RESEARCH COMMUNICATION

Suppression of Prostate Cancer Growth by Resveratrol in The Transgenic Rat for Adenocarcinoma of Prostate (TRAP) Model

Azman Seeni, Satoru Takahashi*, Kentaro Takeshita, Mingxi Tang, Satoshi Sugiura, Shin-ya Sato, Tomoyuki Shirai

Abstract

Research into actions of resveratrol, abundantly present in red grape skin, has been greatly stimulated by its reported beneficial health influence. Since it was recently proposed as a potential prostate cancer chemopreventive agent, we here performed an in vivo experiment to explore its effect in the Transgenic Rat for Adenocarcinoma of Prostate (TRAP) model, featuring the rat probasin promoter/SV 40 T antigen. Resveratrol suppressed prostate cancer growth and induction of apoptosis through androgen receptor (AR) down-regulation, without any sign of toxicity. Resveratrol not only downregulated androgen receptor (AR) expression but also suppressed the androgen responsive glandular kallikrein 11 (Gk11), known to be an ortholog of the human prostate specific antigen (PSA), at the mRNA level. The data provide a mechanistic basis for resveratrol chemopreventive efficacy against prostate cancer.

Key Words: Chemoprevention - prostate cancer - resveratrol - TRAP rats

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Introduction

Prostate cancer has become the most frequently diagnosed cancer and the second leading cause of cancer-related death for men in the United States (Jemal et al., 2007). The conventional treatments available for this disease, such as hormone therapy, chemotherapy or radical prostatectomy, eventually fail to exert control and metastatic disease frequently develops even after surgery and may cause death. Therefore, interest has focused on chemoprevention, suppressing, delaying or reversing carcinogenesis by pharmacologic intervention with naturally occurring or synthetic agents (Sporn and Suh, 2002;Tsao et al., 2004).

Resveratrol, a phytoestrogen, found in grapes and red wine, has been identified as a novel potential cancer chemopreventive agent. Numerous reviews have been published regarding its activity (Jang et al., 1997;Bhat and Pezzuto, 2002; Stewart et al., 2003; Aziz et al., 2003; Aggarwal et al., 2004; Baur and Sinclair, 2006; Delmas et al., 2006) but the molecular mechanisms have yet to be fully defined, especially *in vivo*. Therefore, we explored the effects of resveratrol using the Transgenic Rat for Adenocarcinoma of Prostate (TRAP) model, established in our laboratory using the Simian virus 40 T antigen under control of the probasin gene promoter (Asamoto et al., 2001a; Asamoto et al., 2002). The animals develop high grade prostatic intraepithelial neoplasia (PIN) and well differentiated adenocarcinoma with high incidence in all prostate lobes at 15 weeks of age, all lesions being completely androgen-dependent. The model provides an ideal tool to gain insights into possible mechanisms for prostate cancer prevention (Asamoto et al., 2001b; Cho et al., 2003; Zeng et al., 2005; Kandori et al., 2005; Said et al., 2006; Tang et al., 2007) in the relatively short-term. To our knowledge, the present study provided the first evidence that resveratrol inhibits prostate carcinogenesis in a rat model closely mimicking the human disease. The clues obtained as to the molecular basis of action are of critical importance as the first steps towards human clinical trials.

Materials and Methods

Animals

Male heterozygous TRAP rats were housed three per plastic cage on wood-chip bedding in an air conditioned specific pathogen free (SPF) animal room under standard conditions with food (Oriental MF, Oriental Yeast, Tokyo, Japan) and water *ad libitum*. All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of Nagoya City University Graduate School of Medical Sciences.

Chemicals, reagents and cell lines

Resveratrol was purchased from Sigma, and MG132 and cycloheximide from Calbiochem (EMD Biosciences, Inc., San Diego, CA). Antibodies to cleaved caspase 3, 7, Erk1/2 and phospho-Erk1/2 were purchased from Cell Signaling Technology (Beverly, MA). Anti-AR antibody (PG-21) was from Upstate Technology (Lake Placid, NY), anti-HA-Tag antibody was from BD Sciences Clontech

*Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, 467-8601, Japan. *For Correspondence : E-mail: sattak@med.nagoya-cu.ac.jp FAX: +81-52-842-0817*

(Palo Alto, CA), anti-cyclin D1 was from Oncogene Research Product, anti-Bcl-xL was from Pharmingen, anti-SV40T Ag was from Santa Cruz Biotechnology Inc. and anti-β-actin was from Sigma. COS7 and LNCaP were from the American Type Culture Collection (Manassas, VA). PLS30 cells were established in our laboratory as described previously (Nakanishi et al., 1996; Kato et al., 1998).

Plasmids

To generate pBKCMV-rAR, the rat AR open reading frame (ORF) was amplified by PCR and inserted into pBKCMV (Stratagene). For pGL3-rPBP-luc, a rat probasin promoter fragment (-426 ~ +32) was amplified and inserted into pGL3-basic (Promega). HA-tagged ubiquitin (MT123) was a generous gift from Dr. Dirk Bohmann (University of Rochester Medical Center).

Experimental design

A total of 48 heterozygous transgenic male rats were divided into four equally sized groups. Beginning at the age of three weeks, rats of each group received resveratrol at the concentration of 50, 100 or 200 μg/ml or normal drinking water as the control. Body weights and water consumption were recorded weekly. At 10 weeks of age, all surviving rats were sacrificed and prostates were removed and weighed. Half of each ventral prostate was immediately frozen in liquid nitrogen for storage until processed. The remainder of each prostate was fixed in formalin and routinely processed for embedding in paraffin and sectioning for H&E staining and histopathological evaluation as well as immunohistochemistry. Testosterone and estradiol levels in serum were analysed by radioimmunoassay in a commercial laboratory (SRL, Tokyo, Japan).

Assessment of prostate neoplastic lesion development

Our TRAP rats showed sequential development of prostatic lesions, i.e. low- and high-grade prostatic intraepithelial neoplasias (PINs) to differentiated adenocarcinomas. Low-grade PIN (LG-PIN) were characterized by having with one or two layers of atypical cells with hyperchromatic nuclei and intact gland profiles and high-grade PIN (HG-PIN) showing increased epithelial stratification with nuclear atypia. Adenocarcinomas were characterized by atypical cells fill almost the lumen of the ducts with cribriform structures or solid growth in acini (Figure 1). The relative numbers of acini with the histological characteristics were quantified by counting for every features, e.g. LG-PIN, HG-PIN and adenocarcinoma, from the total acini in each prostatic lobe and calculated the percentages of each lesions by H&E staining and epithelial contents in acinic areas by performed Azan-Mallory histochemical staining to determine the progression of neoplastic lesions. Red staining areas in the prostates were equivalent to viable epithelial lesions and were quantitatively measured with an Image Processor for Analytical Pathology (IPAP, Sumika Technos Co., Osaka, Japan).

Immunohistochemistry

For Ki-67 immunostaining, deparaffinized sections were incubated with diluted rabbit polyclonal Ki-67 antibody (Novocastra). Apoptotic cells were detected using an In situ Apoptosis Detection Kit (TUNEL method) according to the manufacturer's instructions (Takara Bio Co. Ltd). Labeling indices were counted separately in the ventral, dorsal and lateral prostate and expressed as numbers of Ki-67-positive or TUNEL-positive cells per 100 cells.

Western blot analysis

Cells were lysed in RIPA buffer containing 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH8.0), 0.2 mM sodium orthovanadate and Complete Cocktail (Roche). Cell lysates were electrophoresed through SDS-PAGE gels and blotted onto nitrocellulose membranes. Immunoreactive protein bands were visualized using an ECL plus kit (Amersham Pharmacia Biotech, Freiburg, Germany).

Reverse Transcription-PCR and Real Time-RT PCR

Total RNA was isolated using an RNeasy Mini Kit (Qiagen). Total RNAs were reverse-transcribed with the SuperScript First-Strand Synthesis System (Invitrogen Life Technologies) and amplified by PCR (RT-PCR) using specific primers for AR and GAPDH. Primers used were

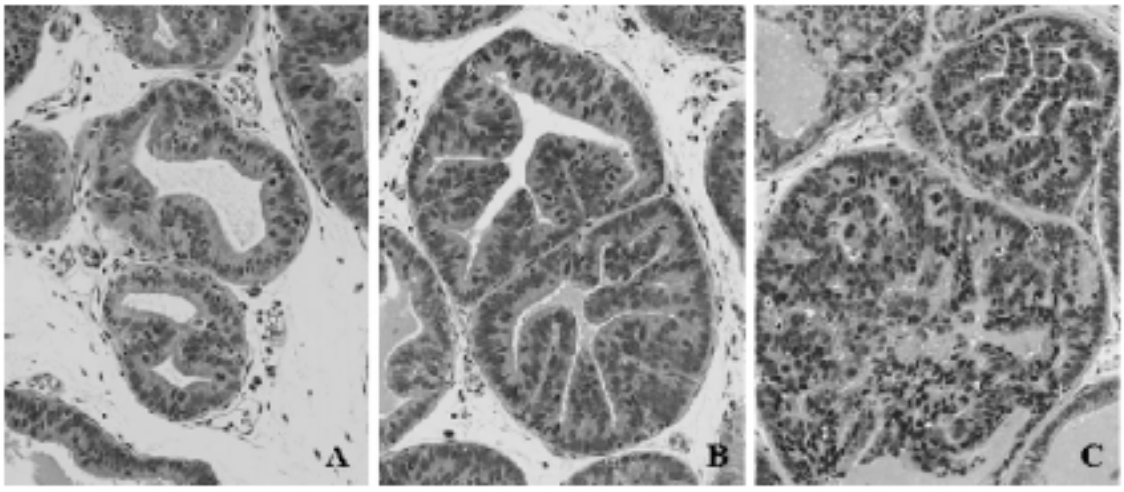

Figure 1. Representative Histopathological Findings for LG-PIN (A), HG-PIN (B) and Adenocarcinoma (C) in Ventral Prostates of TRAP Rats

as follows: (a) AR, forward primer 5-TTGTGAACAGAGTCCCCTAT-3, reverse primer 5-TTCTGGGATGGGTCCTCAGT-3, and (b) GAPDH, forward primer 5-GCGAGATCCCGTCAAGATCA-3, reverse primer 5-CCACAGTCTTCTGAGTGGCAG-3. Real-time quantitative RT-PCR was performed for androgen responsive gene, Gk11 expression using LightCycler (Roche Diagnostics). The primers used to detect GK11 genes are as follows: forward primer 5-GCAGCACCAAACCCCTGGAT-3, reverse primer 5-TGAGATCTGTCACCTTCTCA-3, and primers for rat cyclophilin (used as internal control) are as follows: forward primer 5-TGCTGGACCAAACACAAATG-3, reverse primer 5-GAAGGTGAAAGAAGGCATGA-3.

Reporter gene assay

COS7 cells were transfected with pBKCMV-rAR and pGL3-rPBP-luc using Nucleofector II (Amaxa, Germany). Twenty-four hours after transfection, 10 nM DHT and/or resveratrol was added for another 24 hrs. Cells were lysed with the buffer supplied in the kit 24 hr after transfection. The luciferase assay was conducted using the dual-luciferase reporter assay system (Promega), and the phRL-TK vector (Promega) was used as an internal control. Data shown represent the average and standard deviation of four independent data points.

AR stability assay

COS7 cells were transfected with pBKCMV-rAR, plated into 6-well plate and incubated for 24 hrs. Cells were pretreated with 10 μg/ml cycloheximide for 30 min and then were added 200 μM resveratrol or DMSO. Cells were lysed with RIPA buffer at 0, 1, 2, 4 and 8 hrs after adding resveratrol or DMSO, and cell lysates were subjected to western blot analysis.

Ubiquitylation assay

COS7 cells were transfected with pBKCMV-rAR and MT123 using Nucleofector II, plated into 6-well plates and incubated for 24 hrs. Cells were treated with resveratrol and/or 1 μM MG132 for 24 hrs, and then lysed with IP lysis buffer containing 20 mM Tris-HCl, pH7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM sodium orthovanadate, and Complete Cocktail. Immunoprecipitation was performed using rabbit anti-AR antibodies, and subjected to western blot analysis using anti-HA-Tag antibodies.

AR translation assay

COS7 cells were transfected with pBKCMV-rAR using Nucleofector II, seeded into 6-well plate and incubated for 24 hrs. Cells were pretreated with 100, 200 μM resveratrol or DMSO for 13 hrs, and medium was changed to methionine-, cysteine-free RPMI1640/10% FBS with or without resveratrol at 37°C for 1.5 hrs thereafter. Cells were incubated with methionine-, cysteine-free RPMI1640/10% FBS containing 100 μCi/ml Premix [^{35}S]methionine and [^{35}S]cysteine and unlabeled methionine and cysteine (5 μM each) for 1, 2, 4 and 8 hrs. Cold IP lysis buffer was added to each well to lyse cells. One hundred micrograms of cellular protein were immunoprecipitated with rabbit anti-AR antibodies, and samples were subjected to gel electrophoresis, followed by autoradiographic signal quantitation using NIH image software.

Metastasis assay in nude mice

PLS30 cells ($5x10^6$/animal) were injected into the subcutis of 6-week-old male athymic nude mice of the CD-1 strain (Charles River Japan, Inc, Kanagawa). One week after injection, mice were given resveratrol at concentrations of 100 and 200 μg/ml in their drinking water. Six weeks after injection, mice were sacrificed and examined for numbers of metastatic foci in lungs stained with Indian ink (Wexler, 1966).

Statistical analysis.

Data are expressed as means ± SDs. Differences in means between groups were determined by analysis of variance (ANOVA), followed by the Scheffe's post-hoc test with StatView (version 5.0) software (SAS Institute, Inc., Cary, NC). The Spearman's rank correlation coefficient test was used for analysis of dependent data.

Results

Body weight and water consumption

Resveratrol did not cause mortality or non-significant changes in body and relative organ weights (ventral prostate, liver and kidney) compared to the control group. The groups also did not differ in water consumption. Average resveratrol intake was consistent with the doses given, as shown in Table 1.

Testosterone and estradiol levels in serum

Although serum testosterone levels were significantly reduced in the 50 and 100 microgram/ml resveratrol treatment groups compared to control group values, unfortunately we found that the latter were higher than the normal range, which is about 1 - 2 ng/ml (Zeng et al., 2005; Kandori et al., 2005; Cho et al., 2003; Asamoto et al., 2002) (Table 1). Thus, the results were unexplainable regarding effects of resveratrol on serum testosterone. No

Table 1. Serum Testosterone, Estradiol Levels and the Average Resveratrol Intake of TRAP Rats

Treatment	No. of rats	Testosterone (ng/ml)	Estradiol (pg/ml)	Average resveratrol intake (mg/kg/day)
Control	12	4.28±3.15	4.75±1.11	-
Resveratrol 50 ug/ml	12	1.31±0.81*	4.46±1.47	7.59±1.15
Resveratrol 100 ug/ml	12	0.88±0.40*	3.91±0.99	16.11±2.42
Resveratrol 200 ug/ml	12	4.44±2.27	4.79±1.15	30.05±5.90

Data are means ± SD, *, P<0.01 versus control

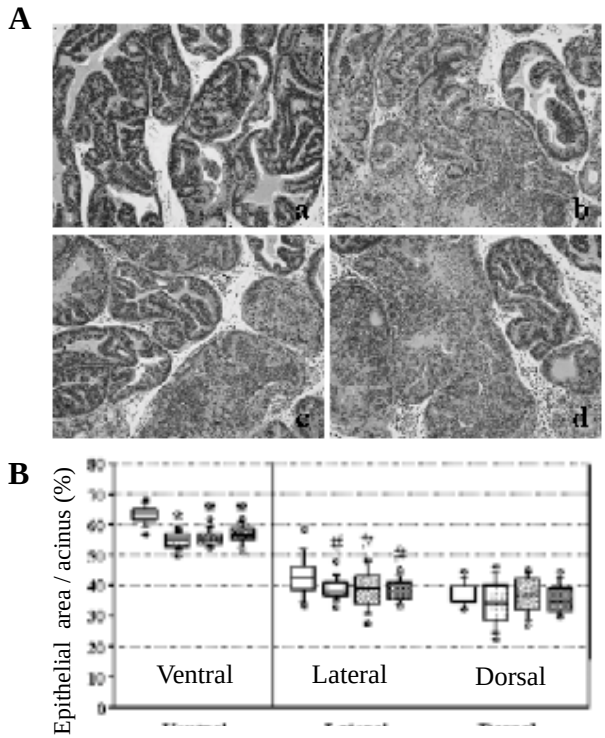

Figure 2. Effects of Resveratrol on Ventral Prostate Adenocarcinomas. (A) Representative histological appearances of (a) control and resveratrol (b) 50 (c) 100 and (d) 200 ug/ml (H &E staining). (B) Quantitative analysis of relative epithelial areas within prostatic acini of TRAP rats. Data are means ± SD for 12 animals. *, P < 0.01 versus control. #, P < 0.05 versus control (Spearman 's rank correlation coefficient test). Boxes left to right: Control; resveratrol 50;100; 200 ug/ml

significant changes were noted in serum estradiol levels compared to controls (Table 1).

Effects of resveratrol treatment on development of neoplastic lesions in the prostate

There were partial pathologic responses to resveratrol treatment as demonstrated by reduction in the content of prostatic neoplastic lesions in TRAP rats (Figure 2A). However, small foci of carcinoma remained, so that there were no significant differences in the incidences of PIN or adenocarcinoma in the prostates of TRAP rats (Table 2). There were no adenocarcinomas in the dorsal and anterior lobes in all groups of rat. As the entire ventral and lateral prostate lobes were occupied with tumor lesions and clear differences in prostate adenocarcinoma incidence were not observed among the groups, we evaluated the areas of epithelium including tumors morphometrically. The results, as summarized in Figure 2B, showed that resveratrol treatment significantly suppressed neoplastic lesion development about 14%, even at low dose (50 μg/ml) in the ventral lobes and also in the lateral lobes with a dose-dependent manner. Furthermore, evaluation of the proportion of preneoplastic and neoplastic lesions showed that resveratrol tended to shift the progression of neoplastic growth by suppressed the number of adenocarcinoma and HG-PIN and consequently, increased LG-PIN in all lobes (Table 3). The numbers of apoptotic cells in the ventral prostate of rats treated with resveratrol were also significantly increased as compared with the controls, while there were no obvious differences in Ki-67 labeling indices (Table 4).

Resveratrol downregulates AR and Gk11 in the ventral prostate

Figure 3A shows that resveratrol clearly suppressed AR protein expression even at low dose of 50 μg/ml and also slightly suppressed SV40 Tag expression at the high dose. RT-PCR analysis of the AR gene showed no obvious differences at the AR mRNA level (Figure 3B), suggesting post-transcriptional downregulation. However, mRNA expression of the androgen responsive gene, Gk11, was significantly suppressed in the ventral prostate (Fig 3C).

Resveratrol affects AR function and its stability in vitro

In COS7 cells transfected with pBKCMV/rAR, resveratrol repressed exogenous AR expression, as well as endogenously in the LNCaP cells (Figure 4A). Subsequent reporter assays clearly demonstrated inhibition of functional AR activity in a dose-dependent manner, this being considered to simply reflect downregulation of AR protein expression by resveratrol. In resveratrol-treated cells, the half-life of AR protein was also slightly reduced, with a one hour difference compared to control cells (Figure 4B), suggesting an influence on AR protein degradation. The suppressive effect of resveratrol was not

Table 2. Incidences of Prostate Adenocarcinomas in TRAP Rats Treated with Resveratrol

Treatment	No. of rats	Incidence of adenocarcinoma (%)			
		Ventral	Lateral	Dorsal	Anterior
Control	12	11 (92)	4 (33)	0	0
Resveratrol 50 ug/ml	12	10 (83)	2 (17)	0	0
Resveratrol 100 ug/ml	12	10 (83)	5 (42)	0	0
Resveratrol 200 ug/ml	12	9 (75)	2 (17)	0	0

Table 3. Quantitative Evaluation of Neoplastic Lesions in Prostate of TRAP Rats Treated with Resveratrol

Treatment	No. of rats	Relative number of acini with histological characteristics(%)								
		Ventral			Lateral			Dorsal		
		LG-PIN	HG-PIN	Carcinoma	LG-PIN	HG-PIN	Carcinoma	LG-PIN	HG-PIN	Carcinoma
Control	12	3.1±1.0	95.3±1.0	1.6±0.5	20.7±12.0	77.6±11.4	1.7±1.6	24.4±10.8	75.6±10.8	-
Resveratrol 50 ug/ml	12	4.8±2.2*	93.9±1.9*	1.3±0.5#	23.5±11.3	75.8±11.0	0.6±0.6*	31.7±20.1	68.3±20.1	-
Resveratrol 100 ug/ml	12	3.7±1.4	95.1±1.5	1.2±0.4*#	18.7± 8.0	80.6± 7.7	0.6± 0.7	28.6±12.0	71.4±12.0	-
Resveratrol 200 ug/ml	12	4.0±1.5	94.9±1.4	1.1±0.4*#	22.9± 9.2	76.5± 9.3	0.6±0.9	30.7±16.7	69.3±16.7	-

Data are mean ± SD *, P<0.05 versus control #, P<0.05 versus control (Spearman's rank correlation coefficient test)

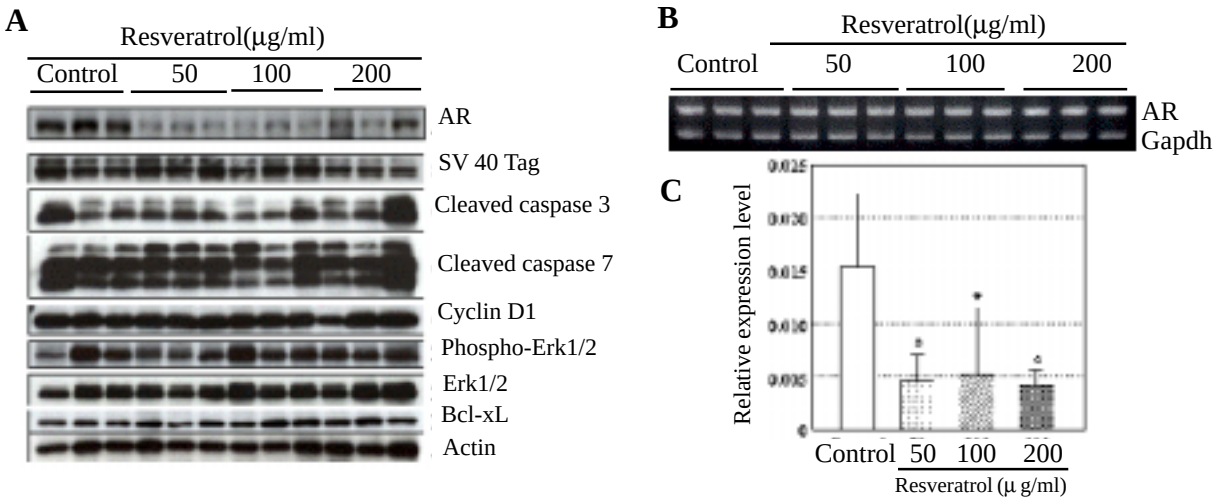

Figure 3. Effects of Resveratrol on AR and Gk11 Expression in the Ventral Prostates of TRAP rats. (A) Western blot analysis for AR, MAPK and apoptosis-related proteins; (B) RT-PCR analysis for AR; (C) Real Time-RT PCR analysis for Gk11 expression normalized to cyclophilin used as an internal control. Data are means ± SD of five animals. *, P < 0.05 versus control

completely blocked by the proteasome inhibitor, MG132 (Figure 4C), suggesting that resveratrol-induced AR protein down-regulation is not mainly via the proteasome-dependent pathway.

Resveratrol suppresses AR translation in vitro

To further determine the possible mechanism involved in the regulation of AR expression at the posttranscriptional level, AR-translation *in vitro* assay was performed to characterize whether resveratrol affects AR protein translation efficiency. Figure 4D shows that treatment with resveratrol reduced AR protein synthesis by about 40% as compared with untreated cells. This inhibitory phenomena might be specific on AR protein translation because resveratrol did not affected the translation of ERK1 and Cyclin D1, which been chosen as control protein, using LNCaP cells (data not shown).

Effects of resveratrol on lung metastasis in nude mice

There were no significant differences in growth of

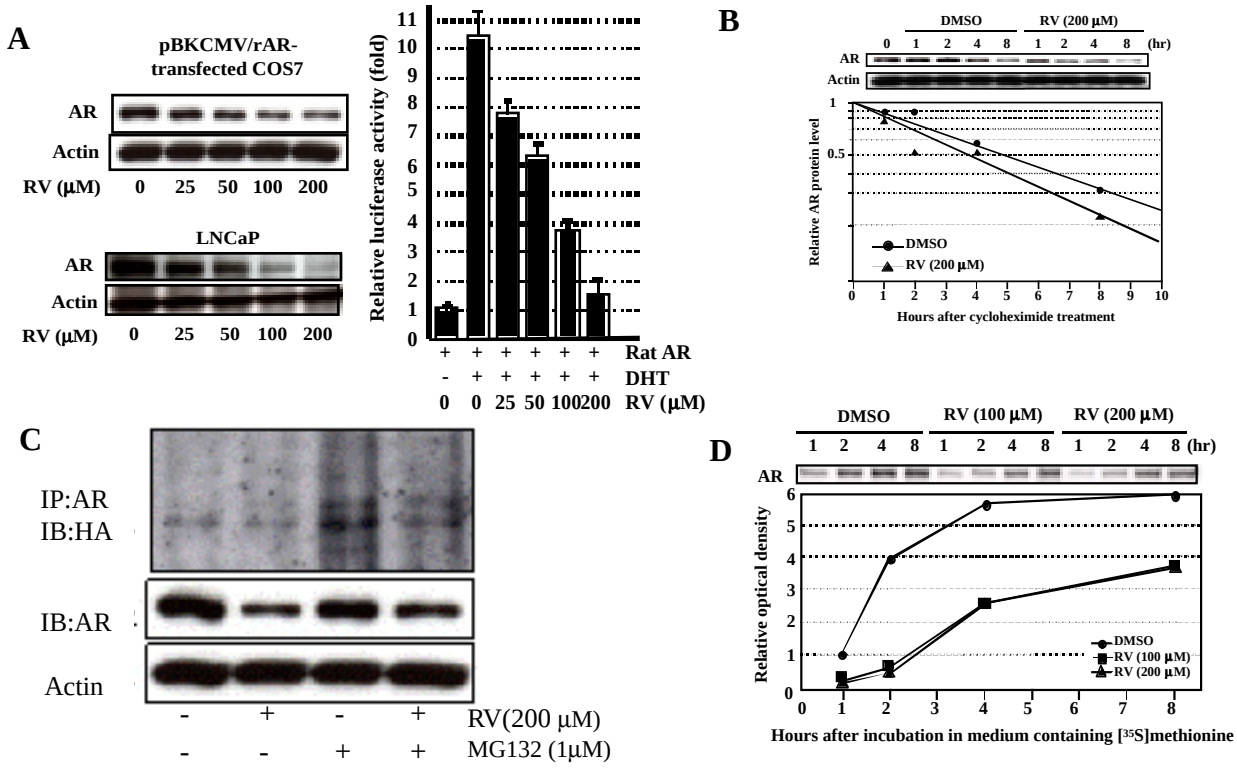

Figure 4. Mechanisms of Down-regulation of AR Protein by Resveratrol. (A) *Left panel*, western blot analysis of AR in transiently pBKCMV/rAR-transfected COS7 cells (top) and in LNCaP cells (*below*). *Right panel*, Reporter gene assay for AR. Means ± SD. (B) AR protein stability assay of COS7 cells transfected with pBKCMV/rAR, pretreated with cycloheximide and then treated with 200 uM resveratrol or DMSO. AR protein levels were determined by western blot analysis and normalized to actin (*top*). The AR expression was quantified and plotted relative to time 0. (C) Ubiquitylation assay for AR in COS7 cells transfected with both pBKCMV/rAR and pMT123 (HA-Ub), treated with resveratrol and/ or MG132. (D) AR translation assay of COS7 cells transfected with pBKCMV/rAR and incubated for 24 hrs. Details are described in the *Materials and Methods*

Table 4. Ki-67 Labeling index (%) and Apoptotic Indices in Prostate of TRAP Rats Treated with Resveratrol

Treatment	No.	Ki-67 labeling			Apoptotic index (%)		
	of rats	Ventral	Lateral	Dorsal	Ventral	Lateral	Dorsal
Control	12	22.6±3.3	23.0±4.8	14.1±3.2	3.6±1.2	2.7±1.1	0.9±0.3
Resveratrol 50 ug/ml	12	23.7±6.0	23.3±6.0	14.8±3.7	5.5±1.9*	2.9±1.0	0.8±0.3
Resveratrol 100 ug/ml	12	22.0±4.4	22.1±3.9	15.1±3.9	5.4±1.9*	3.6± 1.8	0.9±0.3
Resveratrol 200 ug/ml	12	20.6±4.0	26.0±6.7	17.4±3.8	5.4±1.6*	2.4±1.2	0.8±0.2

Data are mean ± SD *, P<0.01 versus control

xenografts or lung metastases after subcutaneous injection of the PLS30 cell line into the flanks of athymic nude mice and treatment with resveratrol (Table 5).

Discussion

There was no report regarding the effectiveness of resveratrol in preclinical animal model of prostate cancer so far (Syed et al., 2007). The present study demonstrated, for the first time to our knowledge, suppressive effects of resveratrol on prostate cancer growth and induction of apoptosis through AR down-regulation in an *in vivo* rat model. This *in vivo* finding is an important step in verifying potential chemoprevention by resveratrol before recommending use in humans. Well-designed *in vivo* animal study plays a critical role between the *in vitro* experiment and clinical trials especially for the optimal dosing and toxicity of the agent. Importantly, the inhibition effect was achieved without any significant change in final body weights, relative liver and kidney weights and water consumption. Lack of signs of toxicity in the present experiment is in line with the earlier finding that oral intake of high dose (20 mg/kg/day) of resveratrol is not harmful to rats (Juan et al., 2002), although the dosage of resveratrol employed in this study was equivalent to 400 - 1,600 times the amount consumed by a person with ordinary wine intake (Gescher and Steward, 2003). The previous report showed that serum concentration of free, 3- and 4'-glucuronide of resveratrol in healthy human with moderate consumption of red wine were up to 26 nM, 190 nM and 2.2 uM, respectively (Vitaglione et al., 2005), and we used the dose of resveratrol that was equivalent to about 100 times concentration compared to these human data in *in vitro* study.

Resveratrol is well known for its phytoestrogenic and antioxidant properties (Baur and Sinclair, 2006) and exerts a variety of beneficial effects in humans, such as protection against the metabolic syndrome (Lagouge et al., 2006),

Table 5. In Vivo Growth and Lung Metastasis of PLS30 rat Prostate Cancer Cells in Nude Mice Treated with Resveratrol

Treatment	No. of mice	Tumor volume of xenograft(cm^3)	No. of metastatic foci in lungs
Control	11	0.96 ± 0.49	50.6 ± 38.9
Resveratrol 100 ug/ml	10	1.10 ± 0.47	35.0 ± 24.5
Resveratrol 200 ug/ml	10	0.85 ± 0.40	38.2 ± 28.5

Data are means ± SD

inflammation and viral infection (Friel and Lederman, 2006). Recently it was also shown to have a possible positive influence on life-expectancy, since food supplementation with resveratrol prolonged lifespan and retarded the expression of age-dependent traits in a short-lived vertebrate (Valenzano et al., 2006; Baur et al., 2006). A population-based case-control study further suggested that consumption of red wine may be associated with a reduction of the relative risk of prostate cancer (Schoonen et al., 2005) and resveratrol is probably one of the main microcomponents of wine responsible. The suppressive effect on AR expression by resveratrol in our study is agreement with earlier *in vitro* findings (Jones et al., 2005; Hsieh and Wu, 2000; Mitchell et al., 1999), as well as the hypothesis that resveratrol results in scavenging of incipient populations of androgen-dependent prostate cancer cells through its influence on the AR (Kyprianou and isaacs, 1988). *In vitro* studies have also indicated that resveratrol has marked antiandrogenic effects, in the androgen-dependent human prostate cell line LNCaP, that involve suppression of AR, the AR-specific co-activator ARA70 and various AR-regulated genes and that these effects are associated with reduced cell-growth and induction of apoptosis (Mitchell et al., 1999).

It is of clear interest that resveratrol not only downregulated the AR in our TRAP model but also suppressed the androgen responsive gene, Gk11, known as the ortholog of human PSA, at the mRNA level. This *in vivo* finding reflect that resveratrol suppressed AR pathway functionally in prostatic lesions of TRAP rats that might similar to affect PSA in human condition, such as the effect of anti-androgen drugs suppressed PSA level that paralleled with cancer growth inhibition by interrupting AR signal pathways in prostate cancer patients.

Resveratrol is also known to suppress late stage processes of carcinogenesis such as angiogenesis and metastasis. For example, resveratrol was able to directly inhibit the gelatinolytic activities of MMP2 and MMP9 which are associated with tumor metastasis (Banerjee et al., 2002). However, effects in cell culture may not directly reflect whole body systems in animal systems and administration of 1-5 mg per kg (body weight) daily of resveratrol in one study failed to affect the growth or metastasis of breast cancer in mice, despite promising *in vitro* results (Bove et al., 2002). In the present investigation, resveratrol similarly did not reduce lung metastasis in mice bearing prostate carcinoma tumors, possibly because the PLS30 cells used are negative for AR protein and show and androgen-independent

phenotype (Nakanishi et al., 1996). Thus, effects of resveratrol are more likely to be AR-dependent. Understanding the molecular mechanisms of resveratrol mediated downregulation of AR signaling may aid in the development of effective chemoprevention since the receptor plays a major role in the initiation and progression of prostate cancer (Sadi et al., 1991). The observed unique effects of resveratrol on AR protein point to possible optimization of chemopreventive effect in future by use in combination with other agents such as vitamin E (Zhang et al., 2002) and selenium (Chun et al., 2006).

In conclusion, our *in vivo* results clearly demonstrated that resveratrol can inhibit prostate carcinogenesis with induction of apoptosis through AR down-regulation, without any signs of tissue-toxicity. Our findings provide support for previous in vitro data as well as population-based case-control study suggesting that resveratrol intake through wine associated with the reduction of the relative risk of prostate cancer. Our findings and the fact that most prostate cancers are initially androgen-dependent suggest that resveratrol warrants further examination with the eventual aim of clinical testing.

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