hemodynamics [3], accumulating evidences have suggested that RAS also participates in the regulation of tumor angiogenesis including that of PCa [4–6]. Angiotensin II (Ang II) is a key biological peptide of RAS. Another major mediator of angiogenesis is vascular endothelial growth factor (VEGF), which is induced by Ang II [7,8]. We recently reported that activation of Ang II leads to potent induction of VEGF in PCa cells [9], and bladder cancer cells [10]. The proangiogenic effects of Ang II suggest that angiotensin II typel receptor (AT1R) blockade (ARB) may protect against cancer by inhibiting angiogenesis.

It is well known that angiogenesis is essential for tumor progression and metastasis [11]. We previously demonstrated that the degree of tumor angiogenesis, including VEGF and microvessel density (MVD), was related to the development and the progression of HRPC, and was accompanied by significantly higher expression of AT1R [9]. On the basis of the correlation between AT1R expression and angiogenesis in HRPC, we tested the effects of candesartan, which was developed as a specific long-acting ARB. Candesartan inhibited VEGF production and dramatically suppressed tumor growth in a xenograft model of human HRPC accompanied by decrease of serum PSA concentration [9]. Furthermore, we previously demonstrated that candesartan inhibited dramatically decreased lung metastasis in a mouse model of murine renal cancer [12] and reduced tumor growth in a xenograft model of bladder cancer [10].

Ang II is a key biological peptide of RAS. The two major subtypes of Ang II receptors are type-1 receptor (AT1R) and type-2 receptor (AT2R). AT1R belongs to the seven transmembrane domain superfamily. AT1R antagonists are used widely as anti-hypertensive agents. Once Ang II binds to AT1R, it activates a series of signaling cascades, which in turn regulate the various physiological effects of Ang II including angiogenesis [3]. Angiogenic factors such as VEGF are regulated by various signaling and transcriptional factors [13]. Hypoxia inducible factor-1 alpha (HIF-1α) has been reported to play a role in VEGF induction by Ang II in vascular smooth muscle cells (VSMC) under normal levels of oxygen [7,8]. However, the status of HIF-1α by Ang II in cancer cells under normal levels of oxygen has not been well characterized yet.

It was recently reported that transcriptional factor Ets-1 was a critical regulator of Ang II-mediated vascular pathophysiology [14]. Moreover, Ets-1 has been shown to regulate genes involved in endothelial function and angiogenesis [15]. We previously demonstrated that the expression of Ets-1 in human renal cell carcinoma (RCC) correlated with VEGF expression and the tumor MVD, and suggested the possibility that Ets-1 was involved in angiogenesis in RCC [16]. However, the status of Ets-1 induced by Ang II in cancer cells has not been investigated yet.

Although the knowledge of downstream mediators of Ang II is critical to the understanding of the pathophysiological roles of RAS and angiogenesis, the status of downstream transcriptional factors induced by Ang II in cancer cells including PCa cells has not been studied yet.

We investigated Ang II-induced changes of HIF-1α and Ets-1 in the nucleus.

MATERIALS AND METHODS

Reagent

Angiotensin II dihydrotestosterone (DHT), and mouse monoclonal antibody for beta-actin were purchased from Sigma (Atlanta, GA). Rabbit polyclonal antibody for Ets-1 and AT1R and mouse monoclonal antibody for HIF-1α were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Candesartan (CV11974) was provided by Takeda Pharmaceutial Co. (Osaka, Japan). CV11974 is the active metabolite of candesartan and is used for in vitro experiments.

Cell Lines and Culture

Two human PCa cell lines (LNCap, C4-2) were routinely maintained in RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% FBS, at 37°C in a humidified 5% CO_2 atmosphere. To examine the androgen-ablated treatment, C4-2 cells were grown in RPMI-1640 containing 10% charcoal-stripped fetal bovine serum, at 37°C in a humidified 5% CO_2 atmosphere. These cells were passaged upon reaching confluence during 6 months. We named this cell line as C4-2AT6 (androgen-ablated treatment for 6 months). The oxygen levels in this work were maintained within normal range.

Measurement of Prostate-Specific Antigen (PSA)

The secretion of PSA in vitro was measured using a commercially available ELISA kit for human total PSA (Quantikine, R&Dsystems, Minneapolis, MN) in LNCaP, C4-2, and C4-2AT6 cells. These three cell lines were suspended in RPMI-1640 containing 10% FBS, plated in 6-well plates and allowed to attach at 37°C in a humidified 5% CO_2 atmosphere. After the medium was replaced with serum-free medium for 24 hr, the cells were incubated with DHT at 10^{-8} M for 48 hr.

WST Assay for Cell ViabilityTreatment With DHTand Angiotensin II

LNCaP, C4-2, and C4-2AT6 were treated with DHT treatment to investigate androgen independence using the WST cell viability assay kit. To examine Ang

II-induced effects on cell growth, the cell lines were incubated with angiotensin II and CV11974. The cell lines were suspended in RPMI-1640 containing 10% FBS, plated in 96-well plates and allowed to attach at 37°C in a humidified 5% CO_2 atmosphere. After the medium was replaced with serum-free medium for 24 hr, the cells were incubated with DHT at various concentrations between 10^{-9} and 10^{-7} M or with Ang II at concentrations between 10^{-9} and 10^{-7} M with RPMI-1640 containing 1% charcoal-stripped fetal bovine serum for 24, 48, or 72 hr. At the end of the incubation period, WST reagents were added to each well and incubated for 1 hr in a humidified 5% CO_2 atmosphere. Cell viability was estimated by colorimetry, reading the color intensity in a plate reader at 570 nm.

Effects of Angiotensin II and AT1R Blockade In Vitro

C4-2 and C4-2AT6 cells were seeded in 100 mm^2 dishes and allowed to attach at 37°C in a humidified 5% CO_2 atmosphere. After 24 hr, the medium was replaced with serum-free medium for 24 hr, and then the cells were incubated with serum-free medium (Ang II 10^{-8} M or CV11974 10^{-7} M, or Ang II 10^{-8} M, and Candesartan 10^{-7} M). Cells were stimulated with the reagents and harvested at 6 hr. The stimulation was stopped by placing the dishes on ice. The supernatant was collected and VEGF was measured using commercially available ELISA kits (Quantikine, R&D Systems). The VEGF protein level was quantified by comparing its optical density with the standard curve for VEGF and normalizing it for the number of the cells.

Cell Extracts and Western Blot Analysis

Whole-cell extracts were obtained using RIPA buffer (50 mM, Tris–HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS), containing protease inhibitors. Nuclear extracts were obtained according to standard methods. Briefly, harvested cells were resuspended in 5 volumes of hypotonic buffer (10 mmol/L HEPES (pH 7.9), 10 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 0.5 mmol/L dithiothreitol) supplemented with proteinase and phosphatase inhibitors (0.5 mmol/L phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mmol/L Na_3VO_4, 1 mmol/L NaF), incubated for 10 min on ice, and sedimented. The cells were then resuspended in 2 volumes of the same buffer, homogenized, and sedimented at 1,000*g* for 10 min, then the pellet (nuclei) was collected. The pelleted nuclei were incubated for 30 min at 4°C in high-salt buffer (20 mmol/L HEPES(pH 7.9), 25% glycerol, 400 mmol/L, NaCl, and 1 mmol/L EDTA) supplemented with proteinase and phosphatase inhibitors. For Western blot analysis, 20 μg of nuclear protein for HIF-1α and Ets-1, and 50 μg of total protein for AT1R were separated by SDS–PAGE (12.5% acrylamide gel) and transferred to a nitrocellulose membrane. Blots were incubated overnight with anti-ETS-1 antibody, anti-HIF-1α antibody, anti-beta-actin, and anti-lamina antibody. After washing, the blots were incubated with peroxidase-labeled secondary antibody (Dako). The signals were detected by enhanced chemiluminescence reagents (ECL Plus Western Blotting Detection System; Amersham Pharmacia Biotech) and analyzed. The intensity was quantified using LAS 3000 system (Fuji Film; Tokyo).

RESULTS

Establishment and Characterization of an Androgen-Independent Subline to C4-2: C4-2AT6

In order to study the relationships between androgen-independency and angiogenesis in HRPC, we generated an androgen-independent subline of C4-2. For this purpose, C4-2 cells were grown in RPMI-1640 containing 10% charcoal-stripped fetal bovine serum, and passaged upon reaching subconfluence. These cells has been cultured successfully through 40 passages for 6 months and named C4-2AT6. C4-2AT6 cells were treated with DHT to investigate the androgen independence (Fig. 1A). DHT did not affect the viability of C4-2AT6 cells at any of the concentrations tested. DHT affected LNCaP cells viability as previously reported by others [17,18]. Similar results were observed at 24 and 72 hr.

To examine the effects on cell proliferation, C4-2AT6 cells were incubated with various concentrations of Ang II or CV11974 (Fig. 1A). Neither Ang II nor CV11974 affected their proliferation. Similar results were observed at 24 and 72 hr. In vitro proliferation assay at clinically achievable concentrations of Ang II (10^{-9} to 10^{-7}) and CV11974 (10^{-7} M) showed no effect on cell proliferation.

The androgen regulation of PSA secretion was analyzed in vitro. LNCaP, C4-2, and C4-2AT6 cells were treated with DHT to induce PSA secretion (Fig. 1B). The level of secreted PSA without DHT was 2.9-fold higher in C4-2AT6 cells (1.72 ng/ml/1,000 cells/48 hr) compared with LNCaP cells (0.6 ng/ml/1,000 cells/48 hr). DHT treatment significantly induced PSA secretion in C4-2AT6 cells (3.05 ng/ml/1,000 cells/48 hr) about 1.8-fold compared with no DHT treatment.

AngII Induces VEGF ProductionThrough AT1R in C4-2 and C4-2AT6, But Not in LNCaP Cells

AT1R expression was demonstrated by Western blot analysis. We investigated AT1R expression in

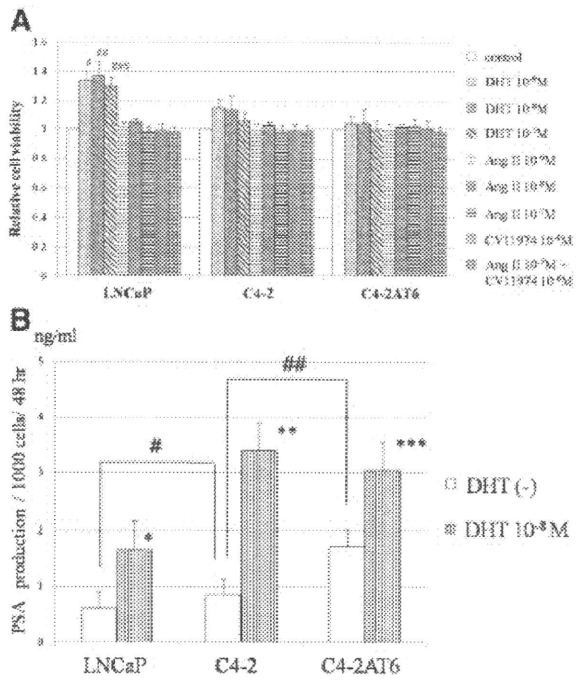

Fig. 1. **A**: Effects of DHT, angiotensin II, ARB on C4-2AT6 cells viability and proliferation. WST analysis after a 48 hr culture showed DHT did not affect C4-2AT6 cells proliferation. Similar results were obtained at 24 and 72 hr. DHT affected LNCaP cells viability. $^{\#}$, $^{\#\#}$, $^{\#\#\#}P < 0.01$, compared to LNCaP cells without DHT treatment. **B**: PSA production in C4-2AT6 cells treated with DHT for 48 hr. C4-2AT6 cells showed significantly higher basal PSA excretion than LNCaP and C4-2 cells. DHT (10^{-8} M) significantly induced PSA production in all three cell lines. $^{\#}P < 0.05$, compared to LNCaP cells without DHT treatment. $^{\#\#}P < 0.01$, compared to C4-2 cells without DHT treatment. *, **, $^{***}P < 0.01$, compared to PSA production without DHT treatment.

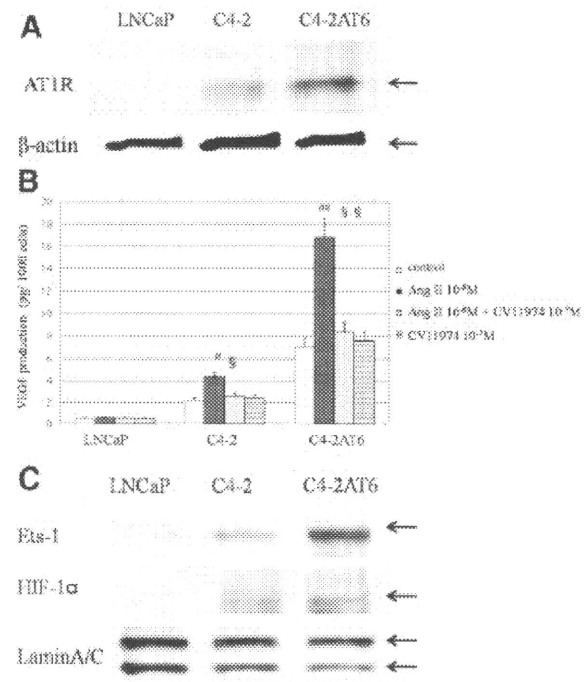

Fig. 2. **A**: HRPC cells showed a higher AT1R expression. C4-2 and C4-2AT6 cells showed higher AT1R expression than LNCaP cells. C4-2AT6 cells showed a prominently high AT1R expression. **B**: VEGF measurement in LNCaP, C4-2, and C4-2AT6 cells after incubation for 6 hr. C4-2AT6 cells showed significantly higher VEGF production than LNCaP and C4-2 cells at the 6-hr time point. In C4-2 and C4-2AT6 cells, Ang II stimulated VEGF production. CV11974 significantly inhibited Ang II-induced VEGF production in these cells. In LNCaP cells, Ang II had no significant effect on VEGF production. $^{\S}P < 0.01$, compared with control C4-2 cells. $^{\S\S}P < 0.01$, compared with control C4-2AT6 cells. $^{\#}P < 0.01$, compared with control LNCaP cells. $^{\#\#}P < 0.01$, compared with control C4-2 cells. **C**: HRPC cells showed higher HIF-1α and Ets-1 expression in the nucleus. Under normal levels of oxygen without any stimulation, C4-2 and C4-2AT6 cells showed higher HIF-1α and Ets-1 expression in the nucleus than LNCaP cells. C4-2AT6 cells showed a prominently high HIF-1α and Ets-1 expression.

androgen-dependant LNCaP cells, and in androgen-independent C4-2 and C4-2AT6 cells. Although the three cell lines expressed AT1R, C4-2 and C4-2AT6 cells showed a significantly higher AT1R expression than LNCaP cells (Fig. 2A). C4-2AT6 cells showed a significantly higher AT1R expression than C4-2 cells.

We assessed VEGF production in LNCaP, C4-2, and C4-2AT6 cells to examine the relationship between angiogenic aggressiveness and androgen independency. To determine the effects of AngII on VEGF production LNCaP, C4-2, and C4-2AT6 cells were exposed to AngII (10^{-8} M) for 6 hr under normal levels of oxygen. At each time point, VEGF in the supernatant was assessed by ELISA (Fig. 2B). C4-2AT6 cells showed significantly higher VEGF production than C4-2 cells and LNCaP cells without any stimulation. AngII induced a further increase of VEGF production in C4-2 and C4-2AT6 cells but not in LNCaP cells, while CV11974 significantly inhibited AngII-induced VEGF production in C4-2 and C4-2AT6 cells. Conversely, AngII did not affect VEGF production in LNCaP cells.

Acquirement of Angiogenic Aggressive Properties in HRPC Cells Through Up-Regulation of AT1R, HIF-1α, and Ets-1 Expression

To examine whether PCa cells acquired the angiogenic aggressiveness property accompanied by up-regulated transcriptional factors under androgen-ablated conditions, we investigated the expression of HIF-1α and Ets-1 in the nucleus under normal levels of oxygen. The three cell lines showed HIF-1α and Ets-1 expression in the nucleus (Fig. 2C). Of the three cell lines, C4-2AT6 cells showed significantly higher

HIF-1α and Ets-1 expression in the nucleus than LNCaP and C4-2 cells without any stimulation.

Ang II Induces HIF-1α in C4-2 and C4-2AT6 Cells, But Not in LNCaP Cells

To determine the effects of AngII on HIF-1α expression in the nucleus, LNCaP, C4-2, and C4-2AT6 cells were exposed to Ang II (10^{-8}M) for 6 hr under normal levels of oxygen. At each time point, nuclear extracts from these cells were obtained and HIF-1α expression was assessed by Western blot (Fig. 3A). In C4-2 cells and C4-2AT6 cells, AngII-induced HIF-1α up-regulation at the nucleus, while CV11974 significantly inhibited AngII-induced HIF-1α expression in C4-2 and C4-2AT6 cells. In LNCaP cells, AngII had no significant effect on HIF-1α expression.

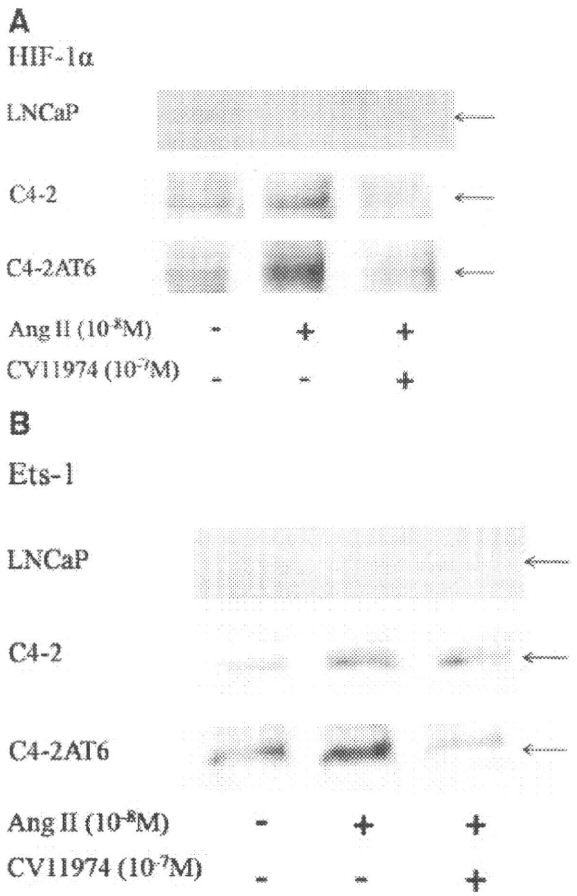

Fig. 3. **A**: Induction of HIF-1α by Ang II in HRPC cells. Ang II-induced HIF-1α expression in C4-2 and C4-2AT6 cells. CV11974 significantly inhibited Ang II-induced HIF-1α expression in these cells. In LNCaP cells, Ang II had no significant effect on HIF-1α expression. **B**: Induction of Ets-1 by Ang II in HRPC cells. Ang II-induced Ets-1 expression in C4-2 and C4-2AT6 cells. CV11974 significantly inhibited Ang II-induced Ets-1 expression in these cells. In LNCaP cells, Ang II had no significant effect on Ets-1 expression.

Ang II Induces Ets-1 in C4-2 and C4-2AT6 Cells, But Not in LNCaP Cells

To determine the effects of AngII on Ets-1 expression in the nucleus, LNCaP cells, C4-2 and C4-2AT6 cells were exposed to Ang II (10^{-8}M) for 6 hr under normal levels of oxygen. At each time point, nuclear extracts from these cells were obtained and Ets-1 expression was assessed by Western blot. AngII-induced Ets-1 expression in C4-2 and C4-2AT6 cells, while CV11974 significantly inhibited AngII-induced Ets-1 expression (Fig. 3B). In LNCaP cells, AngII had no significant effect on Ets-1 expression.

DISCUSSION

In the present study, we demonstrated in HRPC the expression of downstream transcriptional mediators of Ang II were HIF-1α and Ets-1. Furthermore, we showed that HRPC cells acquired angiogenic aggressiveness under androgen-ablated conditions, accompanied by up-regulation of AT1R, HIF-1α, and Ets-1. To clarify these downstream effectors of AngII is critical to the understanding of physiological roles of AngII in PCa. Our results not only provide evidence of protective effects of ARB as an angiogenic inhibitor but also the molecular basis of clinical benefit of ARB in HRPC.

The Ang II and AT1R pathways in angiogenesis are reported to enhance VEGF production. The VEGF/VEGF receptor system plays a central role both in developmental and pathologic blood vessel disease and also in tumor angiogenesis [3,4,7–10,19,20]. Recent studies have shown that VEGF expression in the primary tumor is related to the potential for recurrence and the prognosis of patients with PCa [21,22]. As to angiogenesis in HRPC, we clarified that the specimens from autopsied subjects who died of HRPC showed significantly higher VEGF expression and MVD than androgen-dependant PCa [9]. Additionally, we reported that C4-2 cells showed higher VEGF and MVD than LNCaP cells in vivo [9]. In this study, we found that C4-2AT6 cells without any stimulation showed higher VEGF production than C4-2 cells. Ang II-induced VEGF production in C4-2AT6 cells, while the AT1R blocker significantly inhibited AngII-induced VEGF production. These results indicated that HRPC cells acquire aggressive angiogenic properties under continuous androgen ablation accompanied by AT1R up-regulation, suggesting tumor angiogenesis can be induced through Ang II-dependant pathway.

However, the detailed mechanism of acquired aggressive angiogenic properties in HRPC has been unclear. According to the present results, one of the mechanisms by which HRPC acquires these aggressive angiogenesis is due to up-regulation of transcriptional

factors related to angiogenesis and vascular pathophysiology: HIF-1α and Ets-1, accompanied by up-regulation of AT1R expression. HIF-1α and Ets-1 have been reported to induce some angiogenic factors including VEGF [7,8,14,20,23,24]. Recent studies have shown that one of the downstream effector to induce VEGF production by the Ang II and AT1R pathways is HIF-1α in VSMC. AT1R is activated by Ang II-induced HIF-1α via a protein kinase C-dependant mechanism [7]. Although HIF-1α up-regulation by Ang II has been characterized in VSMC, the effect of Ang II on HIF-1α status in PCa, or other cancer cells has not been clarified yet. We examined HIF-1α expression in the nucleus by Western blot under normal levels of oxygen. We showed that Ang II-induced HIF-1α expression in the nucleus of C4-2 and C4-2AT6 cells, while CV11974 significantly inhibited AngII-induced HIF-1α expression. Moreover, Ang II had no significant effect on the induction of HIF-1α expression in LNCaP cells. These results indicated that the differences in the action of Ang II depended on the degree of AT1R expression.

Ets-1 factors are a family of transcriptional factors that share a highly conserved DNA-binding domain and are involved in the regulation of a wide variety of biological processes [15]. Ets-1 is the prototypical member of this family and is expressed in endothelial cells and VSMCs. It has been reported that Ets-1 regulates the expression of genes involved in angiogenesis, including VEGF, VEGF receptor, and matrix metalloproteinases (MMPs) [14–16,20,23,25,26]. Previous studies have indicated that Ets-1 is related to angiogenesis in a variety of cancers, including uterine endometrial cancer [27,28], ovarian cancer [29], and renal cancer [16]. Recently, Zhan et al. [14] reported that Ets-1 induction is a critical regulator of Ang II-mediated vascular pathogenesis, present especially in VSMC and endothelial cells. The status of Ets-1 by Ang II in PCa remains unclear. We examined Ets-1 expression in the nucleus by Western blot under normal levels of oxygen. We found that Ang II-induced Ets-1 expression in the nucleus in C4-2 and C4-2AT6 cells, while CV11974 significantly inhibited AngII-induced Ets-1 expression. Interestingly, Ang II had no significant effect on Ets-1 expression in LNCaP cells. These results indicate that Ang II does not affect LNCaP cells in the same way as HIF-1α because of the low AT1R expression. The status of HIF-1α and Ets-1 expression induced by Ang II has not been examined yet in cancer, and it is possible that other transcriptional factors are also induced by Ang II in cancer. Based on the present results, we propose a schema to understand the involvement of RAS in PCa (Fig. 4).

The proangiogenic effects of Ang II and pharmacogenic blockade of Ang II signaling may have a significant impact on the treatment of markedly angiogenic tumors including HRPC. Disruption of RAS through

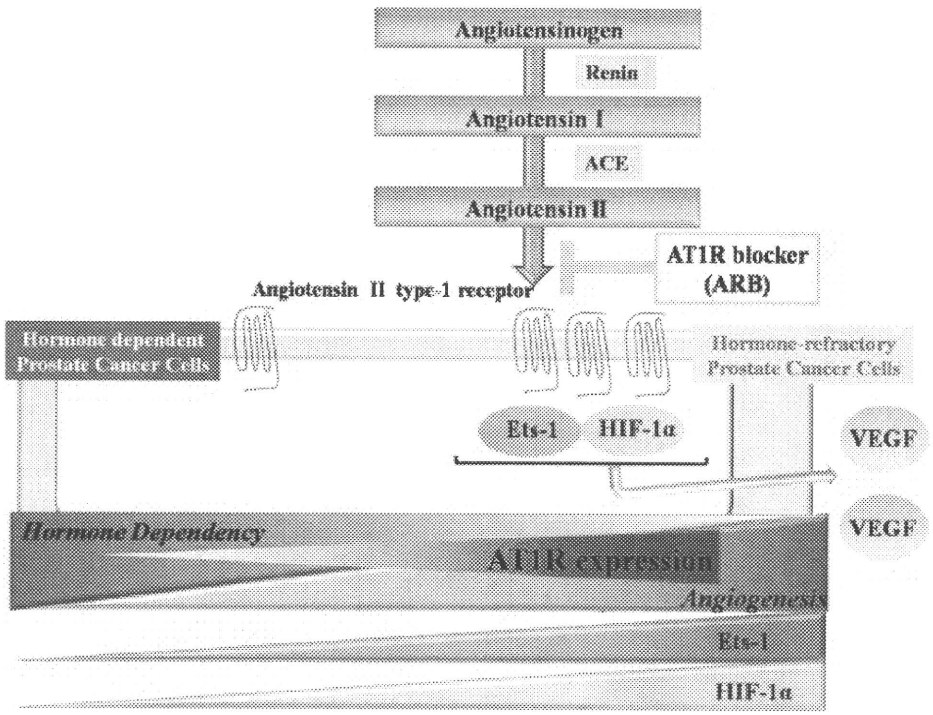

Fig. 4. Simplified schematic representation of how the renin-angiotensin system (RAS) would be involved in the development of prostate cancer, including several points at which hormone-independency and angiogenesis can be associated with the RAS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ARB may not only inhibit tumor angiogenesis but also could increase the efficacy of established chemotherapeutic agents and molecular targeting agents. Because the safety profile of ARB is well established, ARB added to selected cancer therapeutic regimens may serve simultaneously to inhibit tumor angiogenesis and have beneficial effects regarding the prevention of cardiovascular events. Our previous studies confirmed the anti-angiogenic effects of ARB in a HRPC xenograft model [9], a mouse renal cancer lung metastatic model [12], a bladder cancer xenograft model [10], as well as simultaneous effects of ARB and cis-dichlorodiammineplatinum (CDDP) in a bladder cancer xenograft model [30]. These results suggested the clinical application of ARB for urogenital cancer may be promising.

Several reports indicate that AT1R expression is modulated by various factors such as cytokines, growth factors nitric oxide and LDL [31,32], and is regulated by transcriptional, posttranscriptional, and translational mechanisms [31,33]. In this study, we showed AT1R expression was significantly up-regulated in C4-2AT6 at the protein level. However, the detailed molecular mechanisms of AT1R expression up-regulation in HRPC have not been fully elucidated yet. Further investigation is needed to clarify the regulation of AT1R under androgen-ablated conditions.

Our study clearly demonstrates that HRPC acquires aggressive angiogenic properties accompanied by up-regulation of AT1R, HIF-1α, and Ets-1. This study suggests that manipulation of RAS by an AT1R blockade in HRPC may have a significant impact by inhibiting angiogenesis through the inhibition of HIF-1α and Ets-1 expression.

REFERENCES

1. Catalona WJ. Management of cancer of the prostate. N Engl J Med 1994;331(15):996–1004.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008;58(2):71–96.
3. Helin K, Stoll M, Meffert S, Stroth U, Unger T. The role of angiotensin receptors in cardiovascular diseases. Ann Med 1997; 29(1):23–29.
4. Ager EI, Neo J, Christophi C. The renin-angiotensin system and malignancy. Carcinogenesis 2008;29(9):1675–1684.
5. Chow L, Rezmann L, Catt KJ, Louis WJ, Frauman AG, Nahmias C, Louis SN. Role of the renin-angiotensin system in prostate cancer. Mol Cell Endocrinol 2009;302(2):219–229.
6. Khakoo AY, Sidman RL, Pasqualini R, Arap W. Does the renin-angiotensin system participate in regulation of human vasculogenesis and angiogenesis? Cancer Res 2008;68(22):9112–9115.
7. Page EL, Robitaille GA, Pouyssegur J, Richard DE. Induction of hypoxia-inducible factor-1alpha by transcriptional and translational mechanisms. J Biol Chem 2002;277(50):48403–48409.
8. Richard DE, Berra E, Pouyssegur J. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. J Biol Chem 2000;275(35):26765–26771.
9. Kosaka T, Miyajima A, Takayama E, Kikuchi E, Nakashima J, Ohigashi T, Asano T, Sakamoto M, Okita H, Murai M, Hayakawa M. Angiotensin II type 1 receptor antagonist as an angiogenic inhibitor in prostate cancer. Prostate 2007;67(1):41–49.
10. Kosugi M, Miyajima A, Kikuchi E, Horiguchi Y, Murai M. Angiotensin II type 1 receptor antagonist candesartan as an angiogenic inhibitor in a xenograft model of bladder cancer. Clin Cancer Res 2006;12(9):2888–2893.
11. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86(3): 353–364.
12. Miyajima A, Kosaka T, Asano T, Seta K, Kawai T, Hayakawa M. Angiotensin II type I antagonist prevents pulmonary metastasis of murine renal cancer by inhibiting tumor angiogenesis. Cancer Res 2002;62(15):4176–4179.
13. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3(10):721–732.
14. Zhan Y, Brown C, Maynard E, Anshelevich A, Ni W, Ho IC, Oettgen P. Ets-1 is a critical regulator of Ang II-mediated vascular inflammation and remodeling. J Clin Invest 2005;115(9): 2508–2516.
15. Wasylyk B, Hahn SL, Giovane A. The Ets family of transcription factors. Eur J Biochem 1993;211(1–2):7–18.
16. Mikami S, Oya M, Mizuno R, Murai M, Mukai M, Okada Y. Expression of Ets-1 in human clear cell renal cell carcinomas: implications for angiogenesis. Cancer Sci 2006;97(9):875–882.
17. Wu HC, Hsieh JT, Gleave ME, Brown NM, Pathak S, Chung LW. Derivation of androgen-independent human LNCaP prostatic cancer cell sublines: role of bone stromal cells. Int J Cancer 1994;57(3):406–412.
18. Ghosh PM, Malik SN, Bedolla RG, Wang Y, Mikhailova M, Prihoda TJ, Troyer DA, Kreisberg JI. Signal transduction pathways in androgen-dependent and -independent prostate cancer cell proliferation. Endocr Relat Cancer 2005;12(1):119–134.
19. Chua CC, Hamdy RC, Chua BH. Upregulation of vascular endothelial growth factor by angiotensin II in rat heart endothelial cells. Biochim Biophys Acta 1998;1401(2):187–194.
20. Oettgen P. Regulation of vascular inflammation and remodeling by ETS factors. Circ Res 2006;99(11):1159–1166.
21. Ferrer FA, Miller LJ, Andrawis RI, Kurtzman SH, Albertsen PC, Laudone VP, Kreutzer DL. Vascular endothelial growth factor (VEGF) expression in human prostate cancer: in situ and in vitro expression of VEGF by human prostate cancer cells. J Urol 1997; 157(6):2329–2333.
22. Silberman MA, Partin AW, Veltri RW, Epstein JI. Tumor angiogenesis correlates with progression after radical prostatectomy but not with pathologic stage in Gleason sum 5 to 7 adenocarcinoma of the prostate. Cancer 1997;79(4):772–779.
23. Dittmer J. The biology of the Ets1 proto-oncogene. Mol Cancer 2003;2:29.
24. Pearse DD, Tian RX, Nigro J, Iorgulescu JB, Puzis L, Jaimes EA. Angiotensin II increases the expression of the transcription factor ETS-1 in mesangial cells. Am J Physiol Renal Physiol 2008; 294(5):F1094–F1100.
25. Nakayama T, Ito M, Ohtsuru A, Naito S, Nakashima M, Fagin JA, Yamashita S, Sekine I. Expression of the Ets-1 proto-oncogene in human gastric carcinoma: correlation with tumor invasion. Am J Pathol 1996;149(6):1931–1939.
26. Yordy JS, Muise-Helmericks RC. Signal transduction and the Ets family of transcription factors. Oncogene 2000;19(55):6503–6513.

27. Takai N, Miyazaki T, Fujisawa K, Nasu K, Miyakawa I. Expression of c-Ets1 is associated with malignant potential in endometrial carcinoma. Cancer 2000;89(10):2059–2067.
28. Fujimoto J, Aoki I, Toyoki H, Khatun S, Tamaya T. Clinical implications of expression of ETS-1 related to angiogenesis in uterine endometrial cancers. Ann Oncol 2002;13(10):1605–1611.
29. Khatun S, Fujimoto J, Toyoki H, Tamaya T. Clinical implications of expression of ETS-1 in relation to angiogenesis in ovarian cancers. Cancer Sci 2003;94(9):769–773.
30. Kosugi M, Miyajima A, Kikuchi E, Kosaka T, Horiguchi Y, Murai M, Oya M. Angiotensin II type 1 receptor antagonist enhances cis-dichlorodiammineplatinum-induced cytotoxicity in mouse xenograft model of bladder cancer. Urology 2009;73(3):655–660.
31. Nickenig G, Roling J, Strehlow K, Schnabel P, Bohm M. Insulin induces upregulation of vascular AT1 receptor gene expression by posttranscriptional mechanisms. Circulation 1998;98(22): 2453–2460.
32. Xu ZG, Yuan H, Lanting L, Li SL, Wang M, Shanmugam N, Kato M, Adler SG, Reddy MA, Natarajan R. Products of 12/15-lipoxygenase upregulate the angiotensin II receptor. J Am Soc Nephrol 2008;19(3):559–569.
33. Thekkumkara TJ, Thomas WG, Motel TJ, Baker KM. Functional role for the angiotensin II receptor (AT1A) 3′-untranslated region in determining cellular responses to agonist: Evidence for recognition by RNA binding proteins. Biochem J 1998;329(Pt 2): 255–264.

第58回日本泌尿器科学会西日本総会 学術奨励賞

内分泌療法抵抗性前立腺癌におけるDEC療法の局所治療効果

―― Magnetic resonance spectroscopic imaging（MRS）による評価 ――

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MAGNETIC RESONANCE SPECTROSCOPIC IMAGING (MRS) CAN PREDICT LOCAL HISTOLOGICAL RESPONSE TO DOCETAXEL-BASED CHEMOTHERAPY FOR HORMONE-REFRACTORY PROSTATE CANCER

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Conventional MRI hardly illustrates the local treatment efficacy of docetaxcel (DTX)-based chemotherapy for hormone-refractory prostate cancer (HRPC). Three-dimensional proton MR spectroscopic imaging (MRS) is a new tool that provides in vivo biological and metabolic information. We hypothesized that MRS can be substituted for the invasive procedure of systemic sextant prostate biopsy when evaluating the local histological response to DTX-based chemotherapy for HRPC.

A total of 11 HRPC patients treated with DTX-based chemotherapy was employed in this study. Histological response to DTX-based chemotherapy was evaluated by HE staining of systemic sextant prostate biopsy. T2-weighted image (T2WI) on MRI and MRS before and at two, four, eight and twelve months after chemotherapy. T2WI and MRS were performed using pelvic phased-array and/or endorectal coil. After suppression of water and lipids, the ratio of choline to citrate (Cho/Cit) and that of choline to noise (Cho/N) were calculated, and were compared to the histopathological findings in biopsy specimens.

Cho/N levels at pre-therapy and at two, four, eight and twelve months post-therapy were 38.6 ± 15.4, 19.3 ± 16.7, 17.0 ± 41.8, 12.8 ± 19.8 and 13.2 ± 21.2 respectively, showing a stepwise reduction in the Cho/N ratio along with the passage of time after treatment. The Cho/N level was significantly correlated with local histological response to DTX-based chemotherapy analyzed by H and E staining of systemic sextant prostate biopsy. One patient developed local recurrence in spite of DTX-based chemotherapy producing an increase in Cho/N up to 18.2, which preceded PSA elevation. On the other hand, the Cho/Cit level could not be correlated with local histological response to DTX-based chemotherapy, since Cit was not detected at two months after treatment in 10 out of the 11 cases. This finding appeared to indicate the limited use of

the Cho/Cit ratio for predicting local histological response to chemotherapy. The diagnosis-based T2WI was limited by treatment-related changes that included prostatic shrinkage, diffuse low T2-signal intensity in the gland, and an indistinctness of normal zonal anatomy.

MRS, but not T2WI, may be superior to conventional imaging modalities in light of its ability to illustrate local treatment efficacy of DTX-based chemotherapy for HRPC. MRS could be an attractive alternative to the invasive nature of prostate biopsy for predicting local histological response to the treatment.

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key words : hormone refractory prostate cancer, magnetic resonance spectroscopy (MRS), prostate biopsy, docetaxel-based chemotherapy

キーワード：内分泌療法抵抗性前立腺癌，magnetic resonance spectroscopy（MRS），前立腺針生検，DEC療法

緒　　言

欧米における前立腺癌の罹患率は他の固形癌と比較し最も高く，我が国でも前立腺癌の罹患率は顕著に増加している。前立腺癌はPSAスクリーニングの普及に伴い早期に発見される癌の割合が増加し根治可能な癌と認識されるようになった[1,2]。一方，進行期前立腺癌に対しては内分泌療法が主たる治療法となるが，良好な近接効果にも関わらず大多数の症例で再燃を来すことが知られており，内分泌療法に抵抗性を獲得した前立腺癌（hormone-refractory prostate cancer：HRPC）の予後は不良であった[3]。従来よりHRPCは化学療法抵抗性と認識されてきたが，タキサン系抗癌剤にエストラムスチン（EMP）あるいはカルボプラチン（CBDCA）を加えた併用化学療法によりHRPCに対する化学療法は生存期間の延長が期待されるまで発展した[4)~6)]。

前立腺癌に対する治療効果の指標として血液中の前立腺特異抗原（PSA）値の推移は重要で，化学療法施行例でも血清PSA値の変化に基づき治療効果を判断する場合が多い。しかし，血清PSA値の変化から再発巣の同定を行うことは不可能で，また原発巣である前立腺に特化した治療効果を推察することも困難である。一方，内分泌療法あるいは放射線療法後の前立腺の形態・解剖学的変化は，前立腺の萎縮に伴う不鮮明なzonal-anatomyの構成であり経直腸的超音波検査（TRUS）やMRIによる前立腺局所の治療効果判定には細心の注意を払う必要がある[7,8]。それ故，前立腺局所の治療効果を評価する手段としては，前立腺針生検検体による病理組織学的な判断が妥当とされてきた。しかし，前立腺針生検は侵襲的な検査法であり，その病理学的所見のみで原発巣における前立腺癌の活動性を把握することも困難である。

前立腺癌細胞では代謝経路の変化により正常前立腺細胞に豊富に存在するクエン酸（Cit）が低下し，細胞膜の合成と破壊を反映するコリン（Cho）が増加する。この特徴をMRIに応用し，生体内の代謝情報を可視化する技術がmagnetic resonance spectroscopy（MRS）である。MRSの有用性は前立腺癌の局所診断や[9)~13)]，前立腺癌に対する放射線療法あるいは内分泌療法後の局所治療効果判定で注目されている[7,8,14)~16)]。すなわち，内分泌療法や放射線療法施行例の前立腺では，治療直後からChoピークの急激な低下が観察され，治療抵抗性を獲得すれば再度Choピークが上昇することが報告され，Choピークの変化が局所での治療効果を反映する可能性が示唆されている[7,8,14)~16)]。

今回ドセタキセル（DTX），EMPおよびCBDCA併用化学療法（DEC療法）を施行したHRPC症例を対象として，DEC療法後の局所治療効果を評価する上でのMRSの有用性を検証し，その応用性を考察した。

対 象 と 方 法

2004年1月より2006年9月の期間にHRPCと診断され当科でDEC療法を施行した症例のうち，DEC療法前，治療後2，4，8，12カ月にMRSによる前立腺局所の評価が可能であった11例を対象とした（**Table 1**）。年齢は中央値72歳（62-85歳），DEC療法前血清PSA値は中央値78.1 ng/ml（19.7-1592 ng/ml）であった。DEC療法前の画像検査で8例にリンパ節転移を，10例に骨転移に認めた。DEC療法前の前立腺針生検による病理組織学的治療効果はgrade 0a：4例，grade 0b：3例，grade 1：3例およびgrade 3a：1例であった（**Table 1**）。なお今回の対象例は全例ともDEC療法を遂行可能であった（平均10サイクル）。

MRSは1.5 Tesla Signa scanner（GE Medical Systems, Milwaukee, MI, USA）を用いて，phased-array coilとendorectal coilによりpoint-resolved spectroscopy（PRESS）technique を用いて[17)~20)]，sagittalの

T2 強調 fast spin-echo localization image により endorectal coil の位置が適切であることを確認した後，前立腺の基部から尖部までの領域を 3 mm 間隔で各スライス 256 × 192 matrics で撮像した。

前立腺針生検部位に一致する voxel の位置を決定後，MRS で得られた各 voxel の spectrum を NIH-image analyzer program を用いて Cho，Cit および Noise (N) のピーク値を計測し，各 voxel における Cho/Cit 比と Cho/N 比を算出した。今回の検討では Cho/Cit 比あるいは Cho/N 比が 5 以下の場合は metabolic atrophy (MA) として取り扱った。

なお，前立腺針生検は経直腸的に超音波ガイド下で施行し，通常の系統的 6 カ所生検に該当する部位より検体の採取を行った。得られた前立腺組織の HE 染色標本は前立腺癌取扱い規約（第 3 版）の前立腺癌組織学的治療効果判定基準に基づき評価した。

結　　果

T3aN1M0 症例における前立腺局所での MRI，MRS および病理学的所見と血清 PSA 値の各々の経時的推移を **Fig. 1** に示した。本症例は，DEC 療法前の病理組織学的治療効果は grade 0 で，MRS では Cit ピークと比較し高い Cho ピークを示した。治療開始後 2 カ月の組織学的治療効果は grade 2 で治療効果は認めるものの viable な癌細胞群が認められた。一方，MRS では Cho，Cit ともピークを同定できず MA と判断した。治療開始後 4 カ月の組織学的治療効果は grade 3b で，MRS でも MA であった。しかし，8 カ月後には PSA は再上昇し，画像検査では傍大動脈リンパ節の腫脹が認められ DEC 療法抵抗性 HRPC と診断した。この時点の前立腺局所での組織学的治療効果は grade 3a で viable な癌細胞は観察されなかったが，MRS では既に Cho ピークが再上昇しており，転移巣のみならず前立腺局所でも癌が再燃したものと考えられた。本症例の MRI-T2 強調像では前立腺内部は一貫して不均一な低信号を呈しており，経時的な信号強度の変化は認められなかった。

Cit ピークの変化は 11 例中 10 例で DEC 療法開始後 2 カ月，残り 1 例は治療開始後 4 カ月で検出不能となったが，Cho ピークは MA に至るまでの治療期間で 11 例全例で同定可能であった。従来の報告では Cho/Cit 比を治療効果の指標としているが，今回の DEC 療法を対象とした検討では Cho/N 比を用いて前立腺局所の治療効果を評価した。Cho/N 比は DEC 療法前，開始後 2，4，8 および 12 カ月で各々 38.6 ± 15.4，19.3 ± 16.7，17.0 ± 41.8，12.8 ± 19.8 および 13.2 ± 21.2 であった。DEC 療法前の組織学的治療効果が grade 3a の症例においても Cho ピークは検出され，治療開始後は経時的に Cho ピークが低下し，Cho/N 比も治療開始後の時間経過に依存して低下を認めた（**Fig. 2**）。前立腺局所での組織学的治療効果が高くなるに従い Cho/N 比は低下し，grade 0a，0b，1，2，3a および 3b では各々 51.3 ± 8.9，50.4 ± 16.7，28.4 ± 14.9，19.6 ± 13.0，13.8 ± 15.1 および 7.3 ± 13.6 であった。今回の検討症例では，前立腺原発巣での経時的な Cho/N 比の変化は転移巣を含めた病勢を反映する血清 PSA 値の経時的な変化と相関し，DEC 療法開始後血清 PSA 値が低下した後，DEC 抵抗性に至り血清 PSA 値が再上昇した症例では，血清 PSA 値の変動と並行して治療初期に低下した Cho/N 比が再燃時には再上昇していた（**Fig. 3**）。

11 例全例で MRI-T2 強調像による評価も試みたが，今回対象とした HRPC 例では DEC 療法開始前より前立腺内部は不均一な低信号を呈しており，また正常の zonal anatomy が消失していることから前立腺局所の治療効果を評価することは困難であった。

考　　察

治療開始後の前立腺癌では治療に伴い形態学的に大きな変化が生じ，解剖学的な情報に依存する TRUS や MRI を用いた質的な診断，すなわち治療効果を評価することには限界がある。一方，治療に伴う前立腺組織での代謝性変化を評価する MRS では，TRUS や MRI が有する前述の欠点を補うことが期待され，治療開始後の前立

Table I　Patients background

年齢(歳)	PSA(ng/ml)	組織学的治療効果(Grade)	臨床病期
			T3aN1M0　1 例
		0a 4 例	T3aN0M1b　1 例
62-85	19.7-1592.0	0b 3 例	T3aN1M1b　3 例
中央値 72	中央値 78.1	1 3 例	T3bN0M1b　1 例
		3a 1 例	T4N0M1c　1 例
			T4N1M1b　4 例

腺局所における治療効果を経時的な変化として観察することが可能で，質的診断を行う上で有用な撮像法といえる[7][8][14]~[16]。今回，われわれはDEC療法を施行したHRPC症例の前立腺での代謝性変化を経時的にMRSで評価し，同時に施行した前立腺針生検の病理学的所見と比較検討することで，MRSによる前立腺局所での治療効果，すなわちMRSの質的診断能を検証した。

今回の検討では，MRI-T2強調画像による経時的な前立腺原発巣の変化もMRSに併せて評価したが，全例で治療前より一貫して前立腺内部が不均一な低信号を呈し，加えて正常のzonal anatomyの消失により，MRI-T2強調画像による前立腺の質的診断はきわめて困難であった。一方，MRSでは，DEC療法開始後2カ月以上経過すると全例でChoやCitのピークの低下に代表されるspectrumの変化が生じ，前立腺局所でのDEC療法に伴う代謝性変化の検出が可能であった。一般に内分泌療法や放射線治療に伴う前立腺の代謝性変化は，MRSではCho/Cit比を指標として判断される場合が多いが[7][8][14]~[16]，今回のHRPCに対する化学療法施行症例を対象とした検討ではDEC療法開始後早期よりCitのピークが検出不能となり，Cho/Cit比に基づく前立腺局所の治療効果判定では限界があるものと思われた。特にMAの場合Cho，Citともにピークの検出が困難であるため，今回はCho/N比を用いた前立腺局所で

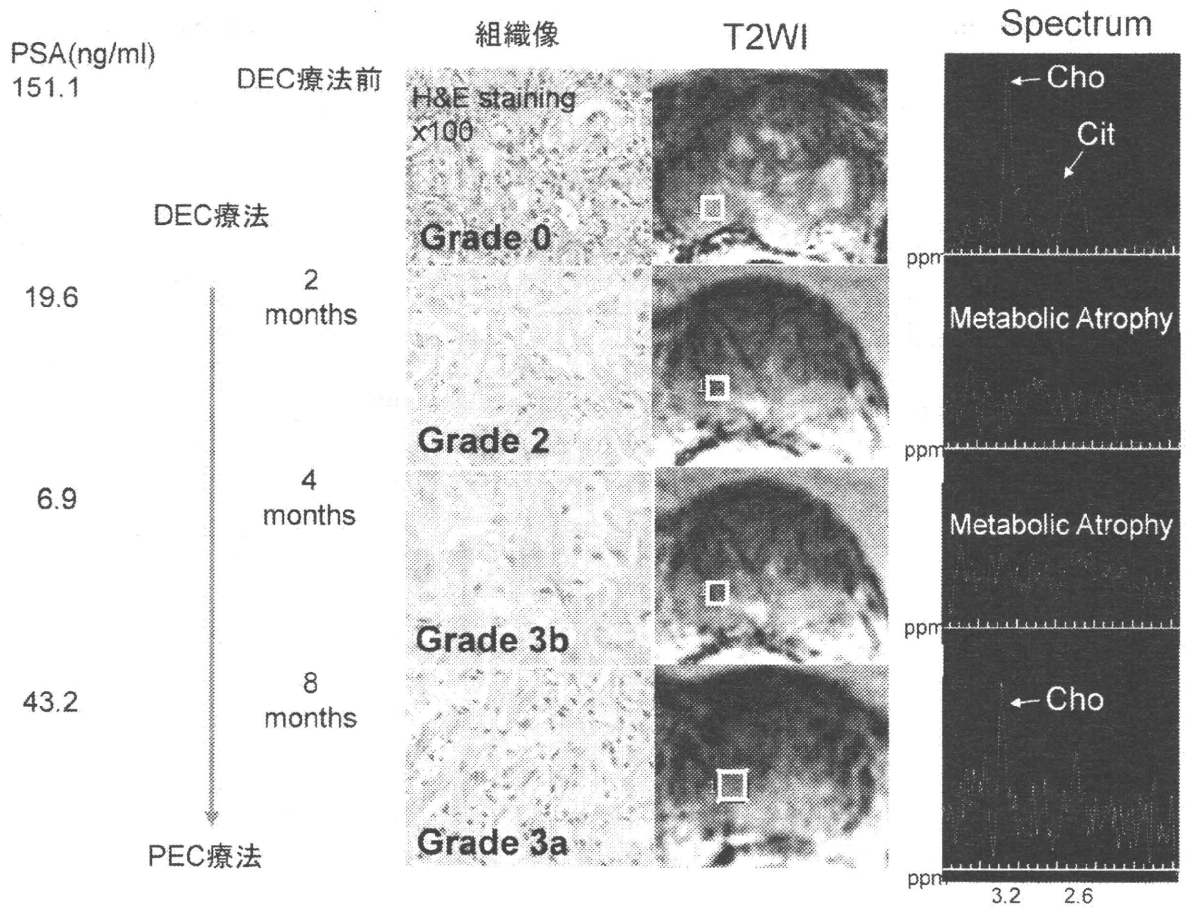

Fig. 1 Alterations of serum PSA value, pathology of the primary site and findings of MRI and MRS during DTX-based chemotherapy are shown in a representative case. Serum level of PSA gradually decreased along with favorable pathological effect after induction of DTX-based chemotherapy. Spectrum of MRS showed rapid reduction of Cho. and Cit. peaks after DTX-based chemotherapy. At 2 months after DTX-based chemotherapy, Cho. and Cit. peaks had already reached an undetectable level, while the pathological effect at the primary site was found to be grade 2. At 4 months, Cho. peak still remained undetectable with the pathological effect of grade 3. At 8 months, serum level of PSA value had increased again up to 43.2 ng/ml, and enlarged lymph nodes were detected on abdominal CT imaging. At this time, the pathological effect at the primary site still remained at grade 3, however, the Cho. peak was found to be detectable on the spectrum again. Prostate cancer foci were hardly depicted persistently with MRI owing to the diffuse low-intensity of the T2 signal and poorly oriented zonal anatomy caused by hormonal manipulation.

の治療効果判定を試みた。Fig. 2 に示すように Cho/N 比は前立腺針生検検体による病理学的所見に基づく局所治療効果と強く相関しており，局所の治療効果が高くなるに従い Cho/N 比は低下し，grade 3b の部位では MA に至る症例もみられた。また，Fig. 3 の如く Cho/N 比の変化は経時的な血清 PSA 値の変化とも強く関連し，転移巣を含めた総合的な前立腺癌の活動性を反映する血清 PSA 値と MRS による前立腺原発巣での癌の活動性

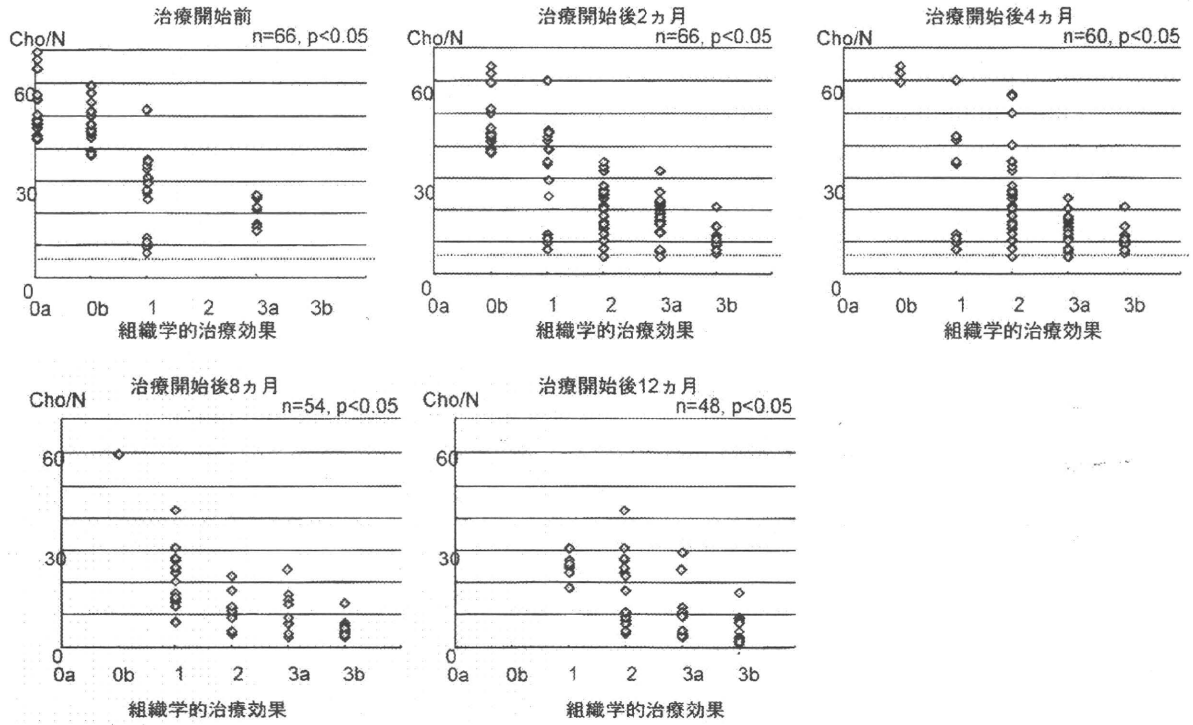

Fig. 2 Relationship of the time-course after DTX-based chemotherapy with pathological effect and Cho/N ratio is shown. Irrespective of time after chemotherapy, the pathological effect was well correlated with the Cho/N ratio.

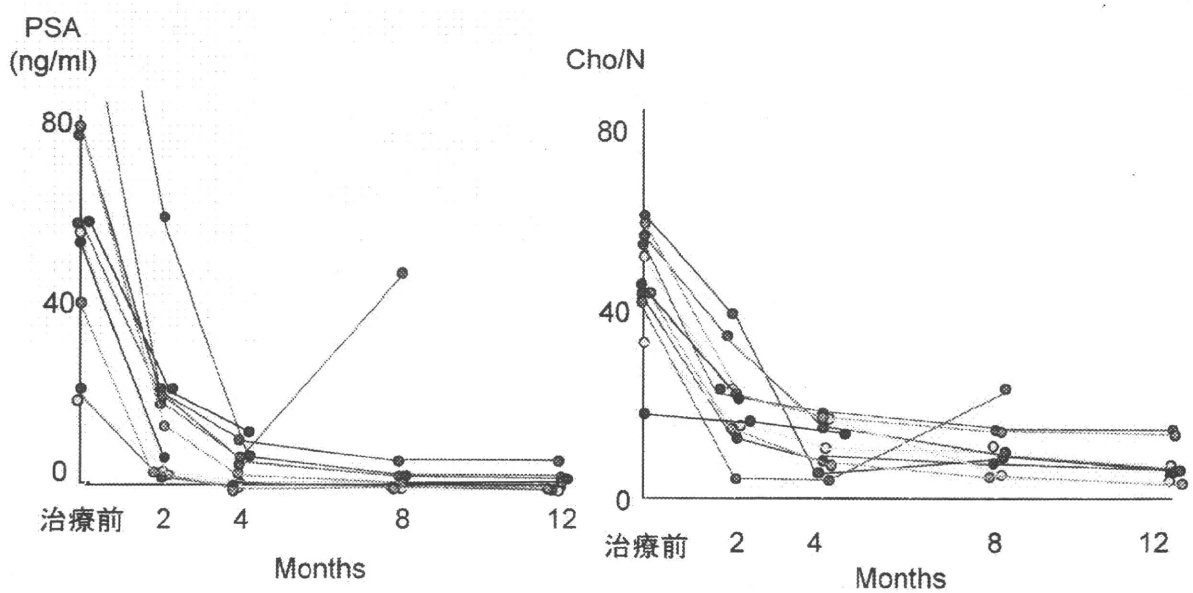

Fig. 3 The time-dependent changes of serum PSA level and Cho/N ratio after DTX-based chemotherapy are shown for each patient. The time-dependent alteration of serum PSA level was closely related to that of the Cho/N ratio.

に関する情報を併用することで，HRPCに対する新たな治療戦略を構築することが可能と思われる。

前立腺針生検の病理学的所見に基づく前立腺局所の治療効果の判定は，前立腺癌のheterogenityあるいは針生検によるsampling errorなどの不確実性に基づく負の要因により必ずしも充分とは断言できず，これが生検検体を用いた評価法の限界と思われる。一方，放射線療法後の再燃予測因子として，前立腺針生検標本でのviableな腫瘍細胞の残存が重要視されており[21]，MRSで陽性であるにも関わらず病理学的には陰性の場合には，viableな癌細胞の残存を疑うべきで，今回の検討でもDEC療法開始後12カ月の時点でgrade 3bと評価された検体中にもCho/N比がMAに到達しない例が認められた。一方で，Fig. 1の症例の如くDEC療法開始後2カ月で組織学的治療効果grade 2の部位で既にCho/N比はMAに至っており，これら事実は病理組織学的変化に先行してMRSが代謝性変化を捉えた可能性があり興味深い。すなわち，MRSで得られる代謝情報を前立腺癌の治療効果判定に応用することで前立腺針生検の欠点を克服することが可能と考えられる。

限局性あるいは進行期前立腺癌に対する治療後の血清PSA値は，前立腺癌の活動性を反映して変動するが，それのみでは活動性病変の局在を同定することは不可能である。MRSによるCho/N比と血清PSA値の両者を比較することで，前立腺原発巣における癌の活動状態を把握できると考えられ，1）Cho/N比が前立腺局所の治療効果に特異的なマーカーとなりうる可能性，2）侵襲的診断手技である前立腺針生検を回避しうるMRSによるvirtual-biopsyの可能性，3）MRSの解析結果に基づいた前立腺癌の高活動性領域に焦点を合わせた限局的な放射線治療（focal therapy）の可能性などMRSは将来性のきわめて高い診断技術として期待される。

結　　語

1）HRPCに対する化学療法後の前立腺局所の治療効果をMRSで評価する際の基本的な情報が得られた。

2）DEC療法の治療効果の判定には従来のMRIよりもMRSが有用であり，特にMRSによるCho/N比を評価することで局所再燃の有無をより早期に推察することが可能と考えられた。

3）Virtual-biopsyやfocal therapyなどMRS技術を応用した新しい診断的あるいは治療的戦略の開発が期待される。

文　　献

1) Johansson, J. E. et al.: Fifteen-year survival in prostate cancer. A prospective, population-based study in Sweden. JAMA. **277**: 467-471, 1997.

2) Pound, C. R. et al.: Natural history of progression after PSA elevation following radical prostatectomy. JAMA. **281**: 1591-1597, 1999.

3) Oefelein, M. G. et al.: Survival of patients with hormone refractory prostate cancer in the prostate specific antigen era. J. Urol. **171**: 1525-1528, 2004.

4) Petrylak, D. P. et al.: Decetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N. Engl. J. Med. **351**: 1513-1520, 2004.

5) Urakami, S. et al.: Docetaxel-based chemotherapy as second-line treatment for paclitaxel-based chemotherapy-resistant hormone-refractory prostate cancer: a pilot study. Urology **65**: 543-548, 2005.

6) Urakami, S. et al.: Combination chemotherapy with paclitaxel, estramustine and carboplatin for hormone refractory prostate cancer. J. Urol. **168**: 2444-2450, 2002.

7) Coakley, F. V. et al.: Endorectal MR imaging and MR spectroscopic imaging for locally recurrent prostate cancer after external beam radiation therapy: preliminary experience. Radiology **233**: 441-448. 2004.

8) Mueller-Lisse, U. G. et al.: Localized prostate cancer: effect of hormone deprivation therapy measured by using combined three-dimensional 1H MR spectroscopy and MR imaging: clinicopathologic case-controlled study. Radiology **221**: 380-390. 2001.

9) Scheilder, J. et al.: Prostate cancer: localization with three-dimensional proton MR spectroscopic imaging-clinicopathologic study. Radiology **213**: 473-480. 1999.

10) Kaji, Y. et al.: Localizing prostate cancer in the presence of postbiopsy changes on MR images: role of proton MR spectroscopic imaging. Radiology **206**: 785-790, 1998.

11) Kurhanewicz, J. et al.: Three-dimensional proton MR spectroscopic imaging of the in situ human

prostate with high (0.24-0.7 cm^3) spatial resolution. Radiology **198**: 795-805, 1996.

12) Kurhanewicz, J. et al.: Citrate as an in vivo marker to discriminate prostate cancer from benign prostatic hyperplasia and normal prostate peripheral zone: detection via localized proton spectroscopy. Urology **45**: 459-466, 1995.

13) Kristen, L. Z. et al.: Transition zone prostate cancer: metabolic characteristics at ^1H MR spectroscopic imaging-initial results. Radiology **229**: 241-247, 2003.

14) Mueller-Lisse, U. G. et al.: Magnetic resonance spectroscopy in patients with locally confined prostate cancer: association of prostatic citrate and metabolic atrophy with time on hormone deprivation therapy, PSA level, and biopsy Gleason score. Eur. Radiol. **17**: 371-378, 2007.

15) Mueller-Lisse, U. G. et al.: Time-dependent effects of hormone-deprivation therapy on prostate metabolism as detected by combined magnetic resonance imaging and 3D magnetic resonance spectroscopic imaging. Magn. Reson. Med. **46**: 49-57, 2001.

16) Pickett, B. et al.: Time to metabolic atrophy after permanent prostate seed implantation based on magnetic resonance spectroscopic imaging. Int. J. Radiat. Oncol. Biol. Phys. **59**: 665-673, 2004.

17) Star-Lack, J. et al.: Improved water and lipid suppression for 3D PRESS CSI using RF band selective inversion with gradient dephasing (BASING). Magn. Reson. Med. **38**: 311-321, 1997.

18) Vigneron, D. B. et al.: An analytical correction of MR images obtained with endorectal or surface coils. J. Magn. Reson. Imaging **3**: 142-145, 1993.

19) Brown, T. R, et al.: NMR chemical shift imaging in three dimensions. Proc. Natl. Acad. Sci. U. S. A. **79**: 3523-3526, 1982.

20) Moyher, S. E. et al.: Surface coil MR imaging of human brain with an analytic reception profile correction. J. Magn. Reson. Imaging **5**: 139-144, 1995.

21) Crook, J. et al.: Postradiotherapy prostate biopsy: what do they really mean? Results for 498 patients. Int. J. Radiat. Oncol. Biol. Phys. **48**: 355-367, 2000.

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Effect of Delayed Maximal Androgen Blockade Therapy for Patients with Advanced Prostate Cancer Who Fail to Respond to Initial Androgen Deprivation Monotherapy

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Objectives: We analyzed the efficacy of additional antiandrogens as second- and third-line treatments after the failure of initial androgen deprivation monotherapy.
Methods: This retrospective study included 53 patients with advanced prostate cancer initially treated with androgen deprivation monotherapy alone. An antiandrogen was added to androgen deprivation monotherapy as the second-line treatment after the failure of the initial androgen deprivation monotherapy. Another antiandrogen, estrogen or steroid was given as the third-line treatment after the second-line treatment failed.
Results: The initial androgen deprivation monotherapy was effective in all 53 patients for a median of 9.6 months. Thirty-three (62.3%) patients showed a prostate-specific antigen response to the second-line treatment for a median of 10.7 months. Of the 46 patients who received the third-line treatment, 16 (34.8%) showed a prostate-specific antigen response for a median of 6.0 months. Patients who responded to the second-line treatment had a significantly higher cancer-specific survival rate than those without a response. In multivariate analysis, a nadir prostate-specific antigen of 4.0 ng/ml or greater during androgen deprivation monotherapy and prostate-specific antigen doubling time of less than 10 months after androgen deprivation monotherapy failure were independent risk factors for prostate cancer death after androgen deprivation monotherapy failure, with hazards ratios of 5.59 and 8.00, respectively. The 5-year cancer-specific survival rates were 100%, 65.0% and 15.5% in patients with 0, 1 and 2 risk factors, respectively ($P = 0.047$).
Conclusions: In this study, the second- and third-line treatments were effective for patients with advanced prostate cancer. Nadir prostate-specific antigen during androgen deprivation monotherapy and prostate-specific antigen doubling time just after the failure of androgen deprivation monotherapy are factors that can predict the prognosis.

Key words: prostate cancer – delayed maximal androgen blockade therapy – androgen deprivation monotherapy – PSA doubling time – PSA nadir

INTRODUCTION

Androgen deprivation therapy (ADT) is the preferred treatment for advanced prostate cancer. After ADT by medical or surgical castration, the majority of patients initially show some evidence of a clinical response (1). However, the disease becomes refractory to hormone treatment in most patients. In those patients, a rising prostate-specific antigen (PSA) level is first observed, followed by progression of clinical symptoms (2).

Androgen deprivation monotherapy (ADMT) consisting of medical or surgical castration alone, or maximal androgen blockade (MAB) consisting of ADMT plus an antiandrogen,

is used to manage patients with advanced prostate cancer as the initial treatment. It is controversial whether MAB should be given initially because of the small survival benefit, adverse effects and cost (3–5). Moreover, the treatment strategy after the failure of the initial ADT is not fully established and there is no consensus about the time when the second-line treatment should be initiated, or which treatment is preferable among alternative antiandrogens, estrogens, steroids and docetaxel-based chemotherapy.

In this study, we analyzed the clinical courses of patients after the failure of the initial ADMT and evaluated the efficacy of additional antiandrogens as the second- and third-line treatments.

SUBJECTS AND METHODS

Patients

We retrospectively analyzed a total of 53 patients with advanced prostate cancer who were diagnosed at Sapporo Medical University Hospital and Sunagawa City Medical Center from 1994 to 2007. Clinical stage was evaluated according to the 1997 TNM classification. T stage was determined by digital rectal examination, computed tomography (CT) and/or transrectal ultrasonography. Pelvic lymph node metastasis was assessed by CT. Bone metastasis was assessed by bone x-ray and radioisotopic bone imaging using 99mTc-methylene-diphosphonate.

The mean age was 74 years (range: 50–83) and serum PSA ranged from 3.7 to 8190 ng/ml at the initial treatment. Gleason score of biopsy were 6 or less in 3 patients, 7 in 13 patients and 8–10 in 37 patients. Clinical stages were T3-4N0M0 in 12, cTanyN1M0 in 9 and TanyNanyM1 in 32 patients. The overall follow-up period from the start of treatment for prostate cancer to the last visit ranged from 8.2 to 147.9 months with a median of 59.1. The initial ADMT was effective in all 53 patients and the median time from the start of ADMT to its failure was 9.6 months (0.8–57.3). The median nadir PSA during the initial ADMT was 2.1 ng/ml (0.0–1230) and the median PSA at the failure of the initial ADMT was 3.1 ng/ml (0.2–1590). Failure was defined as described below.

Treatments

Patients were all initially treated with ADMT by medical or surgical castration alone. Medical castration was achieved using a luteinizing hormone-releasing hormone agonist. Once ADMT failed, the second-line treatment with ADM plus an antiandrogen was initiated. If the second-line treatment failed, the antiandrogen was terminated and antiandrogen withdrawal syndrome (AWS) was observed. When AWS was not found in the subsequent clinical course, another antiandrogen, estrogen or steroid with ADMT was given as the third-line treatment. Each drug used in the second- or third-line treatment was decided based on the preference of the physician.

Evaluation of Response and Definitions of Terms

Changes in the post-therapy PSA level were defined according to the recommendations of the PSA Working Group (6). A 'PSA response' was defined as a 50% or greater decline in the pretreatment PSA level after therapy, and the treatment was evaluated to be effective. A less than 50% decline in the PSA level or a rising PSA level during the treatment was defined as 'no PSA response'. When the PSA failure defined below was found during the treatment, the ongoing treatment was evaluated as having no efficacy. PSA failure was essentially defined as three consecutive increases in the PSA level. The time of PSA failure was defined as the first of the three consecutive increases in the PSA level. The PSA doubling time (PSA-DT) was calculated using the following formula: $PSADT = [\log(2) \times t]/[\log(\text{final PSA}) - \log(\text{initial PSA})]$, where 'log' was the natural logarithm function and 't' was the time from the initial level to the final PSA level (7). To calculate the PSA-DT after the initial ADM failed, the initial PSA was defined as the PSA level at the time of failure of the initial ADMT, and the final PSA level was defined as the PSA value at the time of starting the second-line treatment. Positive AWS was defined as a 50% or greater decrease in the PSA level compared with that at the time when the antiandrogen was discontinued. Cause-specific survival time from the failure of the initial ADMT to death from prostate cancer was calculated.

Statistical Analysis

For the statistical analysis of data, the Kaplan–Meier method, log-rank test and Cox proportional hazards analysis were applied using StatView (SAS Institute, Cary, NC, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

Bicalutamide 80 mg/day was used in 31 patients followed by flutamide 375 mg/day in 18 and chlormadinone acetate (CMA) 100 mg/day in 4. Of the 53 patients, 33 (62.3%) had a PSA response to the second-line treatment. The response was found in 67.7% of patients with bicalutamide treatment and 66.7 of those with flutamide. No patients responded to CMA treatment. The median PSA failure-free survival in the 33 patients having a PSA response was 10.7 months. Of these 33 patients, 5 remained free of any increase in the PSA level at the end of follow-up and 28 showed PSA failure of the second-line treatment. No significant difference was observed in cancer-specific survival after the failure of ADMT between patients with bicalutamide and those using flutamide ($P = 0.053$).

AWS was evaluated in 30 of the 48 patients for whom the second-line treatment finally had no efficacy or who had PSA failure. Only two (6.7%) had PSA decreases of 50% or more, and in three (10.0%) PSA decreased less than 50%.

Of the 48 patients, 46 received third-line treatment (Table 1). They consisted of 27 of the 28 patients who had

PSA failure after the second-line treatment and 19 of the 20 who failed to respond to the treatment. Of the 46 patients, 16 (34.8%) had a PSA response to the treatment. The median PSA failure-free survival for the 16 patients was 6.0 months. Although the estrogen group had a significantly higher response rate than the other antiandrogen group (55.0% vs. 16.0%, $P = 0.026$), there was no difference in cancer-specific survival after failure of the second-line treatment between the two groups ($P = 0.892$). There was no significant difference in the response rates to the third-line treatment between patients with bicalutamide as the second followed by flutamide as the third-line treatment (8.3%), and those with flutamide as the second followed by bicalutamide as the third-line treatment (11.1%, $P > 0.99$).

There were 30 patients (56.6%) who died of prostate cancer. Of the 30, for 22 patients there was no efficacy and for 8 PSA failure in the third-line treatment. No patients received chemotherapy such as docetaxel in this series.

For the 53 patients who received the second- and/or third-line treatments, the 5-year survival rate from the initiation of the second-line treatment was 60.3% and the median follow-up period from the failure to the end of follow-up was 58.8 months (7.8–147.9). Cancer-specific survival in patients who responded to the second-line treatment was significantly higher than for those who did not (Fig. 1).

The risk factors for prostate cancer death after ADMT failure were analyzed. In univariate analysis, a nadir PSA of 4.0 ng/ml or greater during ADMT, duration of ADMT response shorter than 12 months and PSA-DT of less than 10 months after ADM failure were significant risk factors for death (Table 2). In multivariate analysis, a nadir PSA of 4.0 ng/ml or greater during ADMT and PSA-DT of less than 10 months after ADMT failure were independent risk factors for prostate cancer death after ADMT failure, with hazards ratios of 4.41 and 8.00, respectively. Using these risk factors, we determined how they affected the clinical courses of patients with failure of the initial ADMT. The 53 patients were divided into three groups according to the numbers of these two risk factors (Fig. 2). The 5-year cancer-specific survival rates were 100% in patients with no risk factors, 65.0% in those with one risk factor and 15.5% in those with two risk factors ($P = 0.047$). This risk classification predicted the outcomes of the second-line treatment since the response rates to the second-line treatment were 72.7%, 63.0% and 50.0%, and the median durations of responses were 10.7, 4.1 and 1.9 months in the three groups, respectively.

Table 1. PSA responses to the third-line treatment in 46 patients

Second-line	Third-line	Number of patients	Response	Response rate (%)
Bicalutamide	Flutamide	12	1	8.3
	EMP	8	1	12.5
	DES-P	3	2	66.7
	CMA	1	1	100
	DXM	1	1	100
Flutamide	Bicalutamide	9	1	11.1
	EMP	5	4	80.0
	DES-P	3	3	100
CMA	Bicalutamide	3	1	33.3
	DES-P	1	1	100
Total		46	16	34.8

EMP, estramustine phosphate. DES-P, diethylstilbestrol diphosphate. CMA, chlormadinone acetate; DXM, dexamethasone; PSA, prostate-specific antigen.

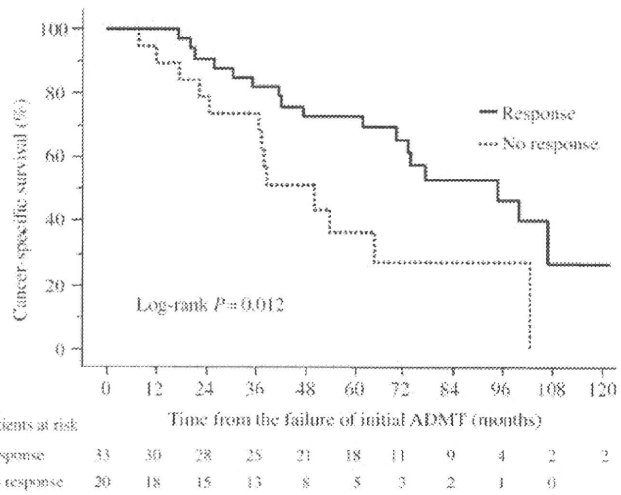

Figure 1. Cancer-specific survival after failure of androgen deprivation monotherapy (ADMT) in patients who responded to the second-line treatment and those who did not.

DISCUSSION

We retrospectively evaluated the effectiveness of the second- and third-line treatments after the failure of the initial ADMT for patients with clinical T3 or more advanced disease. Second-line treatment was effective in 62.3% of patients for a median of 10.3 months. Although there are few reports on the add-on effects of antiandrogens for patients who are initially treated with ADMT, Fujii similarly reported that the second-line treatment using bicalutamide was effective in 65.9% of 44 patients for a median of 9.2 months (8). In another report, which included patients initially treated with not only ADMT but also MAB, add-on or replacement antiandrogen treatment was effective in 22–51% of patients for a median of 6.0–8.6 months (9–11). Thus, adding antiandrogens after the failure of the initial hormonal therapy is thought to be a beneficial treatment option after both ADMT and MAB. In addition, our results suggest that there are few differences between the results obtained with different antiandrogens, as Okihara (10) and Suzuki (11) reported previously.

Table 2. Univariate and multivariate Cox proportional hazards analyses for predicting cancer-specific death after failure of androgen deprivation monotherapy

	Category	*P*-value		HR	95% CI
		Univariate	Multivariate		
Gleason score	≥8 vs. <8	0.28	0.41	1.41	0.62–3.23
PSA at ADMT started (ng/ml)	≥100 vs. <100	0.08			
Clinical M stage	M1 vs. M0	0.14	0.89	1.06	0.47–3.23
Nadir PSA during ADMT (ng/ml)	≥4 vs. <4	0.002	<0.001	4.41	2.04–9.52
Duration of ADMT response (months)	<12 vs. ≥12	0.01			
PSA-DT after failure of ADMT (months)	<10 vs. ≥10	<0.001	<0.001	8.00	2.47–25.74

HR, hazard ratio; CI, confidence interval; ADMT, androgen deprivation monotherapy; PSA-DT, PSA doubling time.

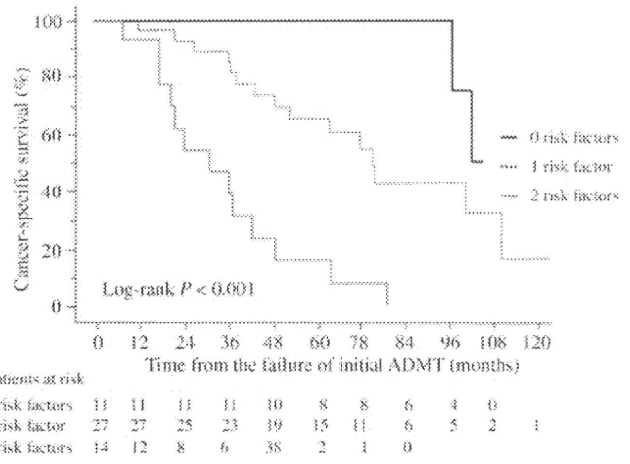

Figure 2. Cancer-specific survival after failure of ADMT in patients divided into three groups according to the number of risk factors.

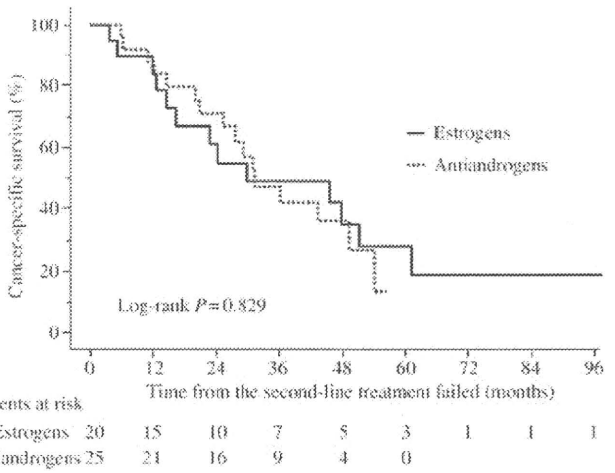

Figure 3. Cancer-specific survival after failure of the second-line treatment in patients who used estrogens and antiandrogens for the third-line treatment.

The response rate to the third-line treatment was only 34.8% in this study. Although estrogens showed the highest response rate, the duration of the response was relatively short. Since cancer-specific survival after the failure of second-line treatment was not significantly different between estrogens and antiandrogens, estrogens were not superior to antiandrogens (Fig. 3).

According to the combination of nadir PSA during ADMT and PSA-DT after the failure of ADMT, we can estimate the cancer-specific survival after the failure of the initial ADMT. These parameters were also predictors for the outcome of the second-line treatment. Thus, parameters obtained before and just after the failure of ADMT contribute to prediction of the outcome of subsequent treatment and the estimation of survival.

Recent reports have shown that docetaxel contributes to the improvement of the overall survival for patients with hormone-refractory prostate cancer (12,13). However, there is no consensus about the timing of the initiation for this chemotherapy. The most important thing to consider when treating advanced prostate cancer is minimizing adverse events due to treatment and maintaining quality of life (QOL). Thus, we should conduct well-designed prospective clinical trials to determine whether the early introduction of this chemotherapy contributes to improvement in patients with hormone-refractory disease.

In this study, we focused on patients who were initially treated with ADMT. There is controversy as to whether patients with advanced prostate cancer should be initially treated with ADMT or MAB. Recent meta-analyses have shown the superiority of initial MAB to ADMT for 5-year cancer-specific survival (3,4,14). In phase 3 trials conducted in Japan, MAB using bicalutamide was superior to ADMT in terms of not only overall survival but also the patients' QOL, as pain and voiding dysfunction especially were improved earlier with MAB (15,16). Although we could not compare the effectiveness of initial MAB and deferred MAB in this study, initial MAB may be indicated for patients with advanced prostate cancer who have the symptoms of locally extended disease and/or metastasis.

CONCLUSIONS

In this study, the response rates to the second- and third-line treatments were 62.3% and 34.8%, respectively. Nadir PSA of 4.0 ng/ml or greater during ADMT and PSA-DT of less than 10 months after the failure of ADMT are factors to predict cancer prognosis after the failure of initial ADM.

Conflict of interest statement

None declared.

References

1. Nelson JB. Hormone therapy for prostate cancer. In: Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, editors. *Campbell-Walsh Urology*, Vol. 3, 9th edn. Philadelphia: Academic Press, 2007;3082–3100.
2. Newling DW, Denis L, Vermeylen K. Orchiectomy versus goserelin and flutamide in the treatment of newly diagnosed metastatic prostate cancer. Analysis of the criteria of evaluation used in the European Organization for Research and Treatment of Cancer-Genitourinary Group Study 30853. *Cancer* 1993;72:3793–8.
3. Samson DJ, Seidenfeld J, Schmitt B, Hasselblad V, Albertsen PC, Bennett CL, et al. Systematic review and meta-analysis of monotherapy compared with combined androgen blockade for patients with advanced prostate carcinoma. *Cancer* 2002;95:361–76.
4. Klotz L, Schellhammer P, Carroll K. A re-assessment of the role of combined androgen blockade for advanced prostate cancer. *BJU Int* 2004;93:1177–82.
5. Lukka H, Waldron T, Klotz L, Winquist E, Trachtenberg J. Maximal androgen blockade for the treatment of metastatic prostate cancer-a systematic review. *Curr Oncol* 2006;13:81–93.
6. Bubley GJ, Carducci M, Dahut W, Dawson N, Daliani D, Eisenberger M, et al. Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group. *J Clin Oncol* 1999;17:3461–7.
7. Shulman MJ, Karam JA, Benaim EA. Prostate-specific antigen doubling time predicts response to deferred antiandrogen therapy in men with androgen-independent prostate cancer. *Urology* 2004;63:732–6.
8. Fujii Y, Kawakami S, Masuda H, Kobayashi T, Hyochi N, Kageyama Y, et al. Deferred combined androgen blockade therapy using bicalutamide in patients with hormone-refractory prostate cancer during androgen deprivation monotherapy. *BJU Int* 2006;97:1184–9.
9. Miyake H, Hara I, Eto H. Clinical outcome of maximum androgen blockade using flutamide as second-line hormonal therapy for hormone-refractory prostate cancer. *BJU Int* 2005;96:791–5.
10. Okihara K, Ukimura O, Kanemitsu N, Mizutani Y, Kawauchi A, Miki T. Clinical efficacy of alternative antiandrogen therapy in Japanese men with relapsed prostate cancer after first-line hormonal therapy. *Int J Urol* 2007;14:128–32.
11. Suzuki H, Okihara K, Miyake H, Fujisawa M, Miyoshi S, Matsumoto T, et al. Alternative nonsteroidal antiandrogen therapy for advanced prostate cancer that relapsed after initial maximum androgen blockade. *J Urol* 2008;180:921–7.
12. Petrylak DP, Tangen CM, Hussain MH, Lara PN, Jr, Jones JA, Taplin ME, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* 2004;351:1513–20.
13. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004;351:1502–12.
14. Schmitt B, Wilt TJ, Schellhammer PF, DeMasi V, Sartor O, Crawford ED, et al. Combined androgen blockade with nonsteroidal antiandrogens for advanced prostate cancer: a systematic review. *Urology* 2001;57:727–32.
15. Akaza H, Hinotsu S, Usami M, Arai Y, Kanetake H, Naito S, et al. Combined androgen blockade with bicalutamide for advanced prostate cancer: long-term follow-up of a phase 3, double-blind, randomized study for survival. *Cancer* 2009;115:3437–45.
16. Arai Y, Akaza H, Deguchi T, Fujisawa M, Hayashi M, Hirao Y, et al. Evaluation of quality of life in patients with previously untreated advanced prostate cancer receiving maximum androgen blockade therapy or LHRHa monotherapy: a multicenter, randomized, double-blind, comparative study. *J Cancer Res Clin Oncol* 2008;134:1385–96.

The Prostate 70:1127–1133 (2010)

Combined Inhibitory Effects of Soy Isoflavones and Curcumin on the Production of Prostate-Specific Antigen

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BACKGROUND. Sustained chronic inflammation in the prostate promotes prostate carcinogenesis. Since an elevated level of prostate-specific antigen (PSA) per se reflects the presence of inflammation in the prostate, intervention to improve the PSA value might potentially have beneficial effects for the prevention of the development of prostate cancer. Isoflavones and curcumin have anti-inflammatory and anti-oxidant properties. We examined the biological effects of soy isoflavones and curcumin on LNCaP cells. After that, we conducted a clinical trial for men who received prostate biopsies, but were not found to have prostate cancer, to evaluate the effects of soy isoflavones and curcumin on serum PSA levels.

METHODS. The expression of androgen receptor and PSA were examined in LNCaP cells before and after treatment of isoflavones and/or curcumin. Eighty-five participants were randomized to take a supplement containing isoflavones and curcumin or placebo daily in a double-blind study. Subjects were subdivided by the cut-off of their baseline PSA value at 10 μg/ml. We evaluated values of PSA before and 6 months after treatment.

RESULTS. The production of PSA were markedly decreased by the combined treatment of isoflavones and curcumin in prostate cancer cell line, LNCaP. The expression of the androgen receptor was also suppressed by the treatment. In clinical trials, PSA levels decreased in the patients group with PSA ≥ 10 treated with supplement containing isoflavones and curcumin ($P = 0.01$).

CONCLUSIONS. Our results indicated that isoflavones and curcumin could modulate serum PSA levels. Curcumin presumably synergizes with isoflavones to suppress PSA production in prostate cells through the anti-androgen effects. *Prostate 70: 1127–1133, 2010.*

KEY WORDS: **prostate cancer; isoflavones; curcumin; PSA**

INTRODUCTION

Prostate cancer is the most common neoplasm in Caucasian men, while the incidence in Asians has been relatively low. Observational studies have suggested that diet is one of the most contributing factors for the lower observed incidence and mortality of prostate cancers in Asia [1]. Environmental factors such as dietary habits may play a major role in the causation of prostate cancer. Besides genetic factors, changes of lifestyle, especially dietary changes, should modify the risk of carcinogenesis. For example, Japanese men who migrated to the U.S. have experienced increasing rates of prostate cancer over consecutive generations [2].

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