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Inhibitory mechanisms of the transcriptional activity of androgen receptor by resveratrol: Implication of DNA binding and acetylation of the receptor

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ABSTRACT

Androgen receptor (AR) is a ligand-dependent transcription factor and plays a key role in the development of prostate cancer. Resveratrol, a polyphenolic compound, inhibits AR function and reduces the level of prostate-specific antigen (PSA), a notable target gene of AR. Here, we investigated the mechanisms by which resveratrol inhibits AR function. Although the protein levels of AR were decreased by resveratrol treatment for 24 h, the decrease could not fully account for the suppression of AR function. The total and the nuclear AR levels were not affected after incubation with 10 μM resveratrol for 3 h, whereas resveratrol inhibited the binding of AR to the enhancer region of PSA and decreased the acetylation of AR even at this early phase. Inhibition of transcription by resveratrol was weaker in the AR acetylation site mutant than in the wild-type. In later phase (24 h) after incubation with resveratrol, the ligand-induced nuclear accumulation of AR was markedly decreased by resveratrol. These data show that resveratrol inhibits DNA binding of AR, presumably by decreasing its level of acetylation and suggest that acetylation of AR is involved in its accumulation in the nucleus.

1. Introduction

The actions of androgens such as testosterone and dihydrotestosterone (DHT) are mediated by the androgen receptor (AR), which is a member of the nuclear receptor superfamily of ligand-dependent transcription factors [1]. Like other nuclear receptors, AR is composed of an N-terminal domain, a DNA-binding domain, a hinge region, and a ligand-binding domain (LBD). Upon ligand binding, AR translocates from the cytoplasm to the nucleus and binds to the androgen response element (ARE) in the target gene promoter to activate transcription. AR function is regulated by post-translational modifications such as phosphorylation [2], ubiquitination [3], and acetylation [4,5]. The AR-mediated DHT signal plays a pivotal role in the development of prostate cancer, the most diagnosed malignant carcinoma among men in western countries [6]. Prostate-specific antigen (PSA), which has some AREs, is the best characterized AR target gene. Serum PSA levels are commonly used for clinical diagnosis of prostate cancer [7,8].

A chemopreventive strategy that uses functional foods is attractive because the development of prostate cancer is considered to be associated with dietary habit [9]. Resveratrol (3,4′,5-trihydroxystilbene), a polyphenolic phytoalexin present in grapes and red wines, has received considerable attention as an anticancer nutrient over the years [10]. Resveratrol dose-dependently decreased the expression of PSA in an LNCaP (androgen-sensitive prostate cancer cell) xenograft in the nude mouse [11] and decreased the expression of glandular kallikein 11 (a rat ortholog of PSA) in a transgenic rat used as a model for adenocarcinoma of the prostate [12]. In LNCaP cells, resveratrol down-regulates approximately half of the genes induced by androgen (including PSA) and inversely up-regulates almost all of the genes repressed by androgen [13]. Thus, resveratrol inhibits AR-mediated androgen signaling. It is speculated that resveratrol suppresses AR function by reducing its expression because resveratrol (50–150 μM) decreased AR mRNA levels by suppressing promoter activity [14] and shortened the half-life of AR at the post-translational stage [15]. However, Hsieh and Wu reported that resveratrol decreased PSA expression even though it did not affect the AR protein level [16,17]. Therefore, the detailed mechanisms by which resveratrol inhibits AR function are not clear.

Here we report that resveratrol reduces the PSA level more efficiently than it reduces the AR level. Accordingly, to understand why

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resveratrol reduces the PSA level more efficiently than it reduces the AR level, we examined the mechanism by which resveratrol inhibits AR function.

2. Materials and methods

2.1. Cell culture

Human prostate cancer LNCaP (AR-positive) and PC-3 (AR-negative) cells were obtained and cultured as described previously [18] unless otherwise indicated.

2.2. Subcellular fractionation

LNCaP cells that had been cultured in phenol red-free RPMI 1640 medium supplemented with 10% dextran-coated charcoal-stripped fetal bovine serum (dFBS) were further incubated in the presence of DHT and/or resveratrol. Subcellular components were fractionated as described previously [18]. In brief, LNCaP and PC-3 cells were suspended in hypotonic buffer [18] and passed through a 23-guage needle. The homogenate (total cell lysates) was separated into cytoplasmic, nuclear, and particulate fractions by differential centrifugation. The proteins in each fraction were analyzed by Western blotting with anti-AR (N-20, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-PSA (C-19, Santa Cruz Biotechnology), anti-α-tubulin (DM1A, Sigma, St. Louis, MO, USA), and anti-lamin B1 (L-5, Zymed Laboratories, San Francisco, CA, USA) antibodies. The band intensity was determined by densitometry using Image J software version 1.41 (National Institutes of Health, Bethesda, USA).

2.3. Chromatin immunoprecipitation (ChIP) assay

A ChIP assay was performed as described previously [19]. LNCaP cells that had been cultured in medium supplemented with 10% dFBS were incubated in the presence of 10 μM resveratrol for 135 min. Cells were further incubated with 10 nM DHT for 45 min in the presence of resveratrol, followed by fixation at a final concentration of 1% paraformaldehyde for 15 min. Cells were lysed in ChIP lysis buffer and sonicated for 10 s for 5 times (Handy Sonic, UR-20P, Tomy Seiko, Tokyo, Japan). The supernatant after centrifugation was diluted with ChIP dilution buffer and precleared with protein A-Sepharose (GE Healthcare) for 1 h. The remaining extract was incubated with 1 μg of control or anti-AR antibodies (N-20) for 16 h and further incubated with protein A-Sepharose for 1 h. Proteins bound to the resin were sequentially washed with low salt wash buffer, high salt wash buffer, and LiCl wash buffer, and further washed twice with TE buffer, followed by elution with ChIP elution buffer containing 10 mM dithiothreitol. The eluted fractions were incubated at 65 °C for 6 h to dissolve cross-linking and further incubated with RNase A and proteinase K. After ethanol precipitation, the PSA enhancer containing ARE (AREIII) was amplified by PCR using a sense primer (5′-TTGGATTGAAAACAGACCTA-3′) and an antisense primer (5′-GTAAAGCAGGCATCCTTGCA-3′) [20] according to the following program: 1 cycle, 95 °C for 1 min; 40 cycle, 95 °C for 15 s, 60 °C for 30 s (for real-time PCR) or 1 cycle, 95 °C for 1 min; 34 cycle, 94 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min; 1 cycle, 72 °C for 10 min (for semi-quantitative RT-PCR). Real-time quantitative PCR was performed with a SYBR Premix Ex Taq (Takara, Shiga, Japan) on Thermal Cycler Dice, TP-800 (Takara). Ct values were transformed into relative quantification data by $2^{-\Delta\Delta Ct}$ method. Signals obtained from the ChIP sample were divided by signals obtained from an input sample and expressed as relative values.

2.4. Immunoprecipitation

Immunoprecipitation was performed as described previously [18], with minor modifications. LNCaP cells grown in phenol red-free RPMI 1640 medium supplemented with 10% dFBS were treated with 10 nM DHT and/or 10 μM resveratrol for 3 h and sonicated in IP buffer [18]. Cell lysates (3 mg) were incubated with monoclonal mouse anti-AR IgG (0.5 μg, AR441, Santa Cruz Biotechnology) and polyclonal anti-AR IgG (N-20, 0.5 μg) for 1 h, followed by addition of protein G-Sepharose (50% slurry, GE Healthcare, Little Chalfont, UK) preequilibrated with IP buffer. The mixture was further incubated for 4 h, and the resin was washed twice with IP buffer. Proteins bound to the resin were analyzed by Western blotting with anti-AR (N-20) and anti-acetylated lysine (Ac-K-103, Cell Signaling Technology, Beverly, MA, USA) antibodies.

2.5. Reporter assay

A luciferase reporter assay was performed as described previously [21]. A mutant AR expression vector (pcDNA3.1-AR(K630T)) was constructed using a QuickChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). PC-3 cells were transiently co-transfected with AR expression vector (pcDNA3.1-AR [18] or pcDNA3.1-AR(K630T)), pARE2-TATA-Luc [18], and pRL-TK for 24 h, and further incubated in the presence or absence of DHT and resveratrol for an additional 24 h. Luciferase activities were determined as described previously [18], and data were expressed as relative light units (RLU).

2.6. Immunofluorescence microscopy

Immunofluorescent microscopy was carried out as described previously [22]. PC-3 cells were transformed by electroporation with pcDNA3.1-ARΔC-Nuc [18], using a Gene Pulser at 950 μF and 220 V (Bio-Rad Laboratories, Hercules, CA, USA). Anti-AR antibody (1/3000, N-20) was used as the primary antibody and was immunoreacted with Alexa Fluor 488-conjugated secondary antibodies (1/5000, Molecular Probes, Eugene, OR, USA). Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, 1 μg/ml), followed by observation using fluorescence microscopy (Nikon, Tokyo, Japan).

3. Results

3.1. Comparison of the levels of AR and PSA

To elucidate whether resveratrol inhibits the transcriptional activity of AR by decreasing the expression of AR, LNCaP prostate cancer cells were incubated with or without DHT (10 nM) for 24 h in the presence of various concentrations of resveratrol, and the expression levels of AR and PSA were compared. The expression level of PSA was increased by DHT and inversely decreased by resveratrol in a dose-dependent manner (Fig. 1A). These results show that resveratrol affects AR function. The band densities normalized to the levels of α-tubulin are shown graphically in Fig. 1B. Resveratrol caused a greater decrease in PSA than in AR. In the cells treated with 10 μM resveratrol, the level of PSA was about 30% of that in the control cells, whereas the level of AR was about 80% of that in the control cells, indicating that there is a significant gap between the decrease of the protein levels of AR and PSA. Therefore, as has been proposed by Hsieh and Wu [16,17], the decrease of the protein level of AR could not fully account for the decrease of the AR function by resveratrol.

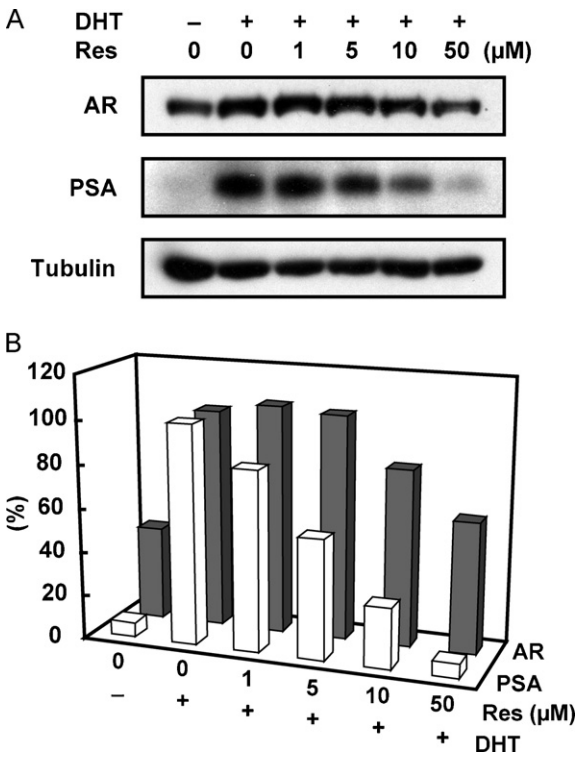

Fig. 1. Expression patterns of AR and PSA in cells treated with various concentrations of resveratrol. (A) LNCaP cells grown in medium supplemented with 10% dFBS were incubated with 10 nM DHT in the presence 0–50 μM resveratrol for 24 h. Proteins (10 μg) in whole cell lysate were subjected to SDS-PAGE, followed by Western blot analysis with anti-AR, anti-PSA, and anti-α-tubulin antibodies. (B) Band intensities were calculated by densitometry. AR and PSA levels that were normalized to the levels of α-tubulin were expressed as a percentage of the intensity of the control (with DHT and without resveratrol). The data are representative of three independent experiments.

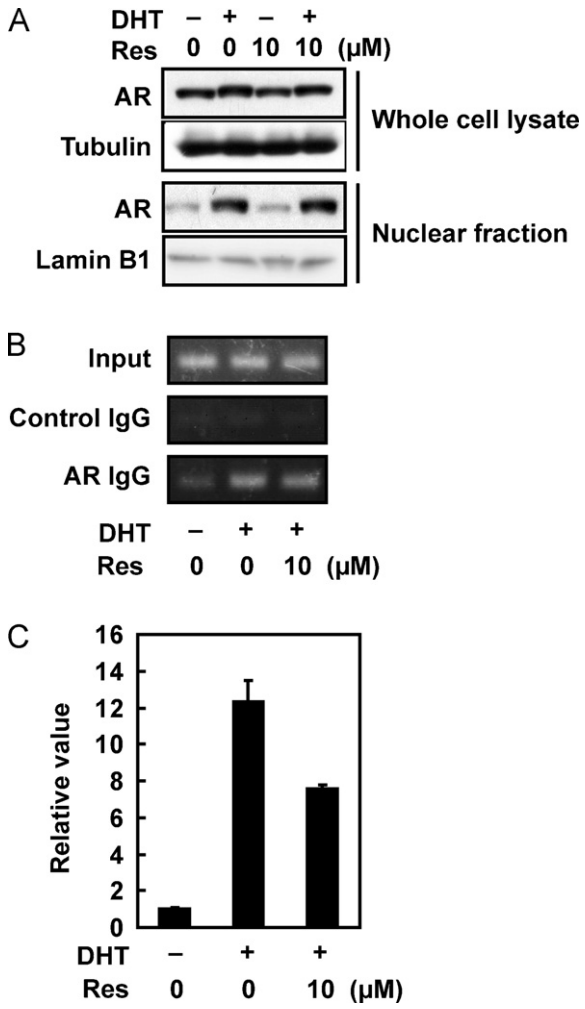

Fig. 2. Subcellular distribution of AR and DNA binding of AR to the PSA enhancer at the early phase. (A) LNCaP cells were incubated in the presence or absence of 10 nM DHT and 10 μM resveratrol for 3 h. The whole cell lysate and nuclear fraction were analyzed by Western blotting with anti-AR, anti-α-tubulin, and anti-lamin B1 antibodies. (B and C) LNCaP cells that had been pretreated with 10 μM resveratrol for 135 min were stimulated by DHT for 45 min. A ChIP assay was performed as described in Section 2. (B) Semi-quantitative PCR was performed and PCR products were analyzed on a 2% agarose gel. (C) Quantitative PCR was performed and signals of ChIP/input were expressed as relative values. Data are expressed as representative of three-independent experiments.

3.2. The binding of AR to PSA enhancer

To account for the decrease of AR function by resveratrol, we investigated mechanisms other than a decrease of AR expression. Resveratrol had its strongest differential effect on the levels of PSA and AR at concentration of 10 μM (Fig. 1B). In the following, we used a concentration of 10 μM. Because ligand-bound AR translocates from the cytoplasm into the nucleus, we examined the effect of resveratrol on the AR level in the nucleus. It was reported that the nuclear AR level peaks after 3 h incubation with ligand [23]. LNCaP cells were incubated in the presence of DHT and 10 μM resveratrol for 3 h, and the AR levels in whole cell lysate and nuclear fraction were analyzed by Western blotting (Fig. 2A). α-Tubulin and lamin B1 were used as internal controls for the whole cell lysate and nuclear fraction, respectively, and AR in the whole cell lysate and nuclear fraction was estimated by densitometry (data not shown). At this early phase, the DHT-induced increases of AR in the whole cell lysate and nuclear fraction were not affected by resveratrol. These results suggest that resveratrol (10 μM) did not affect the nuclear translocation of AR.

Because the AR level was not affected in the presence of 10 μM resveratrol for 3 h, we examined the amounts of AR bound to the ARE on the PSA enhancer with a ChIP assay after incubation with resveratrol for 3 h. LNCaP cells were pre-incubated with resveratrol in the absence of DHT for 135 min and then incubated with resveratrol in the presence of DHT for 45 min. In LNCaP cells treated with 10 μM resveratrol for 3 h, DHT-induced binding of AR to the PSA enhancer was decreased, as shown by semi-quantitative and quantitative PCR (Fig. 2B and C, respectively). Taken together, these results show that resveratrol inhibited the binding of AR to the ARE on the PSA enhancer without affecting the AR level, which resulted in a reduction of PSA expression.

3.3. Acetylation state of AR

The acetylation of AR has been proposed to be important for the DNA-binding of AR [24]. Hence, we examined the level of acetylated AR in LNCaP cells stimulated with DHT for 3 h in the presence or absence of 10 μM resveratrol. In LNCaP cells, using antibodies against AR, equivalent amounts of AR were immunoprecipitated from cells treated with DHT alone or co-treated with DHT and resveratrol for 3 h (Fig. 3, upper panel). The DHT-treatment increased the amount of acetylated AR, but the increase was reversed when the cells were co-incubated in the presence of resveratrol (lower panel).

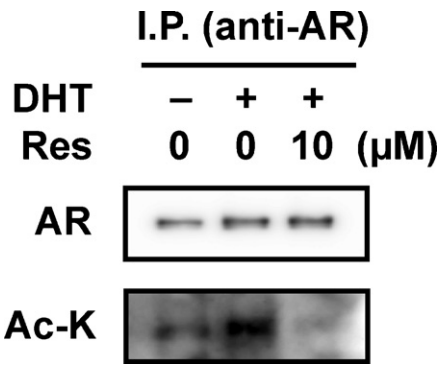

Fig. 3. DHT-induced acetylation of AR. LNCaP cells in medium supplemented with 10% dFBS were treated with 10 nM DHT and/or 10 μM resveratrol for 3 h. Cell lysates were immunoprecipitated with control or anti-AR antibodies and analyzed with Western blotting using anti-AR and anti-acetyl lysine (Ac-K) antibodies. The data are representative of three-independent experiments.

3.4. Acetylation site of AR in resveratrol-decreased transactivation

To understand the biological meaning of the decrease of AR acetylation by resveratrol, we examined the transcriptional activity of mutant AR(K630T), in which Lys is substituted for Thr at position 630, and which is considered as an acetylation mimic form of AR [5]. The expression level of AR(K630T) was lower than that of wild-type AR. As shown in Fig. 4, AR(K630T) exerted stronger transcriptional activity than the wild-type, consistent with a previous study [5]. Resveratrol significantly suppressed the DHT-induced transcriptional activity of wild-type AR, but not that of AR(K630T).

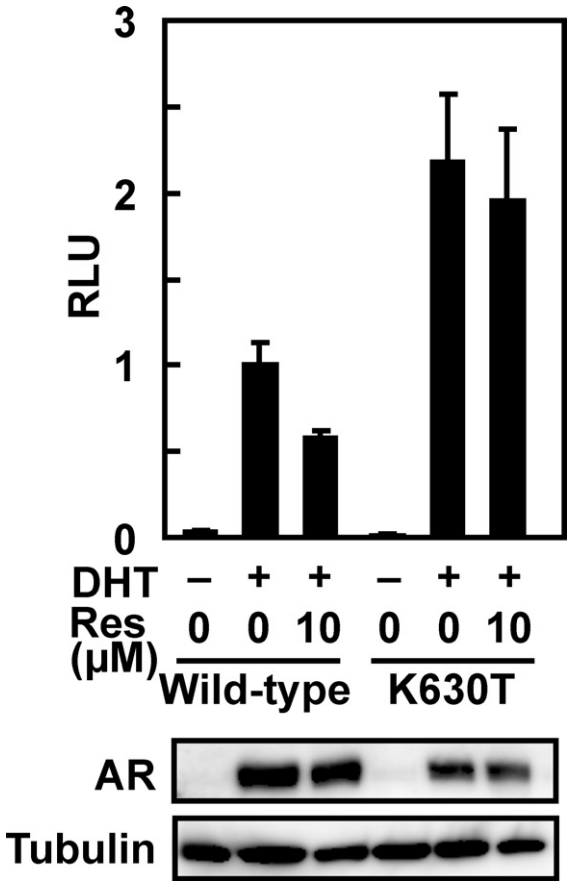

Fig. 4. Effect of resveratrol on transactivation of AR(K630T), an acetylation site mutant. PC-3 cells were co-transfected with AR expression vector (wild-type or K630T mutant), pARE2-TATA-Luc, and pRL-TK for 24 h. Cells were incubated in the presence or absence of 10 nM DHT and 10 μM resveratrol for an additional 24 h, and luciferase activities were measured and expressed as RLU. Protein levels of wild-type AR or AR(K630T) and α-tubulin were determined by Western blotting. Values indicated the means ± SD of triplicate determinations, and the results are representative of two independent experiments.

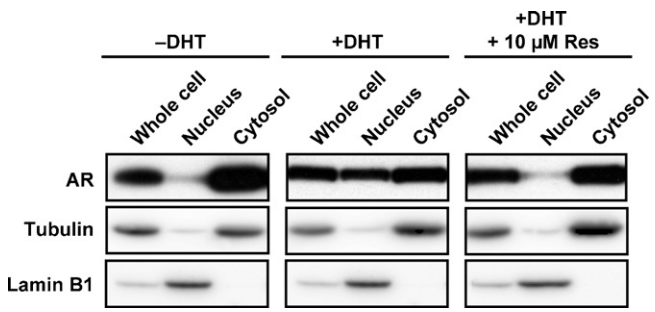

Fig. 5. Accumulation of AR in the nucleus at the later phase. LNCaP cells grown in medium supplemented with 10% dFBS were incubated in the presence or absence of 10 nM DHT and 10 μM resveratrol for 24 h. Whole cell lysate was fractionated into cytosolic and nuclear fractions by differential centrifugation. Proteins (10 μg) in each fraction were subjected to SDS-PAGE, followed by Western blot analysis with anti-AR, anti-α-tubulin (a control for the cytosolic fraction), and anti-lamin B1 (a control for the nuclear fraction) antibodies. The data are representative of three independent experiments.

3.5. Subcellular localization of AR

Resveratrol limited the DNA binding of AR at the early phase after stimulation of DHT. We then examined whether the nuclear AR, which was dissociated from DNA, could be consequently excluded from the nucleus because AR shuttles between the cytoplasm and the nucleus [1]. Because the re-export of AR from the nucleus to the cytoplasm takes over 12 h [25], LNCaP cells were incubated with DHT (10 nM) for 24 h in the presence or absence of resveratrol (10 μM). Cells were separated into nuclear and cytosolic fractions by differential centrifugation, and AR was analyzed by Western blotting. Nuclear-localized AR was markedly increased by DHT, whereas the DHT-dependent increase of nuclear AR was reduced by co-incubation with resveratrol for 24 h (Fig. 5).

3.6. Nuclear localization of ARΔC-Nuc

Mutant AR (ARΔC-Nuc) [18], which lacks the LBD and a partial hinge region, constitutively locates in the nucleus and acts as a constitutively active form of AR even in the absence of DHT [26]. Because the transactivation of ARΔC-Nuc was also down-regulated by resveratrol [15], we examined the localization of ARΔC-Nuc after long-term incubation with resveratrol. ARΔC-Nuc was expressed in PC-3 (AR-negative) prostate cancer cells and incubated with resveratrol (10 μM) for 24 h. Resveratrol decreased the nuclear protein level of ARΔC-Nuc, as shown by subcellular fractionation and Western blotting (Fig. 6A). α-Tubulin and lamin B1 were used as internal controls for the whole cell lysate and nuclear fraction, respectively. In the absence of resveratrol, ARΔC-Nuc was detected by immunofluorescent microscopy within the nucleus in a punctate pattern (Fig. 6B, top left panel). However, in the presence of resveratrol, ARΔC-Nuc was not restricted to the nucleus but was also distributed in the cytoplasm (Fig. 6B, green smear in top right panel). In addition, resveratrol caused ARΔC-Nuc to be localized on the periphery of the nuclear membrane.

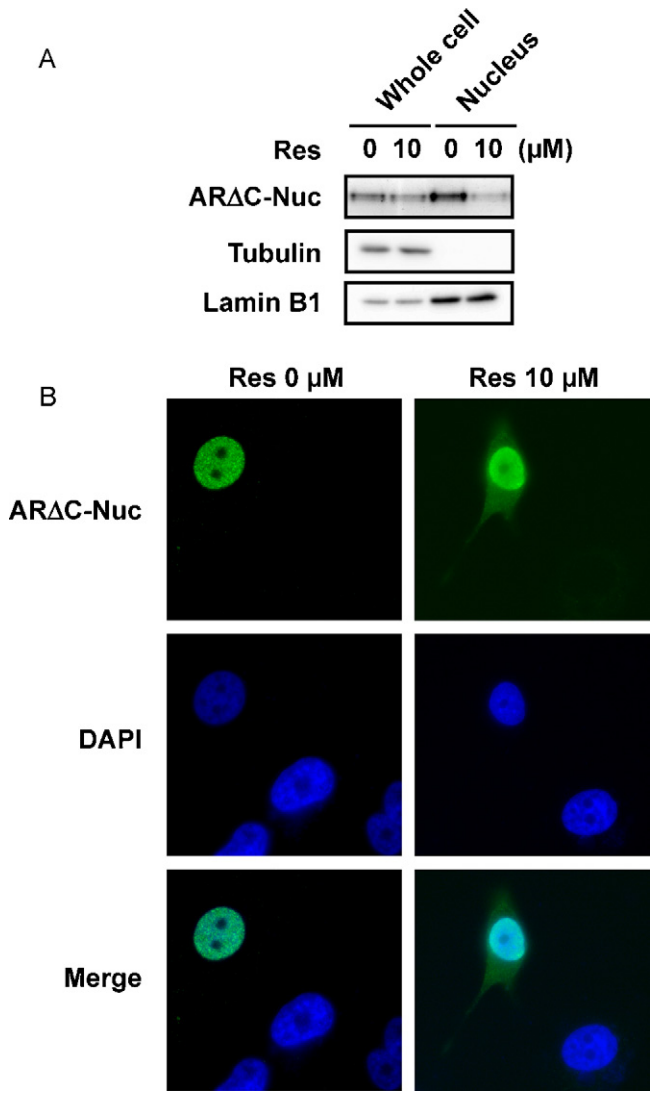

Fig. 6. Subcellular localization of ARΔC-Nuc. Transformed PC-3 cells overexpressing ARΔC-Nuc were incubated in the presence or absence of 10 μM resveratrol for 24 h. (A) After fractionation of the cells, proteins in the total cell lysate and nuclear fraction (each 10 μg) were analyzed by Western blot with anti-AR, anti-α-tubulin, and anti-lamin B1 antibodies. (B) Cells were fixed, permeated, and reacted with anti-AR antibodies, followed by immunoreaction with Alexa Fluor 488-conjugated secondary antibodies. Nuclei were stained with DAPI. The data are representative of two-independent experiments.

4. Discussion

Preventive strategies for prostate cancer have received considerable attention in recent years [10]. Because androgen-dependent AR function plays a pivotal role in the development of prostate cancer, AR is recognized as a preventative and therapeutic target for prostate cancer [9]. Resveratrol inhibits the prostate cancer cells *in vitro* and suppresses the progression of prostate cancer in *in vivo* mouse and rat adenocarcinoma models [12,16,27,28]. In the present study, we identified the mechanism by which resveratrol inhibits the transcriptional activity of AR.

Resveratrol reduces the expression of PSA both *in vitro* and *in vivo*, revealing its inhibitory effect on the androgen axis [11,14]. It also reduces the expression level of AR at both the transcriptional and post-translational stages [14,15]. However, until now, it has not been clear whether resveratrol inhibits AR function by decreasing AR expression. Our results show that AR protein level is suppressed only in the presence of resveratrol at high concentrations (Fig. 1A and B). In contrast, the decrease of the PSA expression was observed even in lower concentrations at which AR levels were not affected, suggesting the existence of other possible mechanisms. These data are consistent with previous studies [14–17]. Together, our results suggest that measuring the levels of total AR protein is insufficient to determine the effect of resveratrol on AR signaling.

After associating with the ligand, AR binds to the ARE on the promoter region and acts as a transcription factor. In addition, AR function is also modified with post-translational modification by cofactor proteins. Resveratrol has little affinity for AR, suggesting that resveratrol does not act as an antagonist of DHT [11]. Moreover, resveratrol dose-dependently decreased the transcriptional activity of ARΔC-Nuc, which lacks the LBD of AR, indicating that resveratrol inhibits AR function even without direct binding to the LBD of AR [15]. Here, we found that resveratrol inhibited the binding of AR to the ARE on the PSA promoter even at an early phase after incubation with resveratrol, when the total and nuclear AR levels were not affected. In addition, DHT-dependent acetylation of AR was also decreased by resveratrol. These data are supported by the findings that the acetylation of AR is important for the binding to DNA [24] and positively affects its transcriptional activity [5].

A recent study suggested that AR acetylation affects the subcellular localization of AR [29]. The nuclear-localized AR was decreased after incubation with 10 μM resveratrol for 24 h, but not for 3 h, suggesting that resveratrol does not inhibit the nuclear import of AR. Because acetylated AR and DNA-bound AR were decreased after only 3 h of exposure to resveratrol, they must have occurred prior to the reduction of nuclear AR. In addition, experiments with the acetylation site mutant AR(K630T) demonstrate the importance of AR acetylation in transcriptional repression by resveratrol (Fig. 4). Taken together, our results suggest that decreasing the acetylation of AR suppresses the binding of AR to the ARE and that non-acetylated AR preferentially translocates from the nucleus to the cytoplasm. Thus, the resveratrol-dependent decrease of nuclear AR might result from augmentation of the nuclear export of AR and not from inhibiting the nuclear import of AR.

Although resveratrol had been considered as an activator of SIRT1, which is a NAD-dependent deacetylase of AR [30], recent results cast doubt on the ability of resveratrol to affect SIRT1 activity [31,32]. In the presence of resveratrol, nicotinamide, a SIRT inhibitor, did not specifically recover AR transactivation (data not shown), suggesting that resveratrol down-regulates the acetylation of target proteins including AR by some mechanism other than enhancement of SIRT1 activity. Because the acetylation site of AR is responsible for the transcriptional up-regulation by PKA and AKT signals [24], these signals may be involved in modulation of the acetylation status of AR by resveratrol. Alternatively, our data may indicate that acetylation of the receptor represents the status of DNA-binding.

Our results demonstrate that resveratrol restricted the association between AR and ARE on the PSA promoter and decreased the acetylation of AR, which in turn suppressed the accumulation of AR in the nucleus. Because these effects were more potent than the decrease of AR protein, they represent a novel mechanism by which resveratrol inhibits AR transactivation.

Acknowledgements

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References

[1] C.A. Heinlein, C. Chang, Androgen receptor (AR) coregulators: an overview, Endocr. Rev. 23 (2002) 175–200.
[2] D. Gioeli, S.B. Ficarro, J.J. Kwiek, D. Aaronson, M. Hancock, A.D. Catling, F.M. White, R.E. Christian, R.E. Settlage, J. Shabanowitz, D.F. Hunt, M.J. Weber, Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites, J. Biol. Chem. 277 (2002) 29304–29314.
[3] L.K. Beitel, Y.A. Elhaji, R. Lumbroso, S.S. Wing, V. Panet Raymond, B. Gottlieb, L. Pinsky, M.A. Trifiro, Cloning and characterization of an androgen receptor N-terminal-interacting protein with ubiquitin-protein ligase activity, J. Mol. Endocrinol. 29 (2002) 41–60.
[4] M. Fu, C. Wang, A.T. Reutens, J. Wang, R.H. Angeletti, L. Siconolfi Baez, V. Ogryzko, M.L. Avantaggiati, R.G. Pestell, p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation, J. Biol. Chem. 275 (2000) 20853–20860.
[5] M. Fu, M. Rao, C. Wang, T. Sakamaki, J. Wang, D. Di Vizio, X. Zhang, C. Albanese, S. Balk, C. Chang, S. Fan, E. Rosen, J.J. Palvimo, O.A. Janne, S. Muratoglu, M.L. Avantaggiati, R.G. Pestell, Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth, Mol. Cell. Biol. 23 (2003) 8563–8575.
[6] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray, M.J. Thun, Cancer statistics, 2008, CA Cancer J. Clin. 58 (2008) 71–96.
[7] P.H. Riegman, R.J. Vlietstra, J.A. van der Korput, A.O. Brinkmann, J. Trapman, The promoter of the prostate-specific antigen gene contains a functional androgen responsive element, Mol. Endocrinol. 5 (1991) 1921–1930.
[8] S.M. Dhanasekaran, T.R. Barrette, D. Ghosh, R. Shah, S. Varambally, K. Kurachi, K.J. Pienta, M.A. Rubin, A.M. Chinnaiyan, Delineation of prognostic biomarkers in prostate cancer, Nature 412 (2001) 822–826.
[9] W.G. Nelson, A.M. De Marzo, W.B. Isaacs, Prostate cancer, N. Engl. J. Med. 349 (2003) 366–381.
[10] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beecher, H.H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275 (1997) 218–220.
[11] T.T. Wang, T.S. Hudson, T.C. Wang, C.M. Remsberg, N.M. Davies, Y. Takahashi, Y.S. Kim, H. Seifried, B.T. Vinyard, S.N. Perkins, S.D. Hursting, Differential effects of resveratrol on androgen-responsive LNCaP human prostate cancer cells in vitro and in vivo, Carcinogenesis 29 (2008) 2001–2010.
[12] A. Seeni, S. Takahashi, K. Takeshita, M. Tang, S. Sugiura, S.Y. Sato, T. Shirai, Suppression of prostate cancer growth by resveratrol in the transgenic rat for adenocarcinoma of prostate (TRAP) model, Asian Pac. J. Cancer Prev. 9 (2008) 7–14.
[13] S.B. Jones, S.E. DePrimo, M.L. Whitfield, J.D. Brooks, Resveratrol-induced gene expression profiles in human prostate cancer cells, Cancer Epidemiol. Biomarkers Prev. 14 (2005) 596–604.
[14] S.H. Mitchell, W. Zhu, C.Y. Young, Resveratrol inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells, Cancer Res. 59 (1999) 5892–5895.
[15] N. Harada, Y. Murata, R. Yamaji, T. Miura, H. Inui, Y. Nakano, Resveratrol downregulates the androgen receptor at the post-translational level in prostate cancer cells, J. Nutr. Sci. Vitaminol. (Tokyo) 53 (2007) 556–560.
[16] T.C. Hsieh, J.M. Wu, Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines, Exp. Cell Res. 249 (1999) 109–115.
[17] T.C. Hsieh, J.M. Wu, Grape-derived chemopreventive agent resveratrol decreases prostate-specific antigen (PSA) expression in LNCaP cells by an androgen receptor (AR)-independent mechanism, Anticancer Res. 20 (2000) 225–228.
[18] N. Harada, R. Yasunaga, Y. Higashimura, R. Yamaji, K. Fujimoto, J. Moss, H. Inui, Y. Nakano, Glyceraldehyde-3-phosphate dehydrogenase enhances transcriptional activity of androgen receptor in prostate cancer cells, J. Biol. Chem. 282 (2007) 22651–22661.
[19] A. Khanna Gupta, T. Zibello, H. Sun, P. Gaines, N. Berliner, Chromatin immunoprecipitation (ChIP) studies indicate a role for CCAAT enhancer binding proteins alpha and epsilon (C/EBP alpha and C/EBP epsilon) and CDP/cut in myeloid maturation-induced lactoferrin gene expression, Blood 101 (2003) 3460–3468.
[20] Y. Shang, M. Myers, M. Brown, Formation of the androgen receptor transcription complex, Mol. Cell 9 (2002) 601–610.
[21] N. Harada, Y. Ohmori, R. Yamaji, Y. Higashimura, K. Okamoto, F. Isohashi, Y. Nakano, H. Inui, ARA24/Ran enhances the androgen-dependent NH_2- and COOH-terminal interaction of the androgen receptor, Biochem. Biophys. Res. Commun. 373 (2008) 373–377.
[22] N. Harada, T. Yokoyama, R. Yamaji, Y. Nakano, H. Inui, RanBP10 acts as a novel coactivator for the androgen receptor, Biochem. Biophys. Res. Commun. 368 (2008) 121–125.
[23] M.D. Sadar, Androgen-independent induction of prostate-specific antigen gene expression via cross-talk between the androgen receptor and protein kinase A signal transduction pathways, J. Biol. Chem. 274 (1999) 7777–7783.
[24] M. Fu, M. Rao, K. Wu, C. Wang, X. Zhang, M. Hessien, Y.G. Yeung, D. Gioeli, M.J. Weber, R.G. Pestell, The androgen receptor acetylation site regulates cAMP and AKT but not ERK-induced activity, J. Biol. Chem. 279 (2004) 29436–29449.
[25] R.K. Tyagi, Y. Lavrovsky, S.C. Ahn, C.S. Song, B. Chatterjee, A.K. Roy, Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells, Mol. Endocrinol. 14 (2000) 1162–1174.
[26] G. Jenster, H.A. van der Korput, J. Trapman, A.O. Brinkmann, Identification of two transcription activation units in the N-terminal domain of the human androgen receptor, J. Biol. Chem. 270 (1995) 7341–7346.
[27] C.E. Harper, B.B. Patel, J. Wang, A. Arabshahi, I.A. Eltoum, C.A. Lamartiniere, Resveratrol suppresses prostate cancer progression in transgenic mice, Carcinogenesis 28 (2007) 1946–1953.
[28] C.E. Harper, L.M. Cook, B.B. Patel, J. Wang, I.A. Eltoum, A. Arabshahi, T. Shirai, C.A. Lamartiniere, Genistein and resveratrol, alone and in combination, suppress prostate cancer in SV-40 tag rats, Prostate 69 (2009) 1668–1682.
[29] M. Shiota, A. Yokomizo, D. Masubuchi, Y. Tada, J. Inokuchi, M. Eto, T. Uchiumi, N. Fujimoto, S. Naito, Tip60 promotes prostate cancer cell proliferation by translocation of androgen receptor into the nucleus, Prostate 70 (2010) 540–554.
[30] M. Fu, M. Liu, A.A. Sauve, X. Jiao, X. Zhang, X. Wu, M.J. Powell, T. Yang, W. Gu, M.L. Avantaggiati, N. Pattabiraman, T.G. Pestell, F. Wang, A.A. Quong, C. Wang, R.G. Pestell, Hormonal control of androgen receptor function through SIRT1, Mol. Cell. Biol. 26 (2006) 8122–8135.
[31] M. Kaeberlein, T. McDonagh, B. Heltweg, J. Hixon, E.A. Westman, S.D. Caldwell, A. Napper, R. Curtis, P.S. DiStefano, S. Fields, A. Bedalov, B.K. Kennedy, Substrate-specific activation of sirtuins by resveratrol, J. Biol. Chem. 280 (2005) 17038–17045.
[32] M.T. Borra, B.C. Smith, J.M. Denu, Mechanism of human SIRT1 activation by resveratrol, J. Biol. Chem. 280 (2005) 17187–17195.