

Molecular Effects of the Herbal Compound PC-SPES: Identification of Activity Pathways in Prostate Carcinoma¹

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Abstract

Clinical trials of the herbal preparation PC-SPES have demonstrated substantial responses in patients with advanced prostate cancer. Biochemical assays and clinical observations suggest that the effects of PC-SPES are mediated at least in part through estrogenic activity, although the mechanism(s) remains largely undefined. In this study, we used cDNA microarray analysis to identify gene expression changes in LNCaP prostate carcinoma cells exposed to PC-SPES and estrogenic agents including diethylstilbestrol. PC-SPES altered the expression of 156 genes after 24 h of exposure. Of particular interest, transcripts encoding cell cycle-regulatory proteins, α - and β -tubulins, and the androgen receptor were down-regulated by PC-SPES. A comparison of gene expression profiles resulting from these treatments indicates that PC-SPES exhibits activities distinct from those attributable to diethylstilbestrol and suggests that alterations in specific genes involved in modulating the cell cycle, cell structure, and androgen response may be responsible for PC-SPES-mediated cytotoxicity.

Introduction

Of the many phytotherapeutic compounds advocated for the prevention or treatment of cancer, the herbal preparation PC-SPES is popular among patients with prostate carcinoma as an alternative to conventional forms of therapy. PC-SPES is available as a dietary supplement and is comprised of extracts from eight different herbs: *Scutellaria baicalensis*, *Glycyrrhiza glabra*, *Ganoderma lucidum*, *Isatis indigotica*, *Panax pseudo-ginseng*, *Dendranthema morifolium* tzel, *Rabdosia rebescens*, and *Serenoa repens*. Analyses of the individual herbs comprising PC-SPES reveal the presence of numerous bioactive compounds that include phytoestrogens, flavonoids, alkanoids, triterpenes, polysaccharides, and trace elements. Several studies have reported the *in vitro* and *in vivo* efficacy of PC-SPES against prostate carcinoma (1). A Phase II trial of 33 patients with AD³ prostate cancer and 37 patients with AI prostate cancer assessed the efficacy and toxicity of PC-SPES in a prospective fashion (2). All AD patients showed declines in PSA levels with a median response duration of 57 weeks. Of patients with AI disease, 54% had a PSA decline with a median time to disease progression of 16 weeks. Despite the clinical use of PC-SPES, few active constituents have been identified, and the mechanisms of antineoplastic activity remain to be determined. Biochemical and clinical studies suggest that the

effects of PC-SPES are mediated at least in part through estrogenic activity (3), and unpublished reports indicate that the synthetic estrogen DES is present in some preparations of PC-SPES.⁴ However, the complexity of the herbal preparation, comprising perhaps hundreds of distinct compounds, implies that other pathways may also be operative. Clinical data raise the possibility that the responses observed with PC-SPES exceed those expected with estrogen alone (2), and studies of the individual herbs comprising PC-SPES report antiproliferative, antimutagenic, and differentiation-inducing activities in multiple tumor types (4–7).

This study was undertaken to determine the molecular mechanism(s) of PC-SPES activity against prostate carcinoma. We used cDNA microarrays to characterize the transcriptional response of LNCaP prostate cancer cells to PC-SPES and compared the gene expression profile with those induced by DES, estradiol, and the synthetic androgen R1881. The transcriptional alterations resulting from these perturbations indicate that PC-SPES exhibits activities distinct from those attributable to DES and suggest that PC-SPES cytotoxicity may be modulated through genes involved in cell cycle control, cell structure, and the AR.

Materials and Methods

Cell Culture and General Methods. DNA manipulations including transformation, plasmid preparation, gel electrophoresis, and probe labeling were performed according to standard procedures (8). Cell lines obtained from the American Type Culture Collection (Manassas, VA) were LNCaP, DU145, and PC3 (each derived from human prostate carcinomas). Cell lines were propagated according to the instructions of the supplier. For experiments determining PC-SPES-mediated temporal gene expression alterations and those comparing PC-SPES with DES, the growth medium was supplemented with 1 or 5 μ M PC-SPES (BotanicLab, Brea, CA), 10 μ M DES, 30 μ M DES (Sigma), or 5 μ M ethanol as control. The PC-SPES lots used for these experiments (lot 5431106 and lot 5431164) do not contain detectable levels of DES as determined by independent laboratory analysis. PC-SPES solubilization was achieved by adding 3.2 g (10 tablets) of PC-SPES to 10 ml of ethanol, incubation for 1 h at 37°C, followed by low-speed centrifugation and filtration with a 0.22 μ m filter. DES (Sigma) was solubilized in DMSO. For experiments comparing PC-SPES with R1881, DES, and estradiol, LNCaP cells were transferred into RPMI 1640 with 10% CS-FBS (Gemini Biosystems, Woodland, CA) 24 h before treatments. This medium was replaced with fresh CS-FBS media or CS-FBS supplemented with the synthetic androgen R1881 (10 nM; New England Nuclear Life Science Products Inc., Boston, MA), 10 μ M 17 β -estradiol (Sigma), 10 μ M DES, 5 μ M/ml PC-SPES, or 5 μ M/ml ethanol as control. Total RNA was isolated at specific time points after cell treatments using Trizol (Life Technologies, Inc.) according to the manufacturer's directions. For the microarray experiments, a reference standard RNA was prepared by combining equal quantities of total RNA isolated from LNCaP, DU145, and PC3 cell lines growing at log phase. RNA derived from a single batch of reference standard was used for every microarray hybridization.

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³ The abbreviations used are: AD, androgen-dependent; AI, androgen-independent; DES, diethylstilbestrol; PSA, prostate-specific antigen; CS-FBS, charcoal-stripped fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; AR, androgen receptor; CDK, cyclin-dependent kinase.

⁴ <http://www.psa-rising.com/medicalpike/pcspes/>.

Microarray Fabrication, Probe Construction, and Hybridization.

cDNA microarrays were constructed as we described previously (9). Briefly, a nonredundant set of 3000 distinct prostate-derived cDNA clones was identified from the Prostate Expression DataBase, a public sequence repository of expressed sequence tag data derived from human prostate cDNA libraries (10). Individual clone inserts were amplified by the PCR, purified, and spotted in duplicate onto Type IV glass microscope slides (Amersham) using a GenII robotic spotting tool (Molecular Dynamics, Sunnyvale, CA; Ref. 9).

Fluorescence-labeled probes were made from 30 μg of total RNA in a reaction volume of 20 μl containing 1 μl of anchored oligo(dT) primer (Amersham); 0.05 mM Cy3-dCTP or Cy5-dCTP (Amersham); 0.05 mM dCTP; 0.1 mM each of dGTP, dATP, and dTTP; and 200 units of Superscript II reverse transcriptase (Life Technologies, Inc.). Reactants were incubated at 42°C for 120 min followed by the hydrolysis of RNA and cDNA probe purification by chromatography (Qiagen, Valencia, CA) as described previously (9). Labeled probes were placed onto a microarray slide with a coverslip, hybridized in a humid chamber at 52°C for 16 h, and washed with SSC gradients. Cy3-labeled cDNA from treated cells was directly compared against Cy5-labeled cDNA from the negative control at each time point. Fluorescent dye labeling was reversed, and a replicate experiment was performed for each sample to control for dye effects.

Image Acquisition and Data Analyses. Fluorescence intensities of the immobilized array targets were measured using a GenII slide scanner (Molecular Dynamics). Quantitative data were obtained with the SpotFinder V 2.4 program.⁵ Local background hybridization signals were subtracted before comparing spot intensities and determining expression ratios. For each experiment, each cDNA was represented twice on each slide, and the experiments were performed in duplicate, producing 4 data points/cDNA clone/hybridization probe. Intensity ratios for each cDNA clone hybridized with treated and control probes were calculated. Gene expression levels were considered significantly different between the two conditions if all four replicate spot ratios for a given cDNA demonstrated a ratio >1.5 or <-1.5 by at least 1 SD, and the average signal intensity was >800 intensity units. Correlation coefficients between array hybridization data sets were calculated in Excel (Microsoft Corp., Redmond, WA) and expressed as *R* values. Selected genes were subjected to hierarchical cluster analysis based on an average linkage clustering algorithm using Gene Cluster software (11). Graphical display of clustered genes was generated by Treeview software (11).

Northern Analysis. Ten μg of total RNA were fractionated on 1.2% agarose denaturing gels and transferred to nylon membranes by a capillary method (8). Blots were hybridized with DNA probes labeled with [α -³²P]dCTP by random priming using the Rediprime II random primer labeling system (Amersham) according to the manufacturer's protocol. Filters were imaged and quantitated by using a phosphor-capture screen and Imagequant software (Molecular Dynamics).

Cell Proliferation Assay. Ninety-six-well microtiter plates were seeded with 5000 cells/well, and cells were allowed to adhere overnight, followed by the addition of test compounds for 24 or 72 h. Cell proliferation was measured by replacing the culture media with RPMI 1640 containing 1 mg/ml MTT. Isopropanol was added after a 4-h incubation, and cells were incubated overnight at 37°C. The conversion of yellow MTT to a blue formazan dye product was measured with a Micro-Quant spectrophotometer at 570 nm. The amount of formazan dye is a direct indication of the number of metabolically active cells in the culture. Each data point represents the average of four separate experiments containing 8 wells for each experimental condition.

Western Analysis. Thirty μg of protein were loaded into a precast 4–12% gel (Invitrogen), run, and transferred according to the manufacturer's instructions using the X Cell mini cell/blotting module (Invitrogen). Ponceau stain was added to confirm equal loading and transfer. The membranes were blocked overnight at 4°C in 5% milk/PBS. Anti-AR antibody (PharMingen) was added at a 1:1000 dilution for 1 h in 3% BSA/PBS. Horseradish peroxidase-conjugated antimouse IgG antibody was added at a 1:2000 dilution for 30 min. Signals were detected with a chemiluminescence kit (Pierce).

Results

PC-SPES-induced Alterations in Prostate Gene Expression.

We performed cDNA microarray analysis to determine alterations in prostate cancer cell gene expression resulting from exposure to PC-SPES. We chose to focus on genes reproducibly exhibiting a ≥ 1.5 -fold change in expression level at any time point after treatment. After 8 h, the transcripts of 19 genes increased, and those of 5 genes decreased. After 48 h, the transcripts of 319 genes were altered, with 144 increased and 175 decreased. It was also apparent that the magnitude of induction or repression increased with time for individual genes (Fig. 1). To assist data interpretation, we placed cDNAs encoding characterized genes into distinct functional categories: cell cycle control, metabolism, apoptosis/cell stress, immune modulation, and androgen regulation (Fig. 1). Genes with other functions and cDNAs encoding uncharacterized genes were not grouped. Hierarchical cluster analysis was performed to determine concordant alterations in gene expression over time in each cohort. The complete list of genes and the measured expression alterations at each time point after PC-SPES exposure are available on the World Wide Web.⁶

PC-SPES treatment decreased the expression of several genes encoding cell structural proteins including α - and β -tubulin, *dystroglycan*, and *collagen 12* (Fig. 1). Transcripts encoding *filamen*, α -*catenin*, α -*tropomyosin*, *vimentin*, and α -1 *collagen 16* were increased. PC-SPES generally inhibited the expression of genes involved in cell cycle regulation. Transcripts encoding *cyclin A*, *cyclin D*, *cyclin E*, *cdc-20 cdc25B*, *cdc28*, *cdc46*, *CDK2*, *MAD2*, and *cdc6-regulated* protein were decreased. However, the expression of quiescin and the CDK inhibitor *p21* increased. PC-SPES markedly inhibited the expression of all known androgen-regulated genes present on the microarray. Transcripts encoding *PSA*, *TMPRSS2*, *NKX3.1*, *prostase*, and *hK2* were decreased after 24 h of treatment and further diminished at the 48 h time point. PC-SPES up-regulated several genes reported to be associated with apoptosis: *p21*, *clusterin/TRPM2*, *PEA15*, *Gadd 34*, *Id1*, *DAD1*, and *thioredoxin reductase*. The cDNA encoding Bcl-2 was not present in our microarray clone set, thus specific alterations in this apoptosis-regulatory gene were not determined. In support of potential immunomodulatory properties of PC-SPES, altered levels of *thymosin- β -4*, *prothymosin- α* , MHC class I genes, *monocyte-specific enhancer factor*, *interleukin 1*, *IFN-regulatory factor 1* and 2, and β 2 *microglobulin* mRNAs were detected in the prostate cells. We did not examine the effects of PC-SPES on other cell types likely to be effectors of an immune response (e.g., lymphocytes).

To confirm the microarray results, we performed Northern analysis for 17 genes exhibiting gene expression alterations after PC-SPES treatment. For each gene studied, the transcript alterations as measured by Northern were concordant with the array findings (Fig. 2). Selecting a suitable gene to serve as a Northern loading control was difficult because PC-SPES had such a dramatic effect on the overall cellular gene expression profile. For example, β -*actin* was induced 1.6-fold as determined by cDNA array measurements at 48 h and was induced by 2.0-fold on the Northern study (data not shown). Another commonly used housekeeping gene, *G3PDH*, was repressed 1.7-fold by cDNA array measurements and decreased 3-fold by Northern analysis. Therefore, we used methylene blue staining of 28S and 18S ribosomal RNAs as the most reliable control for equivalent loading.

Comparative Analysis of Gene Expression Profiles Reflecting Cellular Exposure to PC-SPES and DES. We determined qualitative and quantitative gene expression profiles reflecting prostate cellular responses to different concentrations of the synthetic estrogen DES and compared these results to expression profiles reflecting

⁵ R. Bumgarner, personal communication.

⁶ www.pedb.org/PCSPES/.

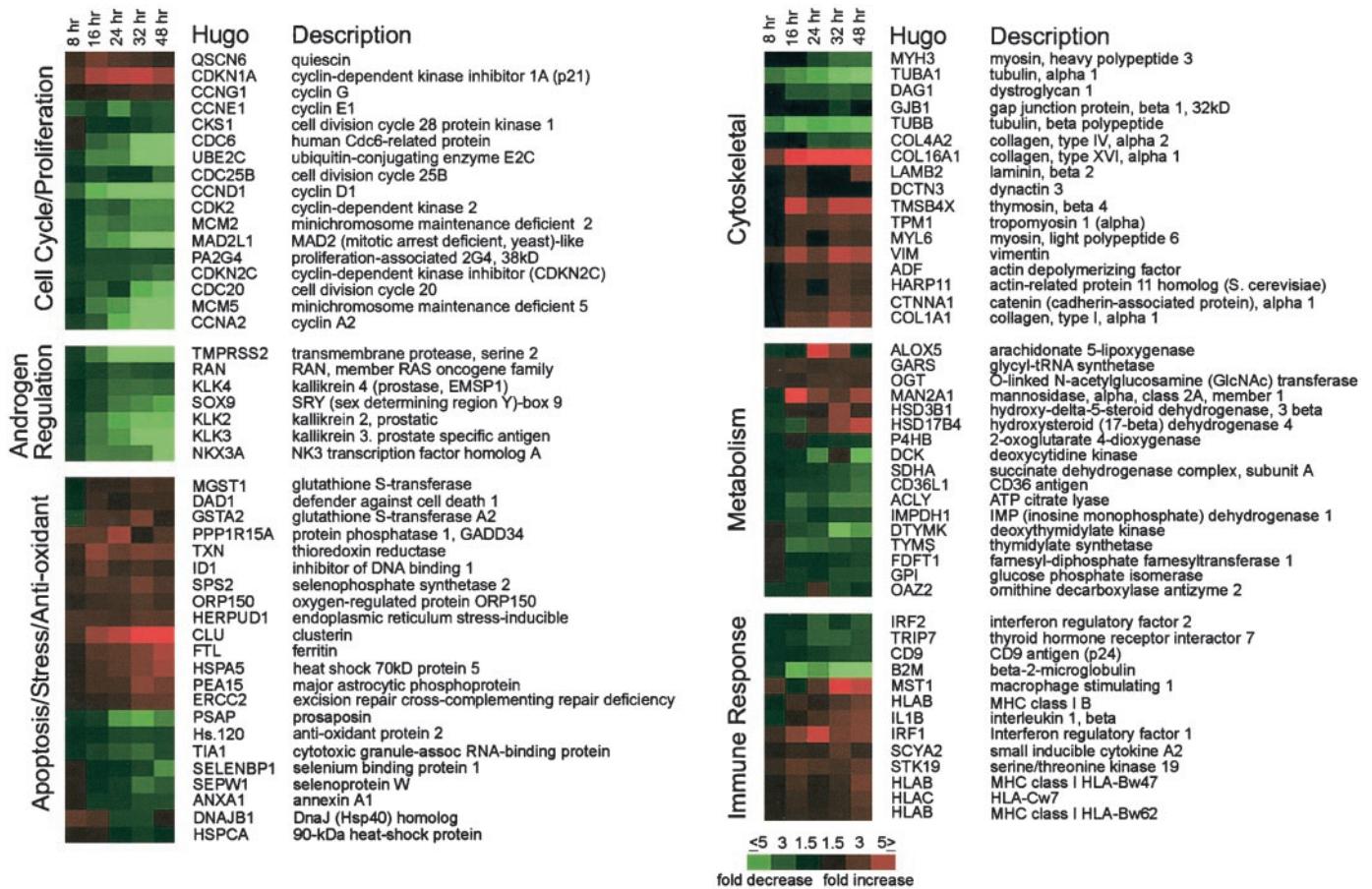


Fig. 1. Temporal alterations in the expression of characterized genes resulting from PC-SPES exposure. Genes are grouped based on known functions and clustered for concordant expression over time. The color scale reflects the experimental fold increase (red) or fold decrease (green) in transcript abundance relative to the corresponding control experiment.

PC-SPES exposure. DES has been shown to induce apoptosis in LNCaP cells at concentrations between 15 and 30 μM (12). Exposure to PC-SPES also induces apoptosis in LNCaP cells, as well as in AI PC3 and DU145 prostate cancer cell lines (13). To determine cytotoxic equivalence of DES and PC-SPES, LNCaP cells were exposed to different compound concentrations, and cell viability was quantitated using a MTT assay that measures mitochondrial respiratory enzyme activity (14). DES concentrations between 10 and 30 μM (Fig. 3A) were equivalent to 3–5 $\mu\text{l/ml}$ PC-SPES in this assay (Fig. 3B).

The comparison of global gene expression changes induced by each treatment was performed by plotting the expression change for each

gene on the microarray after PC-SPES treatment directly against the corresponding expression change induced by DES (Fig. 3, C–F). The experimental variability of the microarray assay was demonstrated by hybridizing probes from two independent PC-SPES treatments to two separate sets of microarrays. The coefficient of correlation between these two hybridizations is $r = 0.86$ (Fig. 3C). This result demonstrates minimal experimental variation attributable to differences in probe labeling, hybridization, and array construction. Exposure of LNCaP cells to 5 $\mu\text{l/ml}$ PC-SPES for 24 h altered the expression of 156 genes relative to untreated cells (Fig. 3D). Treatment with 10 μM DES for 24 h altered the expression of 62 genes. Of these, only six genes (10%) were changed concordantly by PC-SPES. Treatment with 30 μM DES altered the expression of 71 genes, and expression of 12 of these genes (17%) was also changed by PC-SPES. The correlation coefficients between 5 $\mu\text{l/ml}$ PC-SPES and 10 or 30 μM DES are $r = 0.112$ and $r = 0.223$, respectively (Fig. 3, E and F).

In addition to DES, we also compared the PC-SPES gene expression profile with those reflecting cellular responses to the synthetic androgen R1881 and estradiol (results available online⁶ as supplemental data). To simulate the environment of prostate cancer in a castrated host, these treatments were performed on LNCaP cells grown in androgen-depleted media. A concentration of 10 nM R1881 altered the expression of 76 genes after 24 h of exposure. The calculated correlation coefficient of $r = 0.009$ between androgen treatment and PC-SPES is indicative of their highly divergent transcriptional effects. In androgen-depleted media, the correlation between DES and PC-SPES gene expression remained low with a coefficient of $r = 0.117$, a value consistent with experiments per-

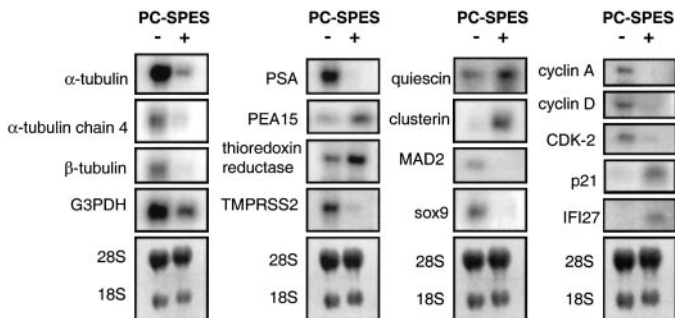


Fig. 2. Northern analysis confirming PC-SPES-mediated gene expression alterations detected by microarray analysis. Equivalent RNA loading is confirmed by methylene blue staining of 28S and 18S RNase. *G3PDH*, glycerol-3-phosphate dehydrogenase; *PEA15*, phosphoprotein enriched in astrocytes 15; *TMPRSS2*, transmembrane protease serine 2; *MAD2*, mitotic arrest-deficient-like 2; *sox9*, SRY box-containing gene 9; *CDK-2*, CDK2; *IFI27*, α -IFN-inducible p27.

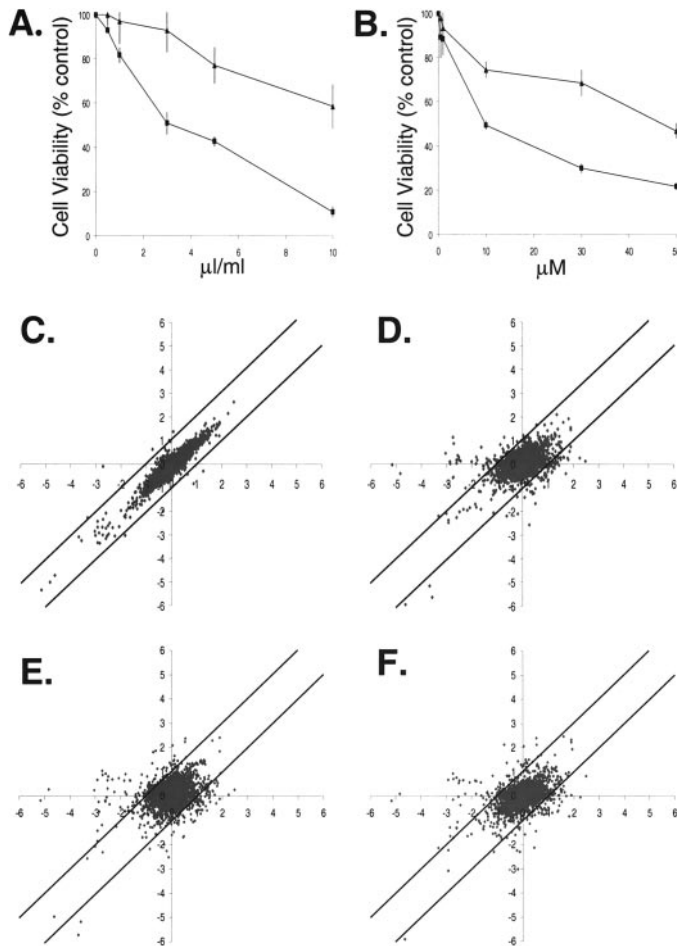


Fig. 3. Comparative analysis of cytotoxicity and gene expression changes resulting from PC-SPES and DES treatment. PC-SPES (A)- and DES (B)-treated cell viability as measured by MTT assay 24 (\blacktriangle) and 72 (\blacksquare) h after treatment. Values are expressed as the percentage of viability of the vehicle control. C–E, comparative scatter plots depicting cellular gene expression ratios of PC-SPES treatment against itself (C), vehicle control (D), 10 μ M DES (E), or 30 μ M DES (F). Each point represents the ratio of expression change for a distinct gene plotted for PC-SPES treatment (X axis) against the comparison treatment (Y axis). Only genes with an average intensity level above background (300 intensity units) are shown.

formed in growth medium containing androgen. Estradiol altered the expression of 49 genes after 24 h when applied at a concentration of 10 μ M. A majority of the genes induced by estradiol were also induced by androgen including *PSA*, *TMPRSS2*, *hK2*, and *KLK4/prostate*. LNCaP cells are known to express an AR with broad steroid specificity including estrogen-mediated activation (15). When compared with PC-SPES, estradiol exhibited a correlation coefficient of $r = 0.026$.

PC-SPES Regulation of AR Expression. The PC-SPES-mediated transcriptional alteration of several genes known to be androgen regulated prompted additional studies to ascertain whether a common mechanism of control was operative. Northern analysis was performed to determine whether the expression of the AR was changed with PC-SPES treatment. AR transcripts decreased 3–4-fold after 16 h of exposure to PC-SPES, and AR transcripts were undetectable after 48 h of treatment (Fig. 4A). The AR message was unchanged over the same time period in the untreated cells. Western blot analysis confirmed that AR protein levels are decreased to undetectable levels 24 and 48 h after treatment of cells with 5 μ l/ml PC-SPES (Fig. 4B). AR message levels were not significantly reduced by treatment with DES or estradiol, and the addition of androgen did not induce AR transcription in the presence of PC-SPES (Fig. 4C). These findings

support the microarray data indicating that PC-SPES exhibits activities operating through mechanisms distinct from those attributable to known estrogens.

Discussion

In vitro and *in vivo* studies suggest that multiple biochemical processes are influenced by PC-SPES. A critical metabolic pathway modulating prostate cellular growth involves the interaction of androgenic hormones with the cellular AR. The administration of estrogenic agents such as DES results in castrate levels of serum testosterone through the suppression of the hypothalamic-pituitary-gonadal axis (16). Estrogenic activities of PC-SPES preparations have been documented using *in vitro* assays (3), and patients taking PC-SPES exhibit clinical features consistent with exogenous estrogen administration (2). Thus, a component of PC-SPES efficacy likely results from the suppression of testosterone to castrate levels, an event that occurs in >90% of PC-SPES-treated men with AD disease (2). However, PC-SPES also exhibits activity against AI prostate cancer. In this report, we have shown that gene expression profiles reflective of PC-SPES activity *in vitro* are distinct from profiles of the estrogenic compound DES. Thus, PC-SPES-mediated tumor responses may result both from estrogen-mediated central androgen suppression and direct cytotoxicity via estrogen-independent mechanisms. This conclusion is supported by reports describing PC-SPES activity against AI cells derived from lymphoma and lung carcinoma (17, 18).

The gene expression profiles representing PC-SPES activity indicate several pathways that could contribute to cellular growth inhibition. PC-SPES altered the expression of several genes involved in cell cycle regulation and cell proliferation. Transcripts encoding *CDK2*, *MAD2*, several orthologues of yeast CDKs, and the G_1 cyclins A, D, and E were significantly reduced. Transcripts encoding *p21*, a protein inhibiting cell cycle progression, were increased by PC-SPES. Taken together, these findings provide further molecular data to support

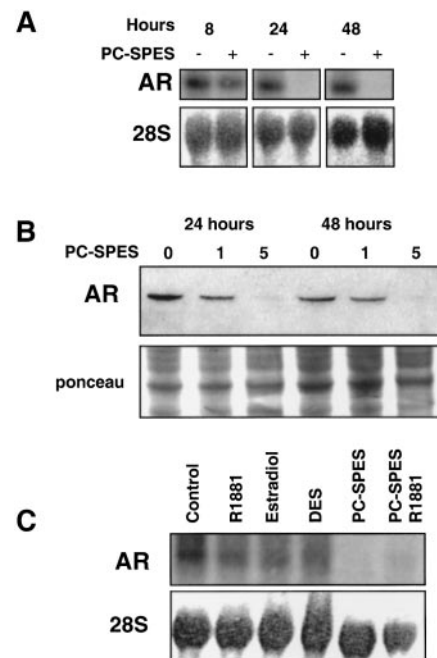


Fig. 4. The effect of PC-SPES on AR expression. A, Northern analysis demonstrating down-regulation of AR transcripts in LNCaP cells after 8, 24, and 48 h of exposure to 5 μ l/ml PC-SPES. B, Western analysis demonstrating down-regulation of AR protein in LNCaP cells after 24 h of treatment with 1 and 5 μ l/ml PC-SPES. Ponceau staining is shown as a control for protein loading. C, Northern analysis demonstrating AR transcript levels in LNCaP cells after 24 h of treatment with vehicle control, the synthetic androgen R1881, estradiol, DES, PC-SPES, and PC-SPES with R1881.

previous reports describing the antiproliferative effects of PC-SPES including up-regulation of p21 expression and growth arrest at the G₂-M phase of the cell cycle (1). In addition to the observed cell cycle alterations, components of PC-SPES have been shown to initiate an apoptotic response in prostate cancer cells. Licochalcone A, an estrogenic flavonoid extracted from licorice root, has been shown to down-regulate Bcl-2 expression and induce apoptosis in leukemia and breast cancer cell lines (19). Although licorice root is used in the formulation of PC-SPES, it represents only a very minor component,⁷ and studies by Kubota *et al.* (1) did not demonstrate alterations of cellular Bcl-2 levels in LNCaP cells treated with PC-SPES. These findings suggest that some mechanisms of PC-SPES cytotoxicity may be cell type dependent.

PC-SPES treatment resulted in the suppression of a large cohort of androgen-regulated genes that included *PSA*, *hK2*, *NKX3.1*, and *TM-PRSS2*. Several clinical trials have reported a reduction of serum PSA levels in patients taking PC-SPES. Whereas this effect could be mediated through a decline in circulating androgens, we have shown that PC-SPES markedly down-regulates expression of the AR. This finding may account for some of the PC-SPES benefits seen in AI cancers. Several reports have described a cross-talk between the AR and signaling networks such as mitogen-activated protein kinase, and protein kinase A and protein kinase C pathways (20). The reduction of cellular AR by PC-SPES could impair these alternative mechanisms of activating AR-responsive processes. Recent studies of baicalin (21), a flavonoid component of PC-SPES, and of quercetin (4), a flavonoid present in tea and red wine, have shown that each agent can independently down-regulate AR expression. Additional studies of these compounds may serve to characterize new forms of antiandrogen therapy.

In addition to modulating the expression of genes in the AR pathway and those directly involved in cell cycle control, PC-SPES markedly decreased the expression of α - and β -tubulins. Tubulin isotypes are structural components of microtubule assemblies that are essential for maintaining cell shape, cell transport, cell motility, and cell division (22). Several chemotherapeutic drugs active against prostate cancer including the taxanes and estramustine function in part through the impairment of microtubule organization and polymerization (23). It is possible that a reduction of cellular tubulins by PC-SPES could provide either a complementary or antagonistic effect toward these and other tubulin-modulating drugs. Additional studies combining PC-SPES with these agents may serve to delineate their optimal use in the clinical setting.

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References

- Kubota, T., Hisatake, J., Hisatake, Y., Said, J. W., Chen, S. S., Holden, S., Taguchi, H., and Koeffler, H. P. PC-SPES: a unique inhibitor of proliferation of prostate cancer cells *in vitro* and *in vivo*. *Prostate*, *42*: 163–171, 2000.
- Small E. J., Frohlich, M. W., Bok, R., Shinohara, K., Grossfeld, G., Rozenblat, Z., Kelly, W. K., Corry, M., and Reese, D. M. Prospective trial of the herbal supplement PC-SPES in patients with progressive prostate cancer. *J. Clin. Oncol.*, *18*: 3595–3603, 2000.
- DiPaola, R. S., Zhang, H., Lambert, G. H., Meeker, R., Licitra, E., Rafi, M. M., Zhu, B. T., Spaulding, H., Goodin, S., Toledano, M. B., Hait, W. N., and Gallo, M. A. Clinical and biologic activity of an estrogenic herbal combination (PC-SPES) in prostate cancer. *N. Engl. J. Med.*, *339*: 785–791, 1998.
- Xing, N., Chen, Y., Mitchell, S. H., and Young, C. Y. Quercetin inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Carcinogenesis (Lond.)*, *22*: 409–414, 2001.
- Matsuzaki, Y., Kurokawa, N., Terai, S., Matsumura, Y., Kobayashi, N., and Okita, K. Cell death induced by baicalin in human hepatocellular carcinoma cell lines. *Jpn. J. Cancer Res.*, *87*: 170–177, 1996.
- Ghosh, D., Wawrzak, Z., Pletnev, V., Erman, M., Duax, W. L., Pangborn, W., Zhu, D. W., Labrie, F., and Lin, S. X. Molecular mechanism of inhibition of steroid dehydrogenases by licorice-derived steroid analogs in modulation of steroid receptor function. *Ann. N. Y. Acad. Sci.*, *761*: 341–343, 1995.
- Wang, S. Y., Hsu, M. L., Hsu, H. C., Tzeng, C. H., Lee, S. S., Shiao, M. S., and Ho, C. K. The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. *Int. J. Cancer*, *70*: 699–705, 1997.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
- Lin, B., Ferguson, C., White, J. T., Wang, S., Vessella, R., True, L. D., Hood, L., and Nelson, P. S. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Res.*, *59*: 4180–4184, 1999.
- Hawkins, V., Doll, D., Bumgarner, R., Smith, T., Abajian, C., Hood, L., and Nelson, P. S. PEDB: the Prostate Expression Database. *Nucleic Acids Res.*, *27*: 204–208, 1999.
- Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA*, *95*: 14863–14868, 1998.
- Robertson, C. N., Roberson, K. M., Padilla, G. M., O'Brien, E. T., Cook, J. M., Kim, C. S., and Fine, R. L. Induction of apoptosis by diethylstilbestrol in hormone-insensitive prostate cancer cells. *J. Natl. Cancer Inst. (Bethesda)*, *88*: 908–917, 1996.
- de la Taille, A., Buttyan, R., Hayek, O., Bagiella, E., Shabsigh, A., Burchardt, M., Burchardt, T., Chopin, D. K., and Katz, A. E. Herbal therapy PC-SPES: *in vitro* effects and evaluation of its efficacy in 69 patients with prostate cancer. *J. Urol.*, *164*: 1229–1234, 2000.
- Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., Abbott, B. J., Mayo, J. G., Shoemaker, R. H., and Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.*, *48*: 589–601, 1988.
- Veldscholte, J., Berrevoets, C. A., Ris-Stalpers, C., Kuiper, G. G., Jenster, G., Trapman, J., Brinkmann, A. O., and Mulder, E. The androgen receptor in LNCaP cells contains a mutation in the ligand binding domain which affects steroid binding characteristics and response to antiandrogens. *J. Steroid Biochem. Mol. Biol.*, *41*: 665–669, 1992.
- Kitahara, S., Yoshida, K., Ishizaka, K., Kageyama, Y., Kawakami, S., Tsujii, T., and Oshima, H. Stronger suppression of serum testosterone and FSH levels by a synthetic estrogen than by castration or an LH-RH agonist. *Endocr. J.*, *44*: 527–532, 1997.
- Hsieh, T. C., Ng, C., Chang, C. C., Chen, S. S., Mittleman, A., and Wu, J. M. Induction of apoptosis and down-regulation of bcl-6 in mutu I cells treated with ethanolic extracts of the Chinese herbal supplement PC-SPES. *Int. J. Oncol.*, *13*: 1199–1202, 1998.
- Sadava, D., Ahn, J., Zhan, M., Pang, M. L., Ding, J., and Kane, S. E. Effects of four Chinese herbal extracts on drug-sensitive and multidrug-resistant small-cell lung carcinoma cells. *Cancer Chemother. Pharmacol.*, *49*: 261–266, 2002.
- Rafi, M. M., Rosen, R. T., Vassil, A., Ho, C. T., Zhang, H., Ghai, G., Lambert, G., and DiPaola, R. S. Modulation of bcl-2 and cytotoxicity by licochalcone-A, a novel estrogenic flavonoid. *Anticancer Res.*, *20*: 2653–2658, 2000.
- Sadar, M. D. Androgen-independent induction of prostate-specific antigen gene expression via cross-talk between the androgen receptor and protein kinase A signal transduction pathways. *J. Biol. Chem.*, *274*: 7777–7783, 1999.
- Ikezoe, T., Chen, S. S., Heber, D., Taguchi, H., and Koeffler, H. P. Baicalin is a major component of PC-SPES which inhibits the proliferation of human cancer cells via apoptosis and cell cycle arrest. *Prostate*, *49*: 285–292, 2001.
- Downing, K. H., and Nogales, E. Tubulin structure: insights into microtubule properties and functions. *Curr. Opin. Struct. Biol.*, *8*: 785–791, 1998.
- Stearns, M. E., and Tew, K. D. Estramustine binds MAP-2 to inhibit microtubule assembly *in vitro*. *J. Cell Sci.*, *89*: 331–342, 1988.

⁷ S. Chen, personal communication.