used to confirm equal loading and transfer efficiency across the lanes (representative blot shown in panel *A*). Data were normalized to band intensity of control cells treated with DMSO vehicle alone and are shown as means ± SEM, $n = 3$. Asterisks indicate different from DMSO control: $^{*}P = 0.019$, $^{**}P = 0.007$

TABLE 1

Concentrations of genistein and daidzein in LNCaP cell culture medium after 48-h exposure to ISF[1]

Isoflavone	ISF, 20 *mg/L*		ISF, 150 *mg/L*	
	Free aglyone	Total	Free aglyone	Total
			μ*mol/L*	
Genistein	4.4	6.6	28	28
Diadzein	6.3	8.2	25	25

[1] Aliquots of medium were analyzed, in duplicate, directly for free aglycone genistein and daidzein, whereas a 2nd aliquot was hydrolyzed with B-glucosidase to determine total (glucoside + aglycone) genistein and daidzein.

TABLE 2

Proliferation, cell cycle control, and/or tumor progression of androgen-regulated genes in LNCaP cells exposed to 150 mg/L of a soy isoflavone concentrate compared with vehicle alone[1]

Gene	Unigene ID	Accession no.	Gene name	Control vehicle, %
SQSTM1	Hs.182248	NM_003900.1	Sequestosome 1 (activation of NF-κB pathway in bone remodeling)	245
MAF	Hs.134859	AF055376.1	V-maf musculoaponeurotic fibrosarcoma oncogene homolog	47
NKX3	Hs.55999	AF247704.1	Homeobox protein NKX3 (transcription factor)	16
KLK3	Hs.171995	NM_001648.1	Kallikrein 3 (prostate specific antigen, proteolysis)	14
KLK2	Hs.181350	AA595465	Kallikrein 2 (prostate specific antigen, proteolysis)	6
Metabolism				
ME1	Hs.14732	AL049699/ NM_002395	Malic enzyme 1, NADP(+)-dependent, cytosolic (lipid synthesis)	410
EHHADH	Hs.432433	NM_001966.1	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase (fatty acid oxidation)	280
HMGCS1	Hs.397729	NM_002130	3-Hydroxy-3-methyl-glutaryl-CoA synthase 1 (cholesterol synthesis)	230
DPYSL2	Hs.173381	NM_001386.1	Dihydropyrimidinase-like2	220
IDI1	Hs.76038	NM_004508.1	Isopentenyl-diphosphate Δ isomerase (cholesterol synthesis)	218
UGDH	Hs.28309	NM_003359.1	UDP-glucose dehydrogenase	211
FDFT1	Hs.191435	AA872727	Farnesyl-diphosphate farnesyl-transferase 1 (cholesterol synthesis)	207
SCD	Hs.119597	AB032261.1	Stearoyl-CoA desaturase (Δ-9) (fatty acid synthesis)	195
MAOA	Hs.183109	NM_000240.1	Monoamine oxidase A	55
PPAP2A	Hs.528702	AB000888.1	Phosphatidic acid phosphatase type 2A	49
UAP1	Hs.21293	S73498.1	UDP-N-acteylglucosamine pyrophosphorylase 1	47
ARG2	Hs.172851	U75667.1	Arginase, type II	32
Apoptosis or stress response				
SELENBP1	Hs.334841	NM_003944.1	Selenium binding protein 1 (cell growth regulation; peroxisome proliferation)	212
FTH1	Hs.448738	NM_002032.1	Ferritin, heavy polypeptide 1	205
DNAJB9	Hs.6790	AL080081.1	DnaJ (Hsp40) homolog, subfamily B, member 9	205
Other cellular functions, transport, trafficking, or signal transduction				
BAMBI/NMA	Hs.348802	NM_012342.1	BMP and activin membrane-bound inhibitor homolog (xenopus laevis); putative transmembrane protein	281
SMA5	Hs.166361	X83301.1	SMA5 (smooth muscle actin, associated with tumor invasion)	280
ATP1B1	HS.78629	BC000006.1	ATPase, Na+/K+ transporting, β 1 polypepti	228
ADAM10	Hs.172028	N51370/ NM_001110/ AU135154	A disintegrin and metalloproteinase domain 10	200
CAMKK2	Hs.297343	AA181179	Calcium/calmodulin-dependent protein kinase kinase 2, β	56
IQGAP2	Hs.373980	NM_006633.1	IQ motif containing GTPase activating protein 2	42
DUSP4	Hs.417962	NM_001394.2	Dual specificity phosphatase 4	41

[1] Genes directly or indirectly regulated by androgens were identified by cross-comparison with other published datasets (23-30). Genes whose expressions were significantly altered by ISF were classified according to their ontological or biological function. Changes in gene expression ≥1.5-fold differed from controls, $P < 0.05$.

TABLE 3

Ontological classification of other genes significantly altered by an isoflavone concentrate in LNCaP cells, as described in Table 2

Gene	Unigene ID	Accession no.	Gene name	Control vehicle, %
INSIG1	Hs.416385	BE300521	Insulin induced gene 1 (restricts lipogenesis)	368
CDKN1A	Hs.370771	NM_000389.1	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	234
CCNB2	Hs.194698	NM_004701.2	Cyclin B2	35
Metabolism				
FACL2/ACSL1	Hs.268012	NM_021122.2	Fatty acid-CoA synthetase long-chain family member 1	224
G6PD	Hs.80206	NM_000402.1	Glucose-6-phosphate dehydrogenase	212
GALNT10	Hs.13785	BE906572	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 10 (malignant transformation)	204
FADS2	Hs.503546	NM_004265.1	Fatty acid desaturase 2	195
PGD	Hs.392837	NM_002631.1	Phosphogluconate dehydrogenase	198
HIBCH	Hs.236642	NM_014362.1	3-Hydroxyisobutyryl-CoA hydrolase	195
PANK3	Hs.388400	AL565516	Pantothenate kinase 3; Human glucose transporter pseudogene	193
LSS	Hs.442223	AW084510	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase) (cholesterol synthesis)	192
DHFR	Hs.83765	BC003584.1	Dihydrofolate reductase	51
GALNT7	Hs.156856	NM_017423.1	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactos-aminyltransferase 7	50
Apoptosis or stress response				
TXNRD1	Hs.434367	NM_003330.1	Thioredoxin reductase 1	259
GCLM	Hs.315562	NM_002061.1	Glutamate-cysteine ligase, modifier subunit, synthesis of glutathione-S transferase	234
PPP1R2	Hs.2267819	NM_006241.1	Protein phosphatase 1, regulatory (inhibitor) subunit 2	182
GSTK1	Hs.390667	NM_015917.1	Glutathione S-transferase subunit 13 homolog	166
Molecular function				
JUN	Hs.78465	BG491844	V-jun sarcoma virus 17 oncogene homolog (avian), regulation of transcription	259
FOXO3A	Hs.14845	N25732	Forkhead box O3A	219
SESN1	Hs. 14125	NM_014454.1	p53 Regulated PA26 nuclear protein (DNA damage response)	213
TAX1BP1	Hs.5437	AF090891.1	Tax1 (human T-cell leukemia virus type I) binding protein 1, oncoprotein	201
JUND	Hs.2780	NM_005354.2/ AI339541	Jun D proto-oncogene	197
PIR	Hs.424966	NM_003662.1	Pirin (iron-binding nuclear protein) (transcription factor associated with proto-oncogene Bcl-3)	195
RAC3	Hs.45002	NM_005052.1	Ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)	59
SAP30	Hs.512813	NM_003864.1/ AW589975/ BF247098	Sin3-associated polypeptide, 30kDa (regulation of gene expression)	56
KIF2C	Hs.69630	AY026505.1	Mitotic centromere-associated kinesin-like family member 2C	52
CDKN2C	Hs.4854	NM_001262.1	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	51
EIF3S8	Hs.388163	NM_003752.2	Eukaryotic translation initiation factor 3 (over-expression associated with prostate cancer progression)	49
ENDOG	Hs.420106	NM_004435.1	Endonuclease G	49
KIF4A	Hs.279766	NM_012310.2	Kinesin family member 4A	48
KIAA0830	Hs.167115	AL573201	KIAA0830 protein (endonuclease)	51
TOP2A	Hs.156346	NM_001067.1	Topoisomerase (DNA) II	47
CEBPD	Hs.76722	NM_005195.1/ AV655640	CCAAT/enhancer binding protein (C/EBP), Δ	46
BTG1	Hs.2559	AL535380/ NM_001731	B-cell translocation gene 1, antiproliferative (negative regulation of cell growth)	24
Other cellular functions, transport, trafficking, or signal transduction				
NQO1	Hs.406515	AI039874	NAD(P)H dehydrogenase, quinone 1	568

Gene	Unigene ID	Accession no.	Gene name	Control vehicle, %
H. sapiens cDNA clone J10784	HS.287525	AV705244	Homo sapiens mRNA; cDNA DKFZp566G0746 (catalytic activity)	269
RDX	Hs.263871	AL137751.1/ NM_002906.1	Radixin (cytoskeleton, cell proliferation and motility)	239
ASPH	Hs.413557	AF306765.1	Aspartate β-hydroxylase (cell migration)	233
ATP2B1	Hs.20952	L14561	ATPase, Ca++ transporting, plasma membrane 1	203
PIK3C2A	Hs.249235	AV682436	Phosphoinositide-3-kinase, class 2, α polypeptide; Homo sapiens mRNA; cDNA DKFZp564L222	202
GABARAPL3	Hs.334497	AF180519.1	GABA(A) receptors associated protein like 3 (intracellular membrane trafficking, interaction with microtubules)	199
GCA	Hs.377894	NM_012198.1	Grancalcin, EF-hand calcium binding protein	194
ITM2B	Hs.446450	NM_021999.1	Integral membrane protein 2B	193
CLDN3	Hs.25640	BE791251/ NM_001306	claudin 3 (integral to membrane, required for tight junctions, upregulated in various tumors)	61
HEBP2	Hs.439081	NM_014320.1	Heme binding protein 2 (SOUL)	58
OK/SW-cl.56	Hs.356729	AF141349.1	β 5-tubulin	53
SLC43A1	Hs.444159	NM_003627.1	Solute carrier family 43, member 1; prostate cancer overexpressed gene 1 (POV1)	52
PRC1	Hs.344037	NM_003981.1	Protein regulator of cytokinesis 1	42
RACGAP1	Hs.23900	AU153848	Rac GTPase activating protein 1 (intracellular signaling cascade)	39
APOBEC3B	Hs.226307	NM_004900.1	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	39
TM4SF1	Hs.351316	AI189753	Transmembrane 4 superfamily member 1	36