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Resveratrol-Induced Gene Expression Profiles in Human Prostate Cancer Cells

Sunita B. Jones[1], **Samuel E. DePrimo**[1], **Michael L. Whitfield**[2], and **James D. Brooks**[1]
[1]Department of Urology, Stanford University School of Medicine, Stanford, California
[2]Department of Genetics, Stanford University School of Medicine, Stanford, California

Abstract

Objective—The transhydroxystilbene resveratrol is found at high levels in red wine and grapes, and red wine consumption may be inversely associated with prostate cancer risk. To gain insights into the possible mechanisms of action of resveratrol in human prostate cancer, we did DNA microarray analysis of the temporal transcriptional program induced by treatment of the human prostate cancer cell line LNCaP with resveratrol.

Methods—Spotted DNA microarrays containing over 42,000 elements were used to obtain a global view of the effects of resveratrol on gene expression. Prostate-specific antigen (PSA) and androgen receptor (AR) expression were determined by Northern blot and immunoblot analyses. Cell proliferation was determinedbythe 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide assay and cell cycle analysis by flow cytometry.

Results—We observed time-dependent expression changes in >1,600 transcripts as early as 6 hours after treatment with resveratrol. Most striking was the modulation of a number of important genes in the androgen pathway including PSA and AR. Resveratrol also down-regulated expression of cell cycle and proliferation-specific genes involved in all phases of the cell cycle, induced negative regulators of proliferation, caused accumulation of cells at the sub-G_1 and S phases of the cell cycle, and inhibited cell proliferation in a time- and dose-dependent manner.

Conclusion—Resveratrol produces gene expression changes in the androgen axis and cell cycle regulators that may underlie its putative anticancer activities in prostate cancer.

Introduction

The most diagnosed cancer among men, prostate cancer will claim an estimated 29,900 lives this year in the United States alone (1). Considerable effort has been devoted to detecting and treating localized prostate cancer, and limited progress has been made in the treatment of recurrent or advanced disease. Epidemiologic evidence and two intervention trials have fueled interest in developing chemopreventive strategies for prostate cancer (http://www.crab.org/select/;2). Thus far, selenium, vitamin E, lycopene, cruciferous vegetables, and antiandrogens have been proposed as potential prostate cancer chemopreventive agents (3,4). The recent inverse association of red wine intake with prostate cancer risk (5) led us to

Requests for reprints: James D. Brooks, Department of Urology, Stanford University School of Medicine, MC 5118, Stanford, CA 94305. Phone: 650-498-4464; Fax: 650-723-0765. jdbrooks@stanford.edu.

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M.L. Whitfield is currently in the Department of Genetics, Dartmouth Medical School, Hanover, NH 03755.

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wonder whether resveratrol, a polyphenol transhydroxystilbene found at high levels in grapes and red wine, might exert biological effects that protect against prostate cancer.

Resveratrol appears in substantial quantities in several foods. Red wine contains between 0.72 and 3.18 mg/L resveratrol (6-8). Resveratrol is rapidly absorbed by the gut and shows excellent tissue bioavailability (9-14). The effects of resveratrol in biological systems are wide-ranging, and several studies have shown that it can inhibit or modulate metabolic pathways, act as an anti-inflammatory agent or antioxidant, and block cell proliferation (15-21). In prostate cancer cell lines, resveratrol has been shown to block proliferation and possibly act as a negative regulator of androgen pathways (22, 23).

DNA microarray technology has provided insights into the molecular taxonomy of human tumors as well as the transcriptional underpinnings of the cell cycle, prostate cellular senescence, cellular response to stress, and androgen action (24-32). To gain insights into the possible mechanisms of action of resveratrol in human prostate cancer, we did DNA microarray analysis of the temporal transcriptional program induced by treatment of the human prostate cancer cell line LNCaP with resveratrol. Based on these findings, we further investigated the effects of resveratrol on androgen pathways and the cell cycle.

Materials and Methods

Cell Culture and Treatments

The LNCaP cell line was obtained from American Type Culture Collection (Manassas, VA) and maintained in RPMI medium with 10% fetal bovine serum (CDT; Hyclone Laboratories, Logan, UT) and penicillin/streptomycin (Mediatech, Herndon, VA) in an environment of 95% air and 5% CO_2 at 37°C. Upon reaching 75% confluence, cells were treated with either DMSO control or purified resveratrol at several concentrations (LKT Laboratories, St. Paul, MN), dissolved in DMSO, and incubated for varying lengths of time. Final concentration of DMSO in media was 0.01%. Total RNA was prepared from cells using TRIzol (Invitrogen Life Technologies, Carlsbad, CA).

Microarray Hybridizations and Data Analysis

Stanford Human cDNA microarrays were used in this study. Each microarray contains 42,000 elements representing ∼30,000 UNIgene Clusters (32). Microarray hybridizations were done as previously described (The Brown Lab: http://brownlab.stanford.edu;33). Briefly, 100 μg total RNA from each sample was reverse-transcribed and labeled with fluorescence-tagged nucleotides (Cy3-dUTP for the DMSO control samples; Cy5-dUTP for the resveratrol-treated samples). Pairs of resveratrol-treated and DMSO control–labeled cDNA were mixed and hybridized to microarray slides for 14 to 18 hours at 65°C. Microarrays were scanned with a GenePix microarray scanner (Axon Instruments, Union City, CA) and analyzed with GenePix software (34). Spots of insufficient quality were excluded from further analysis by visual inspection. All data are stored in the Stanford Microarray Database at http://genome-www.stanford.edu/microarray. Only spots with a fluorescent intensity in each channel >2.0-fold over background were selected for further analysis; data points that did not meet this criteria were excluded. Genes missing >20% of their data points were also excluded from further analysis. Genes were selected that exhibited a ≥2-fold change in expression level relative to the control sample in at least two experiments. Application of different filtering and data selection criteria resulted in highly reproducible gene clustering patterns demonstrating the robustness of the data set. All microarray data can be downloaded at http://genome-www.stanford.edu/microarray/ (35).

Northern Blot

Equal amounts of total RNA were resolved on 1% agarose formaldehyde gel and transferred to a membrane (Hybond N^+, Amersham Biosciences, Piscataway,NJ) and hybridized with ^{32}P-labeled prostate-specific antigen (PSA) cDNA probe. After quantitation of PSA, the membrane was stripped and rehybridized with a ^{32}P-labeled β-actin probe to monitor RNA sample loading and transfer efficiency.

PSA Quantitation

Six-centimeter culture dishes were seeded with 7×10^5 cells/plate and allowed to adhere for 18 hours. Resveratrol or DMSO was added as mentioned above. For each time point, media was collected and secreted total PSA was measured (Immulite 2000, Diagnostic Products, Corp., Randolph, NJ) and normalized by cell density. All experiments were done in triplicate.

Western Blot

Protein extracts were resolved by SDS-PAGE and transferred to a membrane, blocked with 5% nonfat milk in TBS plus Tween 20 overnight at 4°C, and subsequently incubated with a 1:1,000 dilution rabbit polyclonal α-AR primary antibody (Santa Cruz Technologies, Santa Cruz, CA) for 1 hour at room temperature. Bands were visualized with an anti-rabbit, horseradish peroxidase secondary antibody, and a chemiluminescence probe (ECL kit, Amersham Biosciences) following manufacturer's directions. Glyceraldehyde-3-phosphate dehydrogenase was used as the control for protein loading and transfer efficiency (not shown).

3-(4,5-Dimethylthiazolyl-2)-2,5-Diphenyltetrazolium Bromide Assay

Inhibition of cell proliferation was determined using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay kit according to the manufacturer's protocol (ATCC). After treatment with 25, 75, and 150 μmol/L resveratrol or DMSO alone, cells were harvested and assayed at 12, 24, 48, and 60 hours. Ten microliters MTT labeling reagent were added to each well and the plates incubated for 4 hours followed by the addition of 100 μL solubilization solution. The cells were incubated for another 12 hours and absorbance was measured with a microtiter plate reader (Thermoelectron Corporation, Franklin, MA) at a test wave-length of 540 nm and a reference wavelength of 620 nm. The absorbance was calculated as the difference between the absorbance at the reference wavelength and that at the test wavelength. Percent inhibition was calculated as absorbance of control minus absorbance of drug-treated sample divided by absorbance of control minus absorbance of blank times 100 [$I = (C - E) / (C - B) \times 100$].

Cell Cycle Analysis

The cell cycle distribution of LNCaP cells after treatment with resveratrol or DMSO alone was monitored by flow cytometry of propidium iodide–stained cells. Briefly, 6×10^5 cells were plated in 10 cm culture dishes. Upon reaching 75% confluence, cells were treated with DMSO or 25, 75, and 150 μmol/L resveratrol in DMSO. Adherent and floating cells were collected, resuspended in PBS, fixed in 70% ethanol, stained with propidium iodide (20 μg/mL), 30 μg/μL RNase A, and incubated in the dark for 30 minutes. DNA content was measured using a FACScan instrument equipped with a FACStation running CellQuest software (Becton Dickinson, Franklin Lakes, NJ). All experiments were done in duplicate with similar results. Percentage of cells in each phase of the cell cycle was quantified using FlowJo software (Tree Star, Inc., Ashland, OR).

Results

To gain insights into the potential mechanisms through which resveratrol acts as a putative cancer preventive agent, we used DNA microarrrays to assess global gene expression patterns in a prostate cancer cell line, LNCaP, after treatment with resveratrol. As a first test of whether resveratrol could modulate gene expression patterns, LNCaP cells were treated with 10 nmol/L, 100 nmol/L, 1 μmol/L, 10 μmol/L, 25 μmol/L, 40 μmol/L, and 100 μmol/L resveratrol. Total RNA was harvested between 18 and 40 hours. These RNAs (labeled with the Cy-5 fluorophore) were cohybridized with RNA from cells treated in parallel with vehicle alone (labeled with Cy-3) on spotted cDNA microarrays containing 42,264 elements representing ∼27,286 unique genes (estimated by UNIgene clusters). Only minor gene expression changes were noted at low doses (10 nmol/L-1 μmol/L); however, consistent, dose-dependent changes in gene expression were observed starting at 10 μmol/L that increased up to 100 μmol/L (Fig. 1; Supplementary Fig. S1).

Based on the observed expression changes in our dose-escalation experiments, we assessed the genome-wide patterns of gene expression induced after treatment with either 75 or 150 μmol/L resveratrol in LNCaP cells at time points between 0 and 60 hours after treatment (Fig. 1). We selected 1,656 transcripts whose expression levels changed at least 2-fold from the control samples on at least two arrays. These 1,656 transcripts were further analyzed by hierarchical clustering, revealing groups of genes that varied both in their magnitude and temporal patterns of gene expression in a resveratrol-dependent manner. Six hundred fourteen transcripts (37%) were induced and 1,044 transcripts (63%) were repressed following treatment with resveratrol. Changes in transcript levels were detected as early as 1 hour in a few genes and was apparent in most genes by 8 hours. Many transcripts represented named genes, although most were poorly characterized, and 198 (12%) of the genes were uncharacterized expressed sequence tags.

The human prostate cancer cell line LNCaP expresses the androgen receptor (AR) and responds to androgen stimulation (36, 37). DNA microarrays have been used to characterize the transcriptional program induced by treatment of LNCaP cells with dihydrotestosterone and R1881, a synthetic androgen analogue (31, 35, 38). Deprimo et al. (31) reported 567 androgen-responsive genes and, of these, 517 showed a response to resveratrol of ≥2-fold over control in at least one experiment (Fig. 2) as early as 4 hours after resveratrol treatment and more than half of the transcripts were affected reciprocally. Of the 412 genes that showed increased expression after androgen treatment, 210 were down-regulated by resveratrol. These included genes involved in cell proliferation, apoptosis, polyamine biosynthesis, and many well-characterized androgen targets. Interestingly, a majority of these transcripts showed greater repression at 75 μmol/L than at 150 μmol/L. Of the 105 genes normally repressed by androgens, 92 (88%) were induced by resveratrol. A subset of genes (19%) was induced by both resveratrol and androgen and included genes involved in lipid metabolism, protein trafficking, vesicle formation, and stress response.

PSA, a well-characterized androgen-regulated gene, was repressed >2-fold by 12 hours after treatment with resveratrol. In agreement with transcript levels measured on the microarrays, Northern blot analysis on RNA from LNCaP cells treated with either 75 or 150 μmol/L resveratrol showed significant decreases in *PSA* mRNA levels (Fig. 3A). PSA protein levels were measured in the media of LNCaP cells treated with either 75 or 150 μmol/L resveratrol (Fig. 3B). In accord with the gene expression data, resveratrol-treated cells failed to accumulate PSA in the media compared with DMSO-treated control cells. *PSA* is regulated by the AR, and AR transcript levels were reduced >2-fold within 12 hours of treatment in the microarray data set. We tested whether resveratrol modulated AR levels using Western blot analysis and found a decrease in AR protein levels within 24 hours after treatment with

150 μmol/L resveratrol and within 36 hours in cells exposed to 75 μmol/L resveratrol (Fig. 3C). Somewhat surprisingly, however, decreased expression of AR target genes occurred by 12 hours, well before AR protein levels decreased.

In addition to its effects on the androgen axis, resveratrol produced complex temporal changes in the expression of genes involved in the cell division cycle. Whitfield et al. (28) have comprehensively identified genes whose expression varies periodically as synchronized cells traverse the cell cycle. Genes identified as cell cycle regulated were further grouped according to the phase of the cell cycle at which their expression is highest. By comparing the genes that change expression in the resveratrol-treated cells with those periodically expressed in the cell cycle, we identified 442 transcripts that are cell cycle regulated and change at least 2-fold in expression after treatment with resveratrol (Fig. 4A). Changes in expression levels were observed in genes associated with every phase of the cell cycle. Among the 442 genes in the cluster, 78 (18%) were G_1-S phase specific, 81 (18%) S phase, 99 (22%) G_2 phase, 120 (27%) G_2-M phase, and 64 (14%) were M-G_1 phase–specific genes (Fig. 4B). Approximately 20% of the 442 cell cycle–regulated genes showed increased expression after treatment with resveratrol, including several negative regulators of proliferation (*PA26*, *TSG101*, *PCAF*, and *HDAC3*). Most genes (80%) showed decreased expression after resveratrol treatment by 8 hours and remained repressed over the remainder of the time course. Transcript levels were suppressed over the entire time course and include many of the genes necessary for all the basic processes required to duplicate a human cell (cell cycle control, DNA replication, spindle assembly, mitosis; Fig. 4B). Also seen were proapoptotic genes *JUND*, *IPLA2*, *TP53INP1*, *BOK*, *PA26*, *MDM2*, *RRM2B*, *PIGPC1*, *SARS*, *PDCD4*, and *STK17A* (Fig. 1B) that were induced after treatment with 75 and 150 μmol/L resveratrol.

Because resveratrol produced large-scale changes in expression of cell cycle–regulated genes, we tested whether resveratrol affected LNCaP cell growth byusing an MTT assay (ATCC). In agreement with the microarray data, resveratrol produced a dose- and time-dependent inhibition of cell growth (Fig. 5). Resveratrol (25 μmol/L) inhibited cell growth by only 3% after 24 hours and 16% by 60 hours, corresponding well with limited effects on gene expression seen at that dose. More dramatic growth inhibition was seen with 75 and 150 μmol/L doses that produce significant changesingene expression of the cell cycle–regulated genes. Resveratrol (75 μmol/L) inhibited cell growth by 16%at24hours and 83%by 60 hours after treatment, whereas 150 μmol/L inhibited growth by 53% at 24 hours and 86% by 60 hours.

Flow cytometry was used to further characterize the effects of resveratrol on cell growth. As expected, 25 μmol/L resveratrol produced only minor changes in the cell cycle, with the portion of cells in S phase increasing from 12% in control cells to 18% in treated cells by 60 hours (Fig. 6). A much more dramatic effect was evident at 75 and 50 μmol/L resveratrol, where disappearance of the G_2-M peak and accumulation of cells in S phase was observed. By 48 hours, cells in S phase comprised 27% of the total when treated with 75 μmol/L resveratrol and 30% in 150 μmol/L resveratrol-treated cells. A sub-G_1 peak was observed in cells treated with 75 and 150 μmol/L resveratrol by 48 hours, although a corresponding sub-G_1 peak was absent in cells treated with 25 μmol/L resveratrol even after 60 hours. These findings suggest that resveratrol induces apoptosis at high doses and this may, in part, account for the significant effects seen on MTT assay.

Discussion

The effects of resveratrol on the AR and androgen-responsive genes seem to be complex. In LNCaP cells, resveratrol has been shown to suppress secretion of PSA, although controversy

exists as to whether this decrease is due to decreased expression of the AR or is independent of AR signaling pathways (23, 39). The gene expression data suggests that the down-regulation of androgen-responsive genes is not solely because of decreased levels of the AR. Resveratrol treatment produced an early and sustained decreased expression of many androgen-responsive genes (*KLK2*, *KLK3*, *KLK4*, *AIbZIP*, *NKX3*, *FKBP5*, *TMEPAI*) long before AR protein levels were diminished. Furthermore, the decreased expression of androgen-responsive genes occurred even at low doses of resveratrol, whereas the decreases in AR transcript levels occurred only at very high doses. Finally, resveratrol did not oppose all transcriptional changes induced by androgen. A subset of 153 genes was up-regulated by both resveratrol and androgen. However, many of these genes, such as *JUNB*, *HSP40*, *SERP1*, and *STCH* seem to reflect cellular stress. In LNCaP, androgen treatment is known to produce cellular stress by inducing an oxidative burst, and this stress pattern has been observed in other gene expression profiles (31, 40). Resveratrol treatment undoubtedly places these cells under stress because they undergo cell cycle arrest and, at higher doses, apoptosis.

How resveratrol might affect expression of androgen-regulated genes, aside from its effects on AR protein levels, is unclear. It is possible that resveratrol acts, in part, as an androgen-receptor antagonist, or by blocking androgen-signaling pathways downstream of AR. Resveratrol has been shown to have partial agonist effects in estrogen-responsive mammary cancer cells and estrogens have been used to treat prostate cancer (41-43). This raises the possibility that resveratrol exerts its effects through other steroid signaling pathways. Whether resveratrol acts directly as an antiandrogen by binding to the AR, or indirectly through its estrogenic effects, awaits further study. Other putative prostate cancer–preventive agents, including vitamin E, lycopene, and methylselenic acid, an organic selenium compound, have been reported to modulate androgen signaling in LNCaP cells through unknown mechanisms (44-49).

We have found that resveratrol inhibits the proliferation of LNCaP cells in a dose- and time-dependent manner. Cell growth arrest occurred in G_1 and S phase of the cell cycle as shown by flow cytometry combined with a concomitant decrease in the number of cells in G_2-M (Fig. 6). Dramatic gene expression changes accompany this cell cycle arrest and are distributed throughout all phases of the cell cycle. Resveratrol suppressed transcript levels for genes involved in cell cycle control, such as *cyclins D*, *E*, *A*, and *B* (Fig. 4). Negative regulators of proliferation, such as cyclins G_1 and G_2 and *PA26* and *HDAC3* (Fig. 1B), were induced in response to resveratrol and likely contribute to S-phase arrest. Therefore, resveratrol-induced growth arrest seems to be mediated by a complex network of cell cycle regulatory genes. The results observed here confirm other studies of gene expression demonstrating that cell cycle arrest results in decreased expression of the cell cycle–regulated genes (28). The cell cycle–regulated genes seem to be regulated such that expression occurs primarily when cells are dividing and not at any other time.

Considerable work has been published on resveratrol and its effect on the cell cycle and is consistent with the results presented here. Most studies suggest that resveratrol arrest cells in S phase and at the S/G_2 transition. In a study using HL-60 cells, resveratrol exposure resulted in accumulation of cells at G_1 and at the S phases with and an absence of the G_2-M peak (20). This was attributed to an increase in the levels of cyclins A and E along with accumulation of phosphorylated cdc2. Hsieh et al. (50) reported that resveratrol induced NO synthase in pulmonary epithelial cells with suppression of the cell cycle through the S and G_2 phases. Cell cycle arrest was accompanied by a corresponding increase in the expression of p53 and p21 and apoptosis. Inhibition in cell cycle progression by resveratrol by inducing S-phase arrest was also reported in osteoblasts, breast, colon, and prostate cancer cells (51, 52). In breast cancer cells, resveratrol caused an accumulation of cells in the S phase with a

concomitant reduced expression of Rb and increased expression of p53 and bcl-2 proteins (53). Resveratrol-mediated growth inhibition and apoptosis in prostate cancer were observed in androgen nonresponsive cell lines with a disruption in the G_1-S phase transition (54) and S-phase arrest in androgen responsive LNCaP cells (55).

Resveratrol has been reported to induce apoptosis via several pathways including DNA damage (56, 57) through p53-dependent and p53-independent pathways (16, 56-59) and by affecting lipoxygenase and cyclooxygenase activities (60). Narayanan et al. (61), using highly purified resveratrol and a different microarray platform, reported induction of apoptosis at 10 μmol/L in LNCaP that is correlated with modulation of p53-mediated molecular targets. We see similar regulation of *p53* genes, but only at relatively high levels of resveratrol. In addition, we see apoptosis only at the highest concentration used in our study. In our gene expression data set, a few apoptosis genes were modulated, but not enough to elucidate pathways involved in apoptosis. It is, therefore, possible that critical apoptotic signals occur posttranscriptionally.

The microarray results reported here provide additional insights into the mechanisms of action of this compound in prostate cells. Resveratrol produced striking induction of quinone reductase (*NQO1*) transcript levels. Quinone reductase is tightly regulated at the transcriptional level, and has served as a surrogate for phase 2 enzyme responsiveness (62). Indeed, we observe coordinate induction of other phase 2 enzymes (*MGST1*, *TXNRD1*, *GSTA2*, and *PRDX1*) and glutathione synthetic pathways (*UGDH*). Induction of phase 2 enzyme activity by resveratrol has been reported in other model systems, but may be particularly relevant to prostate cancer. From its earliest stages, human prostate cancers lose expression of a critical carcinogen defense enzyme, glutathione *S*-transferase-π or GSTP1, because of extensive methylation of deoxycytidine residues in the 5'-regulatory regions of the *GSTP1* gene (63). Loss of *GSTP1* could render prostate cells susceptible to carcinogenesis by compromising their defenses against endogenous or exogenous electrophilic mutagens. Compensation for loss of *GSTP1* expression by induction of global carcinogen defenses could protect against the DNA damage that contributes to prostate cancer initiation or progression.

We recognize that our findings, whereas provocative, have several limitations. We have analyzed comprehensively the gene expression changes induced in a single, androgen-sensitive prostate cancer cell line, LNCaP, and recognize that these findings should be validated in other model systems.

An important factor in explaining the efficacy of resveratrol is the comparison of dosages used *in vitro* and in animal studies, with dosages that can be expected to be clinically effective in humans. Tissue bioavailability of resveratrol in rat kidney has been reported at 77.75 ng/h/mL (14) following a single administration of red wine containing 28.24 μg resveratrol. Animal and human studies have shown that resveratrol is rapidly absorbed in the gut, attaining highest concentration in the blood in 1 hour, and its accumulation in organs vary (64-66). No information exists regarding resveratrol levels in the prostate tissue for any species. In this study, we have shown changes in gene expression at 10 to 100 μmol/L, physiologically attainable levels in rats (64). However, the levels of resveratrol used in this study that result in gene expression modulation *in vitro* may or may not be attainable in man. At the very least, the relatively low concentration of resveratrol in grapes or wine will likely make it necessary for it to be given as a dietary supplement. Further clinical translational work is urgently needed to clarify the relationship between serum concentrations and levels achieved in the prostate and whether similar gene expression changes are observed *in vivo*.

Our data provides a global view of the potential mechanisms through which resveratrol may act in protecting against prostate cancer and serves as a resource for future investigations into its mechanisms of action. Resveratrol exerts antiandrogenic effects not strictly attributable to repression of AR expression, inhibits the cell cycle, induces apoptosis, and up-regulates enzymes of carcinogen defense. This data set serves as a resource for understanding the effects of resveratrol in the prostate and as a potential source of biomarkers of response *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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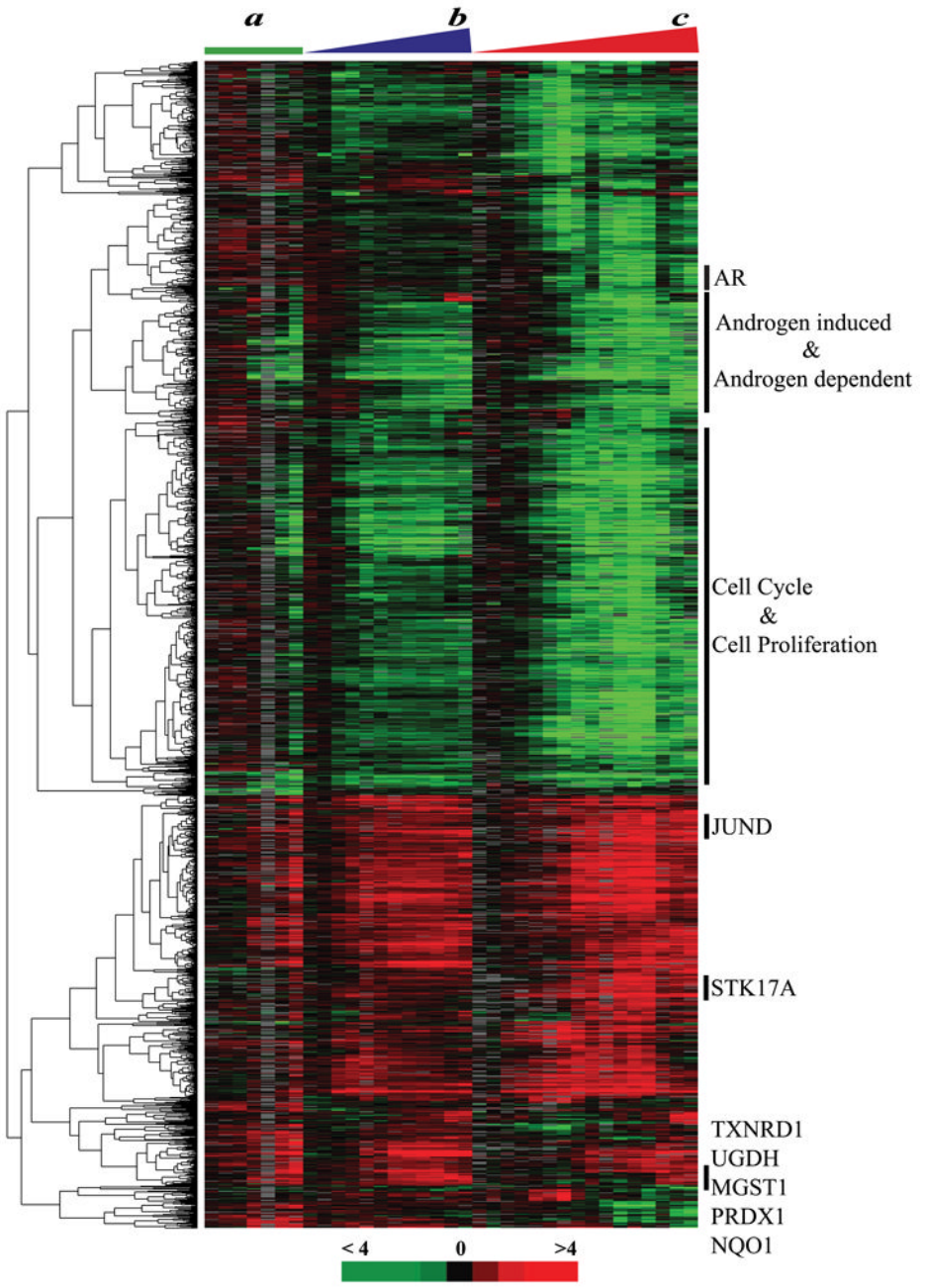

Figure 1.

Hierarchical cluster analysis of transcripts modulated by resveratrol in LNCaP cells. Each column corresponds to a given treatment dose and time. **A**, 10 nmol/L, 100 nmol/L, and 1 μmol/L resveratrol at 43 hours; 10 μmol/L resveratrol at 20 hours; 25 μmol/L resveratrol at 18 hours; 40 and 100 μmol/L resveratrol at 20 hours; **B**, 75 μmol/L resveratrol at 0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 48, and 60 hours; **C**, 150 μmol/L resveratrol at 0, 1, 2, 3, 4, 6, 8, 12, 14, 16, 18, 20, 22, 24, 48, and 60 hours. Columns under the green heading were done using 24,000 element microarrays. Those under the blue and red headings were done using 42,000 element microarrays. *Red squares*, transcripts with increased expression levels compared with DMSO-treated control cells; *green squares*, decreased levels; *black*, levels that were approximately equal in treated and control cells; *gray*, poor quality or missing data. Genes

listed more than once indicate that the microarray contained multiple elements representing that gene. *Scale bar*, color saturation reflects the magnitude of expression ratio. A detailed figure with complete gene names is viewable as Supplementary Fig. S1.

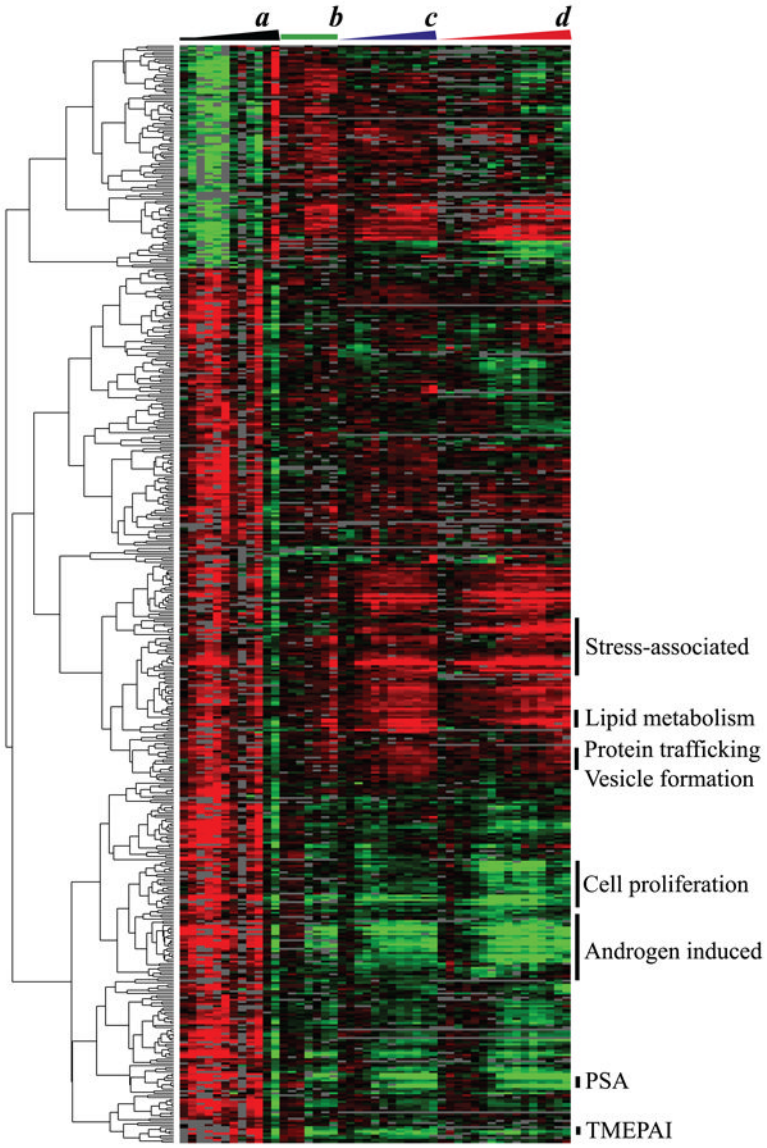

Figure 2.

Expression levels of androgen-responsive genes in LNCaP cells exposed to dihydrotestosterone, R1881, or resveratrol. Gene-expression changes in LNCaP treated with 1 nmol/L R1881 at 7, 9, 18, 24, 50, and 72 hours; 10 nmol/L dihydrotestosterone at 18 and 50 hours; 100 nmol/L dihydrotestosterone and 1 μmol/L dihydrotestosterone at 24 hours; and androgen deprivation at 46 and 70 hours (*A*, controls); 10 nmol/L, 100 nmol/L, and 1 μmol/L resveratrol at 43 hours; 10 μmol/L resveratrol at 20 hours; 25 μmol/L resveratrol at 18 hours; 40 and 100 μmol/L resveratrol at 20 hours (*B*); 75 μmol/L resveratrol at 0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 48, and 60 hours (*C*); 150 μmol/L resveratrol at 0, 1, 2, 3, 4, 6, 8, 12, 14, 16, 18, 20, 22, 24, 48, and 60 hours (*D*). Transcript levels exhibit time- and dose-dependent reciprocal changes between androgen and resveratrol in the majority of the genes. Color bands and saturation scales are as in Fig. 1. A detailed figure with complete gene names is viewable as Supplementary Fig. S2.

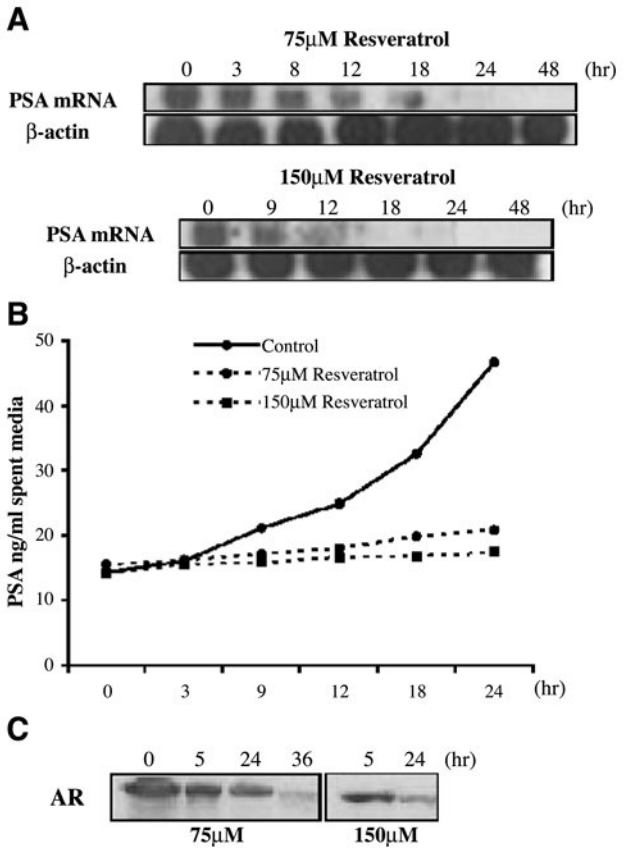

Figure 3.

Effects of resveratrol on PSA and AR. **A**, Northern blot analysis of PSA mRNA in LNCaP cells shows decreased PSA expression over time. Equal loading was confirmed by rehybridization of the stripped membrane with radiolabeled β-actin (*bottom*). **B**, dose- and time-dependent inhibition of accumulation of secreted PSA in media of LNCaP cells. Culture medium was collected at indicated time intervals after addition of resveratrol (75 and 150 μmol/L) for measurement of total PSA. PSA levels were normalized to cell density. **C**, immunoblot analysis of AR protein levels in LNCaP cells shows decreased expression after exposure to resveratrol (25, 75, and 150 μmol/L). Equal loading was determined by glyceraldehyde-3-phosphate dehydrogenase immunoblotting (not shown).

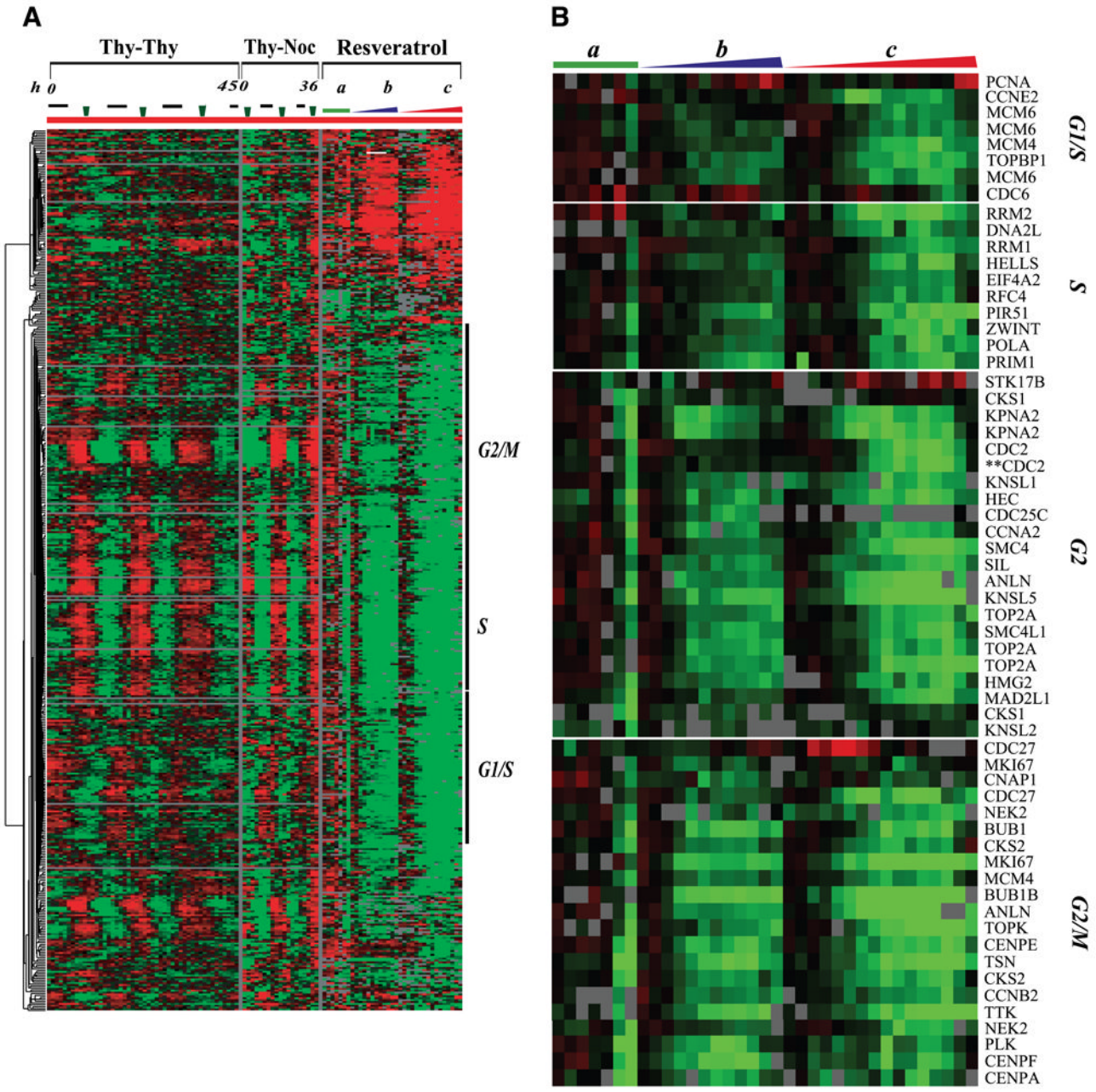

Figure 4.

Expression of cell cycle and proliferation genes in LNCaP cells exposed to resveratrol. *Color bands*, saturation scales and treatment times are the same as in Fig. 1. **A**, overview of the cluster diagram generated by querying microarray data using a gene list containing cell cycle–regulated genes. The full image of this cluster diagram is viewable as Supplementary Fig. S4. **B**, transcript profiles of selected genes involved in each phase of the cell cycle. Resveratrol treatments, color bands, and saturation scales are the same as in Fig. 1. The full image of this cluster diagram is viewable as Supplementary Fig. S4.

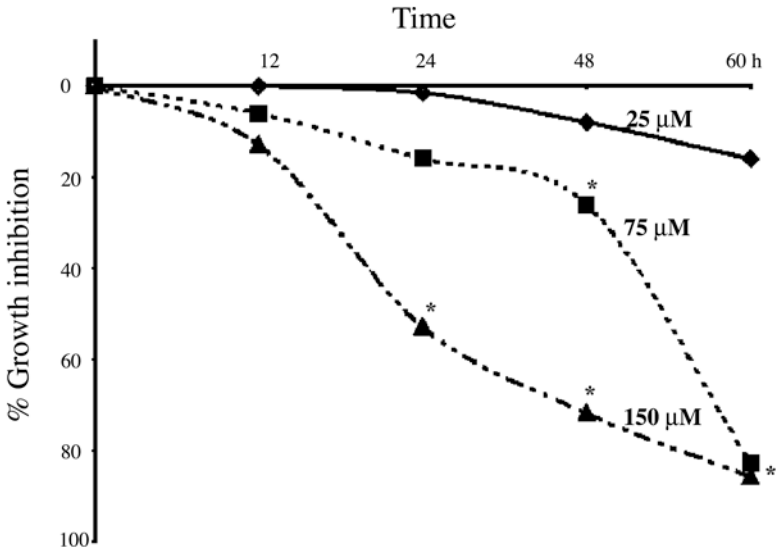

Figure 5.
Time course of resveratrol-induced inhibition of LNCaP cell proliferation determined using the MTT assay. Results are expressed as a percent of inhibition in treated cells compared with control cells treated with the vehicle DMSO only. Data represent means for three separate experiments. *$P < 0.05$ compared with control.

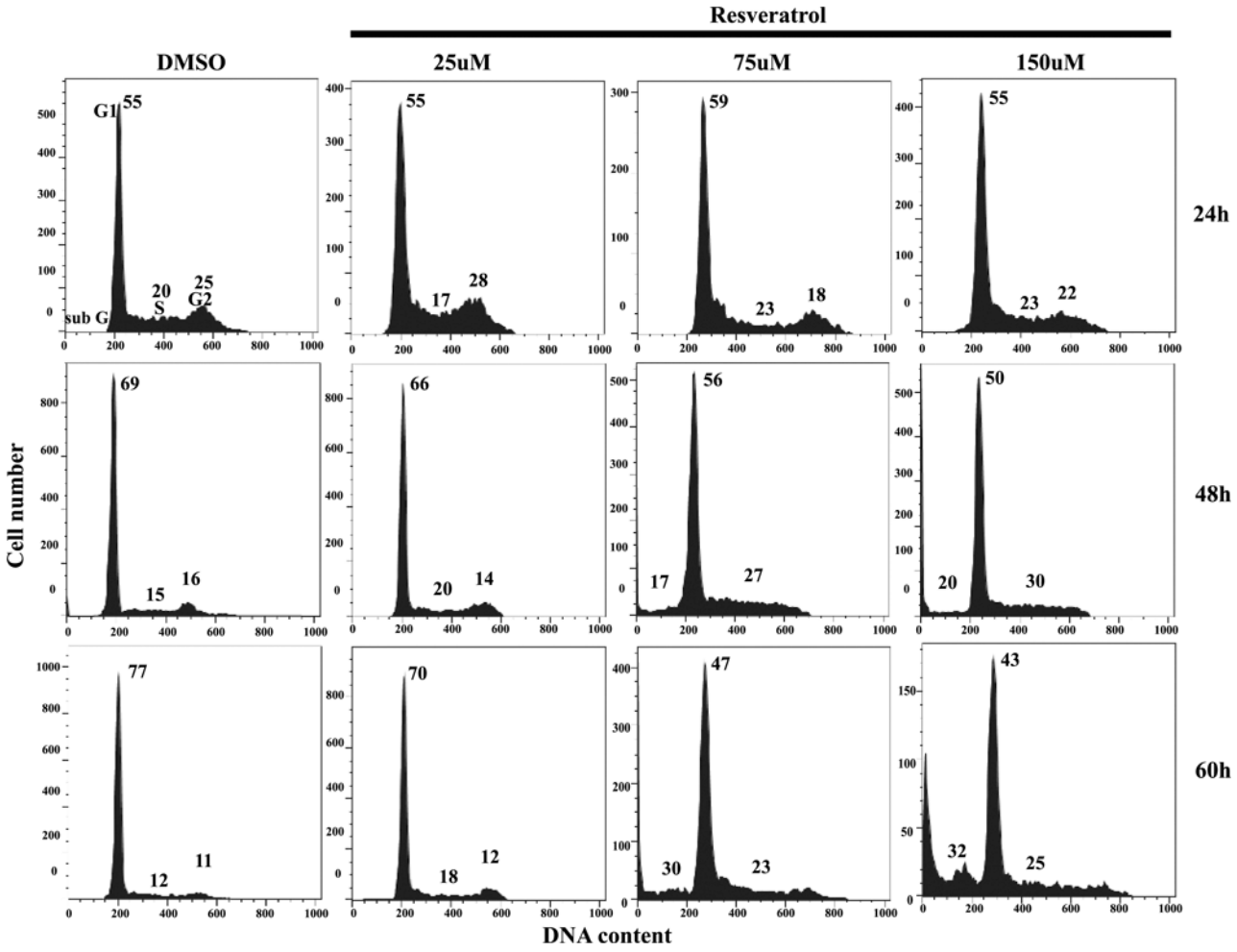

Figure 6.

Fluorescence-activated cell sorting analysis of LNCaP cells after treatment with resveratrol. Cell cycle phase distributions were quantified by staining cells with propidium iodide. Results are expressed as percent of cells in G_1, S, G_2-M, and sub-G_1 phase at each time point after exposure.