Carcinogenesis vol.28 no.9 pp.1946–1953, 2007
doi:10.1093/carcin/bgm144
Advance Access publication August 3, 2007

Resveratrol suppresses prostate cancer progression in transgenic mice

Curt E.Harper[1], Brijesh B.Patel[1], Jun Wang[1], Alireza Arabshahi[1], Isam A.Eltoum[2,3] and Coral A.Lamartiniere[1,2,*]

[1]Department of Pharmacology and Toxicology, [2]UAB Comprehensive Cancer Center and [3]Department of Pathology, University of Alabama at Birmingham, 1670 University Boulevard, Birmingham, AL 35294-0019, USA

*To whom correspondence should be addressed. Tel: 205-934-7139; Fax: 205-934-8240;
Email: coral@uab.edu

Resveratrol, a natural polyphenolic phytochemical, has been reported to act as an antioxidant and provide anticancer activities. We hypothesized that resveratrol would exert a chemopreventive effect against prostate cancer via regulation of sex steroid receptor and growth factor signaling pathways. In the current study, Transgenic Adenocarcinoma Mouse Prostate males were fed resveratrol (625 mg resveratrol per kg AIN-76A diet) or phytoestrogen-free, control diet (AIN-76A) starting at 5 weeks of age. Mechanisms of action and histopathology studies were conducted at 12 and 28 weeks of age, respectively. Resveratrol in the diet significantly reduced the incidence of poorly differentiated prostatic adenocarcinoma by 7.7-fold. In the dorsolateral prostate, resveratrol significantly inhibited cell proliferation, increased androgen receptor, estrogen receptor-β, and insulin-like growth factor-1 receptor, and significantly decreased insulin-like growth factor (IGF)-1 and phospho-extracellular regulating kinase 1 (phospho-ERK 1). In the ventral prostate, resveratrol significantly reduced cell proliferation and phospho-ERKs 1 and 2, but did not significantly alter insulin-like growth factor-1 receptor and IGF-1. Serum total testosterone, free testosterone, estradiol, dihydrotestosterone and sex hormone-binding globulin (SHBG) concentrations and Simian Virus-40 large T antigen expression in the prostate were not altered in resveratrol-treated mice. Total resveratrol concentration in the blood serum of 12-week-old mice treated for 3 weeks with 625 mg resveratrol per kg diet was 52 ± 18 nM. The decrease in cell proliferation and the potent growth factor, IGF-1, the down-regulation of downstream effectors, phospho-ERKs 1 and 2 and the increase in the putative tumor suppressor, estrogen receptor-β, provide a biochemical basis for resveratrol suppressing prostate cancer development.

Introduction

With an anticipated 27 050 deaths in 2007, prostate cancer is second only to lung cancer in cancer-related deaths among men (1). In the prostate, pre-neoplastic lesions start to develop early in life. It has been reported that men of 20–40 years develop low-grade prostatic intraepithelial neoplasia (PIN), with a frequency of 9, 16 and 26%, respectively (2). In addition, high-grade PIN, the precursor to prostate adenocarcinoma is present at a frequency of 0, 5, 10, 41 and 63% of men in their 3rd, 4th, 5th, 6th and 7th decades, respectively (3). According to epidemiological and laboratory data, diet, lifestyle and environmental exposure could play a major role in the etiology of prostate cancer (4–7). Therefore, chemoprevention and the use of dietary supplements without toxicity to reduce cancer risk are extremely pertinent.

Dietary polyphenols have recently gained much attention with respect to disease prevention. Our laboratory has previously shown that the polyphenol, genistein, suppresses prostate cancer in transgenic mice (8). We are also interested in other polyphenols with reported health benefits. Resveratrol (trans-3,4′,5 trihydroxystilbene) is a phytoalexin stilbene found in grape products (e.g. wine and berries). It has been shown to reduce the risk of heart disease (9) and act as an antioxidant (10) and anti-inflammatory agent (11). *In vitro* studies have demonstrated that resveratrol has the ability to halt carcinogenesis at the initiation, promotion and progression stages (12). Although dietary resveratrol has been shown to suppress mammary tumors in 7,12-dimethylbenz(a)anthracene and nitrosylmethylurea induced mammary cancer models (13–15), chemoprevention of prostate cancer has not yet been reported with resveratrol in animal models.

The mechanism of action of resveratrol is not completely elucidated. In cell cultures, resveratrol can activate tumor suppressor gene p53 (16), decrease cyclooxygenase-2 expression (17), reduce nuclear factor-kappa B activation (18) and inhibit protein tyrosine kinase activity (19). Evidence shows that resveratrol may partially act by way of sex steroid receptor and growth factor signaling. Using prostate cancer cells, Gao *et al.* (20) demonstrated the ability of resveratrol to modulate androgen receptor (AR) by way of the Raf–MEK–extracellular signal-regulating kinase (ERK)-signaling pathway. Another report described resveratrol as a mixed agonist/antagonist for estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β) using cytosolic extracts from MCF-7 cells and rat uteri (21). Resveratrol has been shown to alter the insulin-like growth factor (IGF)-signaling pathway at the ligand (22) and receptor (23) levels in breast cancer cells. A plethora of *in vitro* studies have demonstrated a reduction in cell proliferation and an increase in apoptosis in both androgen-dependent and -independent prostate cancer cell lines (16,24–27). *In vivo* studies investigating the potential of resveratrol to alter sex steroid receptor and growth factor protein expression, cell proliferation and apoptosis in the prostate have been limited. It is important to expand on the findings of *in vitro* studies to determine their biological relevance in an animal model prior to using dietary agents in clinical trials.

In this study, we investigated the potential of resveratrol in the diet to protect against spontaneously developing prostate cancer using the Transgenic Adenocarcinoma Mouse Prostate (TRAMP) model, an animal that closely mimics prostate cancer in humans (28). In the TRAMP model, *Simian Virus-40 large T antigen* (SV-40 Tag) is under control of the rat probasin promoter, allowing androgen-regulated protein expression specific to the epithelium of the prostate. *SV-40 Tag* acts as an oncogene by interacting with *retinoblastoma* and *p53* tumor suppressor gene products to disrupt cell cycle regulation leading to uncontrolled proliferation. Already, the TRAMP model has been used in prostate cancer chemoprevention studies with other dietary agents (8,29,30). For this study, we hypothesized that dietary resveratrol would protect against prostate cancer development, inhibit cell proliferation and modulate sex steroid receptor expression and IGF-signaling proteins in the prostate.

Abbreviations: AR, androgen receptor; BSA, bovine serum albumin; DHT, dihydrotestosterone; DLP, dorsolateral prostate; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular regulating kinase; ER-α, estrogen receptor-α; ER-β, estrogen receptor-β; IGF, insulin-like growth factor; IGF-BP3, insulin-like growth factor-binding protein 3; IGF-1R, insulin-like growth factor-1 receptor; PBS, phosphate-buffered saline; phospho-ERK 1, phospho-extracellular regulating kinase 1; PIN, prostatic intraepithelial neoplasia; SV-40 Tag, Simian Virus-40 large T antigen; TRAMP, Transgenic Adenocarcinoma Mouse Prostate; VP, ventral prostate.

Materials and methods

Animals

Animal care and treatments were conducted according to established guidelines and protocols approved by the Animal Care and Use Committee of the University of Alabama at Birmingham. TRAMP male and female breeders were obtained from the National Cancer Institute Mouse Repository (Cancer Research Center, Frederick, MD) and used to develop this colony. Then, heterozygous transgenic females were bred with non-transgenic C57BL/6 males in order to generate heterozygous transgenic male offspring (TRAMP C57BL/6 females × C57BL/6 male breeders). At 3 weeks of age, the offspring were weaned and tails were clipped. DNA was extracted using a DNeasy Tissue Kit

(Qiagen, Valencia, CA), and a polymerase chain reaction-based screening assay was performed to evaluate transgene incorporation (8,28). Dietary treatment with resveratrol was initiated at 5 weeks of age and continued until the animals were killed at either 12 or 28 weeks of age for mechanisms of action or chemoprevention studies, respectively. Control animals received powdered phytoestrogen-free AIN-76A diet (Harlan Teklad Global Diets, Wilmington, DE). The treatment group received 625 mg resveratrol per kg diet; a dose extrapolated from the resveratrol mammary cancer chemoprevention study carried out by Bhat *et al.* (14). Body weights were monitored at 6, 12, 18, 24 and 28 weeks of age. For cell proliferation analysis and protein biomarkers, animals were killed at 12 weeks of age and dorsolateral prostate (DLP) and ventral prostate (VP) and testes were excised, weighed, flash frozen in liquid nitrogen and stored at −80°C until time of analysis. For the determination of resveratrol concentration in blood serum, 9-week-old mice were treated with 625 mg resveratrol per kg diet for 3 weeks and killed between 9:00 and 10:00 AM.

Chemicals

Resveratrol, extracted from Rhizoma Polygoni Cuspidati (Xi'an Sino-Dragon Import & Export Co., China) and tested as 98% pure by High-performance liquid chromatography was mixed at 625 mg resveratrol per kg AIN-76A diet by the laboratory of Dr Clinton Grubbs (University of Alabama at Birmingham Chemoprevention Center).

Histopathology

At time of necropsy in the chemoprevention studies (28 weeks of age), all organs were examined for gross abnormalities in control ($n = 53$) and resveratrol-treated ($n = 29$) animals. We evaluated macrometastasis to the bone, abdominal wall, lymph nodes, liver, kidney and lung. Also, we monitored prostate, testes, seminal vesicle and tumor weights. The entire urogenital tract containing the DLP, VP and urethra were placed in cassettes, immersed in 10% formalin, dehydrated in a series of alcohol dilutions, fixed in xylene, embedded in paraffin wax, sliced into 5 μm sections and placed on SuperfrostPlus (Fisher Scientific, Pittsburgh, PA) microscope slides as described by Folkvord *et al.* (31). Sections were stained with hematoxylin and eosin. Dr Isam Eltoum, a Board Certified Pathologist, blindly scored each coded sample using the following grading scale developed specifically for rodents as described by Wechter *et al.* (32): Grade 1 (non-cancerous), Grade 2 (low-grade PIN), Grade 3 (high-grade PIN), Grade 4 (well-differentiated lesion), Grade 5 (moderately differentiated lesion) or Grade 6 (poorly differentiated lesion).

Immunohistochemistry

Immunohistochemistry was employed to measure Ki-67 antigen as a marker for proliferating cells in DLP and VP of 12-week-old TRAMP mice (six controls, six resveratrol treated). SV-40 Tag was evaluated immunohistochemically to determine if the effects of resveratrol was due to an indirect effect caused by a reduction in transgene expression or a direct biological effect on the prostate. Briefly, paraffin-embedded tissue sections on glass slides were deparaffinized in xylene and rehydrated in a gradient of alcohols. Samples were boiled in Antigen Unmasking Solution (Vector, Burlingame, CA) for 20 min and then allowed to cool to room temperature. Endogenous peroxidase activity was blocked by incubating specimens in 3% H_2O_2 at room temperature for 10 min. Blocking was done using ready-to-use 2.5% normal horse serum from the ImmPRESS™ Reagent Kit (Anti-Mouse Ig; Vector). Next, monoclonal mouse anti-rat Ki-67 antigen, Clone MIB-5 antibody (DakoCytomation, Carpinteria, CA) or SV-40 Tag (Santa Cruz Biotechnology, Santa Cruz, CA, SC-147) diluted in phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) was applied to the specimens for 30 min followed by washes in PBS. ImmPRESS™ Reagent secondary antibody was then applied to the samples for 30 min followed by washes in PBS. Chromogen, diaminobenzidine (Vector) was applied to samples for 10 min followed by a wash in tap H_2O for 5 min. To counterstain, hematoxylin QS (Vector) was applied to the specimens for 1 min, followed by a wash in tap H_2O. Specimens were immersed in a series of graded alcohols, placed in xylene and mounted with coverslips using Mounting Media (Vector). The slides were viewed using a Nikon Labophot-2 microscope (Nikon Corporation, Tokyo, Japan) and digitally recorded using a Nikon 8.0 Mega Pixels CoolPix 8700 Digital Camera (Nikon). For cell proliferation, epithelial cells were counted using Image J software (Image J, National Institute of Health). The DLP and VP were analyzed separately (a minimum of 2000 cells counted per lobe per slide). The epithelial cells staining positive (brown) for Ki67 as well as the non-proliferative epithelial cells (stained blue) were counted. The proliferative index was defined as the number of positively stained cells divided by the total number of cells counted × 100. SV-40 Tag expression was semi-quantitated and localization was evaluated as described previously (33).

Immunoblot analyses

When possible, protein expression levels of sex steroid and growth factor receptors and their ligands were measured by western blot analysis. Briefly, tissues were homogenized in radioimmuno precipitation assay lysis buffer (Upstate, Lake Placid, NY) and protease/phosphatase inhibitors (Sigma, St Louis, MO). Protein concentrations for each sample were determined using the Bradford Protein assay (Pierce, Rockford, IL). Protein supernatant from each sample was added at a ratio of 1:1 to sample buffer containing the following: 0.5 M Tris, pH 8.8, glycerol, 10% sodium dodecyl sulfate, 1% bromophenol blue and β-mercaptoethanol. Prior to the analysis of the samples, antibody conditions were optimized using mice DLP and VP tissue and positive and negative controls in order to correctly identify the band of interest. When analyzing our samples via western blot analysis, we occupied all 26 lanes in the gel with eight samples per group (24 total samples) and two molecular weight ladders (Kaleidoscope and Pre-stained Broad Range (Bio-Rad, Hercules, CA) per gel in order to correctly identify the band of interest. Equal concentrations of protein were electrophoresed and transferred to nitrocellulose membranes. The membranes were blocked with 5% non-fat milk and incubated overnight with the appropriate primary antibodies. After a series of washes, the proper secondary antibody conjugated with horseradish peroxidase was applied. After an additional series of washes, SuperSignal West Dura Chemiluminescence (Pierce) was applied to detect the proteins of interest. The relative intensity of the bands was measured using VersaDoc Imaging System (BioRad). Antibodies were purchased from commercial sources and detailed as follows: AR (Santa Cruz, SC-816), ER-α (Santa Cruz, SC-542), insulin-like growth factor-1 receptor (IGF-1R) (Santa Cruz, SC-712), insulin-like growth factor-binding protein 3 (IGF-BP3) (Santa Cruz, SC-9028), phospho-extracellular regulating kinases 1 and 2 (phospho-ERKs 1 and 2) (Cell Signaling Technology, Danvers, MA, #9101S) and total-extracellular regulating kinases 1 and 2 (total ERKs 1 and 2) (Cell Signaling, #9102). Positive protein controls purchased from the supplier of the corresponding antibodies and the use of Kaleidoscope Precision Plus Protein and Pre-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis Broad Range standards (BioRad) were employed in order to identify the protein of interest.

Enzyme-linked immunosorbent assay

IGF-1 and ER-β proteins were quantitated via enzyme-linked immunosorbent assay (ELISA) (34) because reliable antibodies to evaluate these proteins by western blot analysis were not available. Kinetic curves were set up for each protein to establish zero order kinetics. For each sample, 1 μg of protein was diluted in 100 μl of coating solution (10 mM PBS, pH 7.2) and applied to a 96-well Nunc-Immuno Plate (Nalge Nunc International, Rochester, NY). Next, overnight incubation at room temperature and a series of washes with 1× PBS + 0.05% Tween 20 (BioRad) were implemented. PBS + 1% BSA was applied for 1 h to block extraneous binding sites and then washed. The appropriate primary antibody, IGF-1 (Santa Cruz, SC-9013) or ER-β (Upstate, #06-629), diluted in 10 mM PBS + 1% BSA was added and incubated for 2 h. After washing with PBS, horseradish peroxidase-conjugated secondary antibody diluted in PBS + 1% BSA was incubated for 4 h at room temperature. Following a series of washes, the reaction was incubated with the ImmunoPure TMB Substrate Kit (Pierce) and halted using 2 N H_2SO_4 as a stopping solution. Samples were run in duplicate and the absorbance at 450 nm was read in an OPTI max Microplate reader (Molecular Devices, Sunnyvale, CA). For the IGF-1 assay, mouse liver with and without IGF-1 primary antibody served as positive and negative controls, respectively. Rat prostate with and without ER-β primary antibody was used as positive and negative controls for the ER-β assay.

Sex steroid blood serum concentrations

Serum total testosterone (bound and unbound), free testosterone (unbound), serum-hormone-binding globulin (SHBG), dihydrotestosterone (DHT) and estradiol concentrations were measured from 12-and 28-week-old control and resveratrol-treated transgenic animals using radio-immunoassays (Diagnostic Systems Laboratories, Webster, TX) as described by the manufacturer. All samples were run in duplicate with eight samples per group by Dr John Mahan (obstetrics/gynecology Department, University of Alabama at Birmingham, Birmingham, AL).

Resveratrol blood serum concentrations

Since rodents are nocturnal animals and eat predominately at night, blood samples were collected in the morning. Immediately following blood collection, serum was pooled (five mice per sample), centrifuged at 1200*g* for 15 min and stored at −80°C until processed for analysis. Resveratrol stock solution was prepared in 80% methanol in water at a concentration of 1.0 mg/ml. The lower limit of quantification using these methods was at a signal-to-noise ratio of 10:1 (5 nM resveratrol). Interassay coefficients of variation were <30% at

lower limit of quantification. Standard working solutions (0.05–25 μM) containing chrysin, phenolphthalein and 4-methylumbelliferone sulfate (internal standards, all 100 nM, as markers of effective hydrolysis) were prepared by appropriate dilution with 80% methanol in water. Mice serum samples (0.2 ml) were diluted into ammonium acetate buffer, pH 5, and reacted with *Helix pomatia* β-glucuronidase-sulfatase at 37°C for 4 h and the aglucones were extracted twice with 2 ml ethyl acetate by vortexing for 1 min in a 5 ml glass test tube. Chrysin was added as a recovery marker for the aglucones. The ethyl acetate phases were separated by centrifugation for 10 min at 3000*g*. The extracts were evaporated to dryness under nitrogen and then reconstituted in 100 μl of 80% aqueous methanol. Aliquots (20 μl) of the reconstituted extract were injected onto a 2.0 × 100 mm Luna 3 μm Phenyl-hexyl reverse-phase column (Phenomenex, Torrance, CA). The mobile phases were (A) 10 mM NH_4OAc and (B) acetonitrile 10 mM NH_4OAc. The column was pre-equilibrated in 20% B:80% A. After injection, the concentration of B in the mobile phase was increased at 5%/min to 100% B over a 0–5 min period. Then, the concentration of B was decreased to 0% at 50%/min for 1 min and then held isocratically for another 4 min. The total cycle time was 10 min per sample. The mobile phase flow rate was 0.2 ml/min. The column eluate was passed into the chemical ionization interface of a MDS-Sciex 4000 Qtrap triple quadrupole mass spectrometer (MDS Sciex, Ontario, Canada). The interface was operated in the negative mode with a source temperature of 400°C and a nebulizing current of 3 amp. Resveratrol was detected by selecting its $[M-H]^-$ molecular ions in the first quadrupole, causing collisional dissociation with nitrogen gas and selecting a specific daughter ion fragment in the third quadrupole. Selected fragment ions for resveratrol were used for specific and quantitative measurements using a multiple reaction ion monitoring mass spectrometry approach. The cycle time for the complete group was 0.93 s. All sera were independently analyzed in duplicate. Individual ion chromatograms representing each compound were analyzed by Analyst 1.4.1 and the area under the peak eluting in the correct time window was determined. This was normalized to the chrysin internal standard peak and the relative peak area was plotted against concentration of the standard. After fitting the response curve to a polynomial function, the relative areas for the unknowns were calculated by interpolation.

Statistics

Fisher's exact test was implemented to evaluate histopathological grade frequencies among treatment groups. For biochemical data generated from immunoblot analysis and ELISA, statistical comparisons were performed using two-sample Student's *t*-test assuming unequal variances. We considered $P < 0.05$ to be significant.

Results

Resveratrol in the diet suppressesd prostate cancer development in TRAMP mice. By 28 weeks of age, TRAMP mice fed control diet developed high-grade PIN (Grade 3) and prostate cancer (Grades 4–6) at a frequency of 34 and 67%, respectively (Table I). None of the animals on control diet demonstrated normal (Grade 1) or low-grade PIN (Grade 2). Resveratrol in the diet significantly reduced the incidence of poorly differentiated prostatic adenocarcinoma (Grade 6 lesions) from 23 to 3% (7.7-fold) and delayed the progression of well differentiated (Grade 4 lesions) from 42% in controls to 62% in resveratrol-treated mice. There was no statistical change in latency, number of tumors per animal, tumor weight or number of liver, kidney, lung or lymph node metastases between the control- and resveratrol-treated animals. Following necropsy, all animals were confirmed to be transgenic by a second round of tail clipping and polymerase chain reaction.

Table I. Histopathological analysis of the urogenital tract of 28-week-old TRAMP mice fed control AIN-76A diet or AIN-76A diet supplemented with 625 mg resveratrol per kg diet starting at 5 weeks of age

Treatment	*n*	Grade level					
		1	2	3	4	5	6
Control	53	0%	0%	34%	42%	2%	23%
Resveratrol	29	0%	0%	31%	62%	3%	3%*

Samples were given a score of 1 (no tumor), 2 (low-grade PIN), 3 (high-grade PIN), 4 (well-differentiated tumor), 5 (moderately differentiated tumor) and 6 (poorly differentiated tumor) depending on the presence and progression of lesions. Results are the percentage of mice as a function of the pathological score.
*$P = 0.027$ compared with control treatment.

To determine if the effect of resveratrol was directly on the probasin promoter, we measured SV-40 Tag expression. There was no difference in SV-40 Tag expression in the prostate epithelium of resveratrol-treated mice compared with controls (Figure 1A and B).

Resveratrol in the diet was well tolerated and there was no evidence of toxicity. There was no significant change in body weights in resveratrol-treated mice compared with controls at 6, 12, 18, 24 and 28 weeks of age (data not shown). Likewise, there was no significant difference in food or water intake in resveratrol-treated mice when compared with controls. Furthermore, there was no significant change between treatment groups in the following tissue weights at 12 and 28 weeks of age: testes, DLP, VP and tumor weight. Testes to body weight ratios did not differ between resveratrol and control-treated mice (data not shown).

Resveratrol decreased cell proliferation in prostates of TRAMP mice (Figure 1C and D). We elected to carry out mechanistic studies at 12 weeks because TRAMP mice develop premalignant lesions by 10–12 weeks of age. Hence, this is a good sampling time to investigate cell proliferation and biomarkers as mechanisms of action prior to the time that tumors become large and compromise oxygen and nutrients to the tissue. Furthermore, at 12 weeks, these animals have been exposed to the resveratrol for 7 weeks and that is shortly after PIN is measurable, but with no tumors. Using the Ki67 assay, we found that resveratrol significantly decreased cell proliferation in the DLP by 50%, in the VP by 31% and in the entire prostate (DLP + VP combined) by 43% (Figure 1E).

The sex steroid receptors, AR and ER-β, but not ER-α, were regulated by dietary resveratrol. In the DLP, resveratrol in the diet caused a 2.6-fold (160%) increase in AR and a 1.65-fold (65%) increase in ER-β protein expressions, but no significant change in ER-α (Figure 2). In the VP of resveratrol-treated TRAMP mice, AR and ER-α protein levels were not significantly different from those of controls. Because of limited sample volume, ELISA for ER-β in the VP was not possible.

The IGF-1-signaling proteins were differentially regulated by resveratrol in the DLP and VP. Resveratrol treatment significantly reduced IGF-1 in the DLP, but not in the VP (Figure 3). On the other hand, resveratrol resulted in up-regulated IGF-1R in the DLP, but down-regulated IGF-1R in the VP. The expression of IGF-BP3, the most abundant binding protein, was not significantly changed in the DLP or VP (data not shown). In addition, we investigated the effect of resveratrol on the major site of IGF-1 production, the liver. IGF-1, IGF-1R and IGF-BP3 protein expression did not differ in the liver between control and treated animals (data not shown).

Resveratrol decreased the protein levels of phosphorylated ERKs 1 and 2. The protein kinases, ERK-1 (p44 MAPK) and ERK-2 (p42 MAPK) belonging to an extensively studied group of mitogen-activated protein kinases were measured. Phospho-ERK 1 (activated form) was decreased 51% in the DLP and phospho-ERKs 1 and 2 in the VP were down-regulated 34 and 43%, respectively (Figure 4). On the other hand, total-ERKs 1 and 2 (phosphorylated plus unphosphorylated) remained unchanged in DLP and VP (data not shown).

Resveratrol did not alter sex steroid concentrations in the blood serum. We quantified sex steroid concentrations in the blood serum of 12- and 28-week-old TRAMP mice fed control or resveratrol-containing diet. Total testosterone, free testosterone, estradiol, DHT and SHBG did not differ significantly between resveratrol- and control-treated mice (data not shown). Resveratrol concentrations were determined from blood serum of control and resveratrol-exposed mice. Total resveratrol (free and conjugated) concentration in the blood serum of 12-week-old mice treated for 3 weeks with 625 mg resveratrol per kg diet was 52 ± 18 nM.

Discussion

To our knowledge, this is the first study to show that resveratrol in the diet suppresses spontaneously developing prostate cancer in an animal

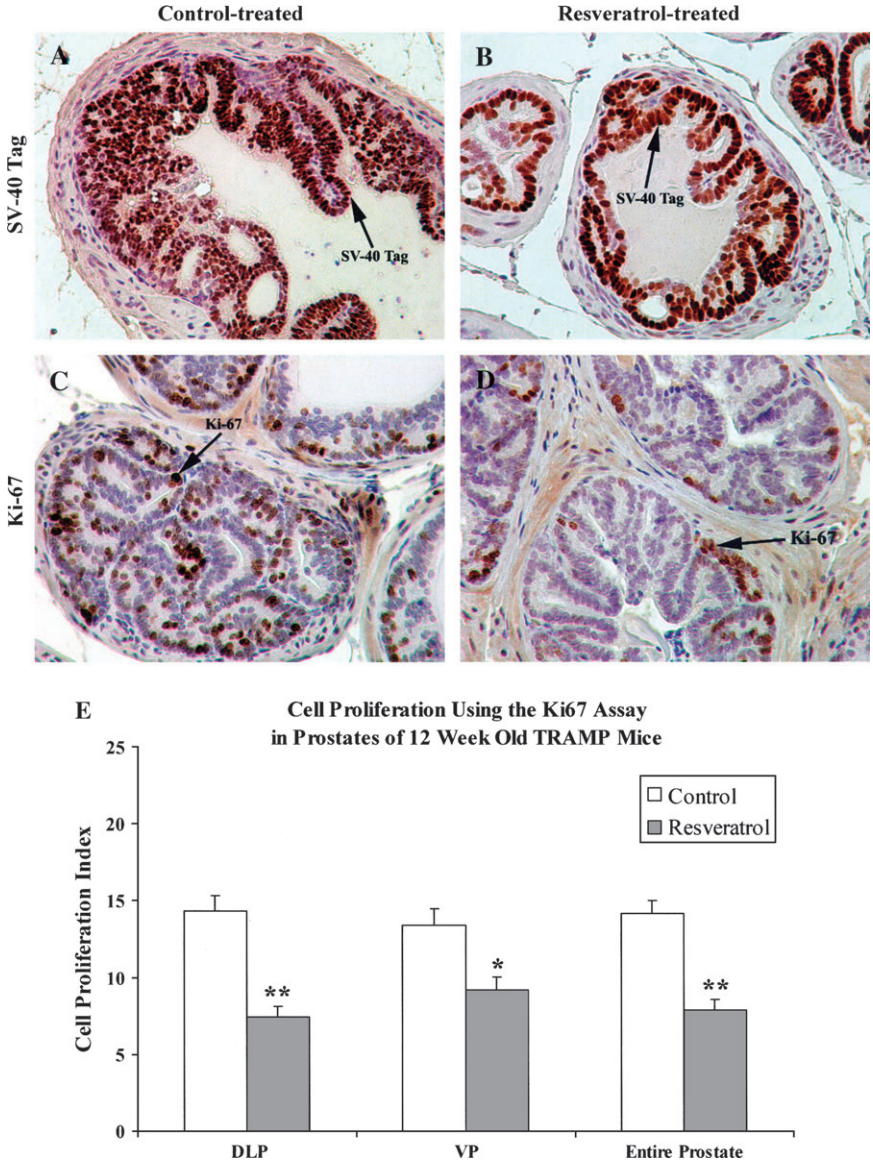

Fig. 1. SV-40 Tag and Ki67 expressions in the DLP of 12-week-old TRAMP mice fed AIN-76A diet (control) or AIN-76A diet supplemented with 625 mg resveratrol per kg diet starting at 5 weeks of age. Sections (**A** and **B**) represent immunohistochemical staining of SV-40 Tag in control- and resveratrol-treated mice prostate cells, respectively, (×40) and (**C** and **D**) Ki67 in control- and resveratrol-treated mice prostate cells, respectively (×40). Arrows point to positively stained epithelial cells. (**E**) Cell proliferation in the DLP, VP and entire prostate. $^{*}P < 0.05$ and $^{**}P < 0.001$ compared with control treatment.

model. There was an 87% decrease (from 23 to 3%) in poorly differentiated prostate tumors in resveratrol-fed mice compared with controls. To accompany this, there was a 42% to 62% increase in well-differentiated tumors, indicating that prostate cancer was arrested at this stage, i.e. the progression of prostate tumor development was slowed down. The chemopreventive potential of resveratrol is supported by the early report of Jang *et al.* (12), where resveratrol induced human promyelocytic leukemia cell differentiation, inhibited the development of pre-neoplastic lesions in 7,12-dimethylbenz(a) anthracene-treated mouse mammary glands in culture and inhibited tumorigenesis in a mouse skin cancer model. In addition, three reports have shown that resveratrol suppressed chemically induced mammary cancer in Sprague Dawley rats (13–15).

The ability to delay the onset or diminish the progress of slowly developing cancers such as prostate cancer by chemoprevention can have a huge impact on human lives. Clinicians and scientists are aware that men are predisposed for prostate cancer since high-grade PIN is found in 5, 10 and 41% of men in their 4th, 5th and 6th decades, respectively (3). Although we may not wipe out prostate cancer, we do hope to suppress the progression of prostate cancer so men can extend their lives and quality of life. Supporting our chemoprevention finding is the mechanistic data that resveratrol can regulate cell proliferation, sex steroid receptor protein expression and specific growth factor signaling proteins in the prostate.

A decrease in cell proliferation often accompanies a delay in cancer progression. In young transgenic mice that demonstrate high-grade PIN, we observed a 43% reduction in the cell proliferation index in the prostate of resveratrol-treated TRAMP mice. In comparison, other studies have illustrated the anti-proliferative effects of resveratrol (25,27) in both androgen-responsive (LNCaP) and -independent (DU 145 and PC-3) human prostate carcinoma cells lines. In our study, the reduction in proliferation most probably plays a major factor in the chemopreventive action of resveratrol. It is important to note that resveratrol in the diet did not alter the expression of SV-40 Tag in the prostates of TRAMP mice, since SV-40 Tag was readily detectable in both resveratrol- and control-treated mice. These results demonstrate

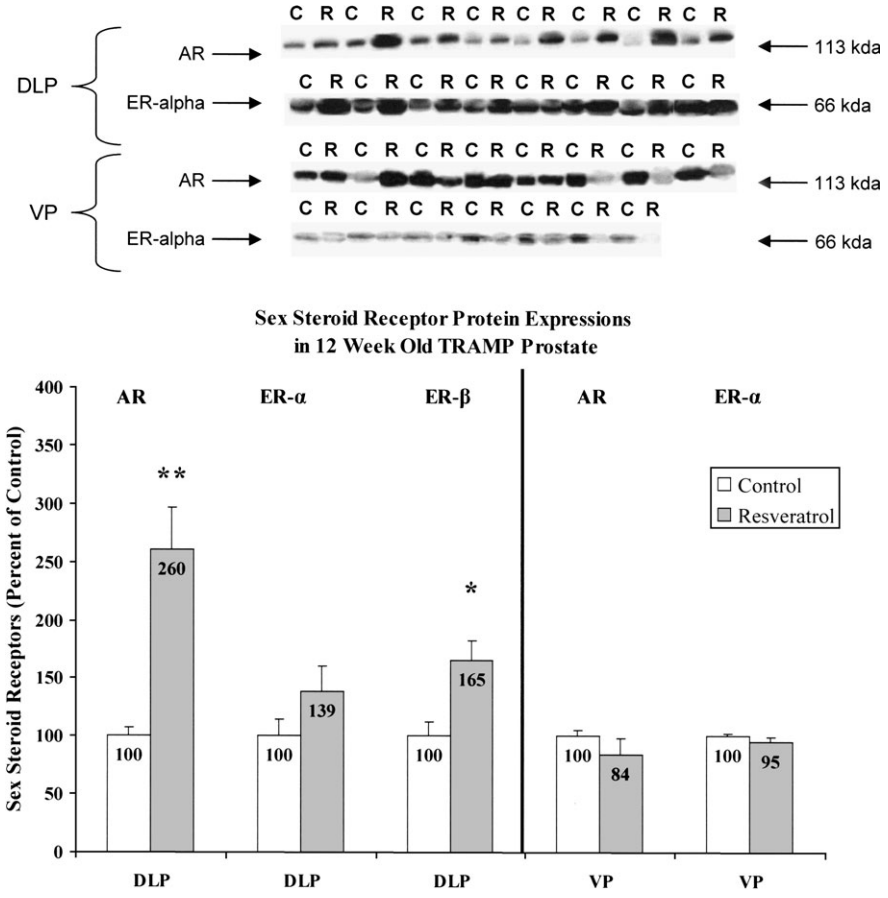

Fig. 2. AR, ER-α and ER-β protein expressions in DLP and VP of 12-week-old TRAMP mice fed AIN-76A diet (control) or AIN-76A diet supplemented with 625 mg resveratrol per kg diet starting at 5 weeks of age. Upper bands depict western blots for AR and ER-α (C, control and R, resvertrol treatments) and the lower figure is a graph of densitometry measurements from these western blot analyses plus ELISA for ER-β. Each sample consisted of three pooled prostates and each group contained seven to eight samples. Densitometry values for control mice were set at 100. There was inadequate sample to perform ELISA for ER-β in the VP. $^{*}P < 0.05$ and $^{**}P < 0.001$ compared with control treatment.

that the observed effects of resveatrol is a 'direct' biological effect on the prostate and not simply through the down-regulation of the transgene. After noting these biological changes, we shifted our focus to determining underlying mechanisms responsible for the changes in cell proliferation.

Androgen signaling mediated by the ligand-activated transcription factor, AR and its corresponding ligands, testosterone and DHT, are believed to play a major role in prostate development and the etiology of prostate cancer. Historically, down-regulation in AR has been associated with a decrease in prostate cancer risk. In contrast, we showed a 2.6-fold increase in AR in the DLP and no change in the VP of 12-week-old TRAMP mice. Interestingly, Gao *et al.* (20) found that resveratrol enhanced AR transactivation at lower concentrations and inhibited AR transactivation at higher concentrations in LNCaP and PC-3 prostate cancer cells. Our data suggest that resveratrol exerts its action, in part, by modulating the function and expression of AR in a lobe-specific manner. In regards to this tissue specificity, Prins *et al.* (35) reported differential regulation of AR in the separate lobes of the rodent prostate. Also, it has been reported that spontaneously developing tumors in TRAMP mice originate first and more extensively in the DLP than the VP (36). Two plausible causes of the up-regulation of AR in the DLP by dietary resveratrol could be a consequence of resveratrol down-regulating androgen levels or a compensation for reduced growth factor signaling (i.e. IGF signaling) in the prostate. However, our measurement of total testosterone, free testosterone, estradiol, DHT and SHBG did not differ significantly between resveratrol- and control-treated mice. Therefore, the up-regulation of AR in the DLP by resveratrol does not seem to account for the chemoprevention by resveratrol.

The increase in ER-β by resveratrol may provide protection against prostate cancer and insight into the mechanism of action of resveratrol. Our data compliment a previous study using MCF-10A and MCF-7 breast cancer cell lines that showed increased ER-β messenger RNA levels after resveratrol treatment (37). Moreover, the loss of ER-β expression is associated with the progression from normal prostate epithelium to PCa (38) and older ER-β knockout mice develop prostatic hyperplasia (39). Because of the frequent loss of ER-β expression in prostate cancer (38), it has been hypothesized that ER-β may act as a tumor suppressor (40). It has been suggested that ER-β may be an attractive therapeutic candidate for prostate cancer intervention (41,42). According to Pak *et al.* (43), the androgen metabolite, 5α-androstane-3β, 17β-diol, binds to ER-β and suppresses the growth of the prostate. Also, ER-α and ER-β are postulated to have different functions and are expressed at different levels and location in the prostate. ER-α, expressed solely in prostate stroma, has been shown to induce cell proliferation and to be elevated in prostate cancer cell lines (44). On the other hand, ER-β, expressed in both normal stroma and secretory prostate epithelium, has been reported to exert a protective effect by inhibiting proliferation in cancer cells (45). It is plausible that resveratrol is binding to ER-β in both the prostate stroma and epithelium or altering the levels of 5α-androstane-3β, 17β-diol and subsequently eliciting chemopreventive effects in the TRAMP model by regulating cell proliferation and differentiation. Unlike many polyphenols, resveratrol binds ER-α and ER-β with

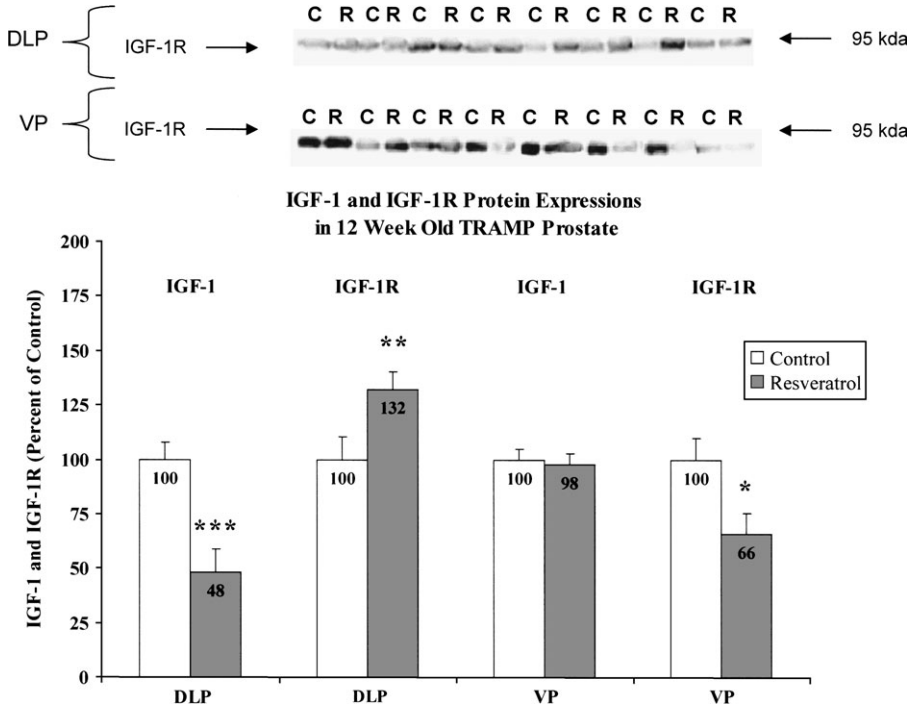

Fig. 3. IGF-1 and IGF-1R protein expressions in DLP and VP of 12-week-old TRAMP mice fed AIN-76A diet (control) or AIN-76A diet supplemented with 625 mg resveratrol per kg diet starting at 5 weeks of age. IGF-1 protein expression was determined via ELISA. Upper bands depict western blots for IGF-1R (C, control and R, resvertrol treatments) and the lower figure is a graph of the densitometry measurement from the western blot analysis. Each sample consisted of three pooled prostates and each group contained eight samples. Densitometry values for control mice were set at 100. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with control treatment.

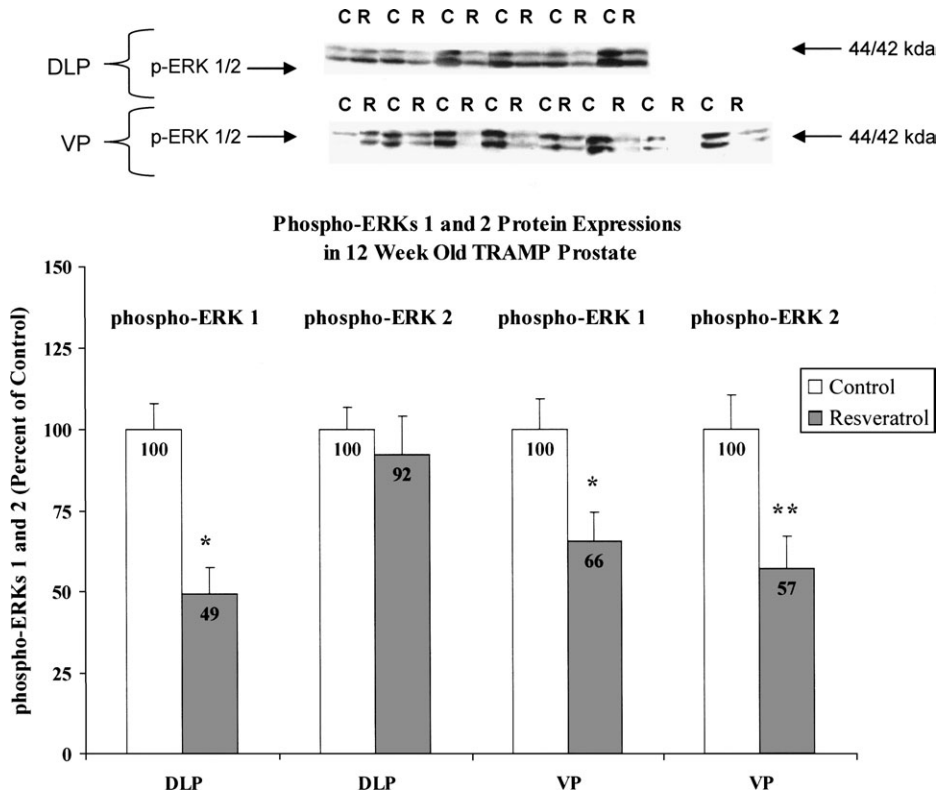

Fig. 4. Phospho-ERKs 1 and 2 protein expression in DLP and VP of 12-week-old TRAMP mice fed AIN-76A diet (control) or AIN-76A diet supplemented with 625 mg resveratrol per kg diet starting at 5 weeks of age. Upper bands depict western blots for phospho-ERKs 1 and 2 (C, control and R, resvertrol treatments) and the lower figure is a graph of densitometry measurements from these western blot analyses. Each sample consisted of three pooled prostates and each group contained six to eight samples. Densitometry values for control mice were set at 100. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with control treatment.

comparable relative binding affinities (46). According to Bowers *et al.* (21), resveratrol acts as an antagonist for ER-α but an agonist for ER-β. Interestingly, resveratrol-liganded ER-β has higher transcriptional activity than estradiol-liganded ER-β. Therefore, resveratrol has the potential to decrease cell proliferation by antagonizing ER-α and aid in suppressing prostate cancer by activating ER-β.

Because the IGF-1-signaling pathway has been associated with prostate cancer progression, we investigated if resveratrol could regulate key proteins in this pathway. In the DLP, IGF-1 was reduced by 52%, but remained unchanged in the VP of resveratrol-treated TRAMP mice. This decrease in IGF-1 suggests a means of protecting the prostate by reducing the potential for androgen-independent growth often associated with the IGF-signaling pathway. This down-regulation of IGF-1 in the DLP by resveratrol is supported by similar regulation of IGF-1 messenger RNA levels and IGF-2 in breast cancer cells (21–23,27). At the receptor level, resveratrol displayed differential regulation of IGF-1R in the prostatic lobes, up-regulating expression in the DLP and down-regulating expression in the VP. This reinforces the idea that rodent prostate lobes are physiologically different and may react differently to bioactive chemicals. Up-regulation in the DLP may be a compensatory effect due to the diminished presence of IGF-1 in that lobe. Although IGF-BP3 protein levels were not altered, IGF-BP3 has the opportunity to bind higher percentage of IGF-1 in the DLP because IGF-1 is decreased, thus leading to less activation of available IGF-1R and a reduction in growth factor signaling. In contrast, it has been reported that IGF-1 may act as a negative feedback signal to repress expression of IGF-1R because high IGF-1 levels result in a decline in IGF-1R (47). On the other hand, resveratrol does down-regulate IGF-1R in the VP, an action that can be viewed as a positive effect against cell proliferation and prostate cancer.

In addition to the modulation of IGF-1 signaling at the ligand and receptor level, downstream effector proteins (ERKs) were also regulated by resveratrol. We found a significant decrease in phospho-ERK 1 in the DLP and VP and a significant decrease in phospho-ERK 2 in the VP. Activation of ERKs 1 and 2 occur concomitant with prostatic epithelial cell proliferation and the initiation of cancer in the TRAMP model (48). According to Stewart (49), resveratrol antagonized EGFR-dependent ERKs 1 and 2 activation in androgen-independent prostate cancer cell lines. Mitogen-activated protein kinase signaling can be initiated by several growth factor ligand/receptor complexes including IGF and EGF signaling, and ultimately leads to the activation of ERKs 1 and 2 (50). Upon phosphorylation, ERKs 1 and 2 translocate to the nucleus and phosphorylate a wide array of transcription factors leading to the transcription of genes involved in proliferation, cell survival and differentiation. In TRAMP mice, resveratrol appears to regulate the IGF-signaling pathway at the ligand (IGF-1) and receptor (IGF-1R) level, thus reducing ERKs 1 and 2 activation. It appears that the down-regulation of IGF-1 plays a bigger role than the up-regulation of IGF-1R in the DLP since the downstream effector, phospho-ERK 1, is also down-regulated. It appears that the decrease in the cell proliferation is a result of the decreased signaling via the mitogen-activated protein kinase pathway. Also, the increase in AR protein expression in the DLP may be a compensation for reduced IGF-1 signaling in that particular lobe. Despite the uncertainty in the significance of up-regulated AR, resveratrol ultimately displayed decreases in phospho-ERKs 1 and 2, downstream effectors of not only the IGF-signaling pathway but also androgen signaling (51).

Our data demonstrate the selective actions of resveratrol and the differential regulation of the DLP and VP in the TRAMP model. As we expected, the most significant changes in protein biomarkers (IGF-1, ER-β, phospho-ERK) occurred in the DLP, where decreases in proliferative index were the most profound. Although the mechanism of action of resveratrol within each lobe varies, it is possible that interaction between the lobes could play a factor in the development and prevention of prostate cancer in this model. Likewise, the various zones within the human prostate may interact and contribute to tumorigenesis. In comparing the human prostate to the rodent prostate, the former is composed of three 'zones' including the central zone, periurethral transition zone and the peripheral zone (52). On the other hand, the rodent prostate can be divided into the ventral, anterior and dorsolateral lobes. In the TRAMP model, prostate cancer is reported to initially develop in the DLP (36). It has been shown that prostate cancer develops to a lesser extent and at a later date in the VP (36,53). In a study conducted by Wikstrom (53), prostate cancer (including well-differentiated, moderately differentiated and poorly differentiated tumors combined) developed 75% less in the VP than in the DLP at 36 weeks of age. Historically, it had been reported that the DLP of the rodent prostate is embryologically homologous to the peripheral zone of the human prostate, where the majority of prostate adenocarcinomas and PIN occurs (54). However, it was recently concluded by a panel of expert pathologists that definitive data does not exist to conclude that one lobe in the rodent model is more homologous to the peripheral zone in the human than another (52). Therefore, we focused our attention on both the DLP and VP. It is fundamentally important to differentiate between the actions of resveratrol in both lobes of the rodent. Future work should determine the similarities and differences in biochemistry between rodent prostate 'lobes' and human prostate 'zones' in order to correctly correlate animal studies with the clinical manifestations of human prostate cancer.

In the current study, the treatment group received 625 mg resveratrol per kg diet, a dose extrapolated from the resveratrol mammary cancer chemoprevention study carried out by Bhat *et al.* (14). This dose is equivalent to the consumption of approximately one 750 ml bottle of red wine per day. This was a proof of principle study and future studies will be designed to use lower concentrations of resveratrol as well as in combination with other polyphenols (i.e. genistein). It appears that when resveratrol is included in the diet, low nanomolar blood serum concentrations may be expected, as indicated by the 52 ± 18 nM concentration reached in our study. This is in agreement with a previous study that showed blood plasma levels reached 75 nM in mice with dietary resveratrol (23 mg/l) (55). Micromolar concentrations of peak total resveratrol have been reported 2.5–5 min post-gavage treatment, but concentrations measured at later time points (1–2 h) were low nanomolar or undetectable (55,56). Interestingly, the concentrations observed in mice were significantly lower than concentrations measured by Whitsett *et al.* (15) in which female rats were fed 1000 mg resveratrol per kg diet. Differences in metabolism and bioavailablity may contribute to the differences in blood serum resveratrol concentrations observed between mice and rats.

In summary, this is the first study to demonstrate that resveratrol in the diet can suppress spontaneously developing prostate tumors in a transgenic animal model. Up-regulation of the tumor suppressor ER-β and decrease in the IGF-1/ERK signal transduction pathway may play a significant role in the prostate chemopreventive actions of resveratrol. Down-regulation of phospho-ERK, a downstream effector of sex steroid receptor and growth factor signaling, is a significant finding and provides tremendous insight into the mechanism of action of resveratrol. Furthermore, resveratrol's ability to modulate, but not completely disrupt, multiple signaling pathways is consistent with components of natural products protecting against the progression of cancer, yet not causing toxicity.

Funding

The Department of Defense grant (DOD DAMD PC 17-03-1-0153) to C.A.L. National Cancer Institute Cancer Prevention and Control Training Program (NCI Grant CA 47888) to C.E.H. University of Alabama at Birmingham's Mass Spectrometry facility (NIH-P30 CA-13148-34).

Acknowledgements

We acknowledge Dr Stephen Barnes and University of Alabama at Birmingham's Mass Spectrometry facility, for assistance in measuring polyphenol concentrations. We thank Dr John Mahan, University of Alabama at Birmingham OB/GYN, for blood serum analysis of hormone concentrations and Dr Mark Carpenter, Auburn University, for his assistance with statistical analysis.

Conflict of Interest Statement: None declared.

References

1. American Cancer Society (2006) *Cancer Facts and Figures 2006*. American Cancer Society, Atlanta, GA.
2. Sakr,W.A. *et al*. (1993) The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. *J. Urol.*, **150**, 379–385.
3. Sakr,W.A. *et al*. (2000) Epidemiology and molecular biology of early prostatic neoplasia. *Mol. Urol.*, **4**, 109–113; discussion 115.
4. Ames,B.N. *et al*. (1998) The causes and prevention of cancer: the role of environment. *Biotherapy*, **11**, 205–220.
5. Weisburger,J.H. (2002) Lifestyle, health and disease prevention: the underlying mechanisms. *Eur. J. Cancer Prev.*, **11** (suppl. 2), S1–S17.
6. Wogan,G.N. *et al*. (2004) Environmental and chemical carcinogenesis. *Semin. Cancer Biol.*, **14**, 473–486.
7. Wolk,A. (2005) Diet, lifestyle and risk of prostate cancer. *Acta Oncol.*, **44**, 277–281.
8. Mentor-Marcel,R. *et al*. (2001) Genistein in the diet reduces the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice (TRAMP). *Cancer Res.*, **61**, 6777–6782.
9. Pace-Asciak,C.R. *et al*. (1995) The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin. Chim. Acta*, **235**, 207–219.
10. Kimura,Y. *et al*. (1985) Effects of stilbene derivatives on arachidonate metabolism in leukocytes. *Biochim. Biophys. Acta*, **837**, 209–212.
11. Chanvitayapongs,S. *et al*. (1997) Amelioration of oxidative stress by antioxidants and resveratrol in PC12 cells. *Neuroreport*, **8**, 1499–1502.
12. Jang,M. *et al*. (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, **275**, 218–220.
13. Banerjee,S. *et al*. (2002) Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloprotease 9. *Cancer Res.*, **62**, 4945–4954.
14. Bhat,K.P. *et al*. (2001) Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. *Cancer Res.*, **61**, 7456–7463.
15. Whitsett,T. *et al*. (2006) Resveratrol, but not EGCG, in the diet suppresses DMBA-induced mammary cancer in rats. *J. Carcinog.*, **5**, 15.
16. Lin,H.Y. *et al*. (2002) Resveratrol induced serine phosphorylation of p53 causes apoptosis in a mutant p53 prostate cancer cell line. *J. Urol.*, **168**, 748–755.
17. Kundu,J.K. *et al*. (2006) Resveratrol inhibits phorbol ester-induced expression of COX-2 and activation of NF-kappaB in mouse skin by blocking IkappaB kinase activity. *Carcinogenesis*, **27**, 1465–1474.
18. Manna,S.K. *et al*. (2000) Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J. Immunol.*, **164**, 6509–6519.
19. Jayatilake,G.S. *et al*. (1993) Kinase inhibitors from Polygonum cuspidatum. *J. Nat. Prod.*, **56**, 1805–1810.
20. Gao,S. *et al*. (2004) Modulation of androgen receptor-dependent transcription by resveratrol and genistein in prostate cancer cells. *Prostate*, **59**, 214–225.
21. Bowers,J.L. *et al*. (2000) Resveratrol acts as a mixed agonist/antagonist for estrogen receptors alpha and beta. *Endocrinology*, **141**, 3657–3667.
22. Vyas,S. *et al*. (2005) Resveratrol regulates insulin-like growth factor-II in breast cancer cells. *Endocrinology*, **146**, 4224–4233.
23. Serrero,G. *et al*. (2001) Effect of resveratrol on the expression of autocrine growth modulators in human breast cancer cells. *Antioxid. Redox Signal.*, **3**, 969–979.
24. Aziz,M.H. *et al*. (2006) Resveratrol-caused apoptosis of human prostate carcinoma LNCaP cells is mediated via modulation of phosphatidylinositol 3′-kinase/Akt pathway and Bcl-2 family proteins. *Mol. Cancer Ther.*, **5**, 1335–1341.
25. Kim,Y.A. *et al*. (2003) Antiproliferative effect of resveratrol in human prostate carcinoma cells. *J. Med. Food*, **6**, 273–280.
26. Morris,G.Z. *et al*. (2002) Resveratrol induces apoptosis in LNCaP cells and requires hydroxyl groups to decrease viability in LNCaP and DU 145 cells. *Prostate*, **52**, 319–329.
27. Sgambato,A. *et al*. (2001) Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage. *Mutat. Res.*, **496**, 171–180.
28. Greenberg,N.M. *et al*. (1995) Prostate cancer in a transgenic mouse. *Proc. Natl Acad. Sci. USA*, **92**, 3439–3443.
29. Gupta,S. *et al*. (2004) Suppression of prostate carcinogenesis by dietary supplementation of celecoxib in transgenic adenocarcinoma of the mouse prostate model. *Cancer Res.*, **64**, 3334–3343.
30. Gupta,S. *et al*. (2001) Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. *Proc. Natl Acad. Sci. USA*, **98**, 10350–10355.
31. Folkvord,J.M. *et al*. (1989) Optimization of immunohistochemical techniques to detect extracellular matrix proteins in fixed skin specimens. *J. Histochem. Cytochem.*, **37**, 105–113.
32. Wechter,W.J. *et al*. (2000) E-7869 (R-flurbiprofen) inhibits progression of prostate cancer in the TRAMP mouse. *Cancer Res.*, **60**, 2203–2208.
33. Nyska,A. *et al*. (2003) Slowing tumorigenic progression in TRAMP mice and prostatic carcinoma cell lines using natural anti-oxidant from spinach, NAO—a comparative study of three anti-oxidants. *Toxicol. Pathol.*, **31**, 39–51.
34. Crowther,J.R. (2000) The ELISA guidebook. *Methods Mol. Biol.*, **149**, III–IV1–413.
35. Prins,G.S. (1989) Differential regulation of androgen receptors in the separate rat prostate lobes: androgen independent expression in the lateral lobe. *J. Steroid Biochem.*, **33**, 319–326.
36. Gingrich,J.R. *et al*. (1999) Pathologic progression of autochthonous prostate cancer in the TRAMP model. *Prostate Cancer Prostatic Dis.*, **2**, 70–75.
37. Le Corre,L. *et al*. (2004) Effects of resveratrol on the expression of a panel of genes interacting with the BRCA1 oncosuppressor in human breast cell lines. *Clin. Chim. Acta*, **344**, 115–121.
38. Horvath,L.G. *et al*. (2001) Frequent loss of estrogen receptor-beta expression in prostate cancer. *Cancer Res.*, **61**, 5331–5335.
39. Krege,J.H. *et al*. (1998) Generation and reproductive phenotypes of mice lacking estrogen receptor beta. *Proc. Natl Acad. Sci. USA*, **95**, 15677–15682.
40. Iwao,K. *et al*. (2000) Quantitative analysis of estrogen receptor-beta mRNA and its variants in human breast cancers. *Int. J. Cancer*, **88**, 733–736.
41. Ho,S.M. (2004) Estrogens and anti-estrogens: key mediators of prostate carcinogenesis and new therapeutic candidates. *J. Cell. Biochem.*, **91**, 491–503.
42. Imamov,O. *et al*. (2004) Estrogen receptor beta regulates epithelial cellular differentiation in the mouse ventral prostate. *Proc. Natl Acad. Sci. USA*, **101**, 9375–9380.
43. Pak,T.R. *et al*. (2005) The androgen metabolite, 5alpha-androstane-3beta, 17beta-diol, is a potent modulator of estrogen receptor-beta1-mediated gene transcription in neuronal cells. *Endocrinology*, **146**, 147–155.
44. Bonkhoff,H. *et al*. (1999) Estrogen receptor expression in prostate cancer and premalignant prostatic lesions. *Am. J. Pathol.*, **155**, 641–647.
45. Weihua,Z. *et al*. (2002) An endocrine pathway in the prostate, ERbeta, AR, 5alpha-androstane-3beta,17beta-diol, and CYP7B1, regulates prostate growth. *Proc. Natl Acad. Sci. USA*, **99**, 13589–13594.
46. Mueller,S.O. *et al*. (2004) Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ERalpha) and ERbeta in human cells. *Toxicol. Sci.*, **80**, 14–25.
47. Yu,H. *et al*. (2000) Role of the insulin-like growth factor family in cancer development and progression. *J. Natl Cancer Inst.*, **92**, 1472–1489.
48. Uzgare,A.R. *et al*. (2003) Differential expression and/or activation of P38MAPK, erk1/2, and jnk during the initiation and progression of prostate cancer. *Prostate*, **55**, 128–139.
49. Stewart,J.R. *et al*. (2004) Resveratrol antagonizes EGFR-dependent Erk1/2 activation in human androgen-independent prostate cancer cells with associated isozyme-selective PKC alpha inhibition. *Invest. New Drugs*, **22**, 107–117.
50. Putz,T. *et al*. (1999) Epidermal growth factor (EGF) receptor blockade inhibits the action of EGF, insulin-like growth factor I, and a protein kinase A activator on the mitogen-activated protein kinase pathway in prostate cancer cell lines. *Cancer Res.*, **59**, 227–233.
51. Nguyen,T.V. *et al*. (2005) Androgens activate mitogen-activated protein kinase signaling: role in neuroprotection. *J. Neurochem.*, **94**, 1639–1651.
52. Shappell,S.B. *et al*. (2004) Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the bar harbor meeting of the mouse models of human cancer consortium prostate pathology committee. *Cancer Res.*, **64**, 2270–2305.
53. Wikstrom,P. *et al*. (2005) Characterization of the autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) as a model to study effects of castration therapy. *Prostate*, **62**, 148–164.
54. Price,D. (1963) Comparative aspects of development and structure in the prostate. *Natl Cancer Inst. Monogr.*, **12**, 1–27.
55. Asensi,M. *et al*. (2002) Inhibition of cancer growth by resveratrol is related to its low bioavailability. *Free Radic. Biol. Med.*, **33**, 387–398.
56. Yu,C. *et al*. (2002) Human, rat, and mouse metabolism of resveratrol. *Pharm. Res.*, **19**, 1907–1914.

Received October 25, 2006; revised June 11, 2007; accepted June 11, 2007