Inhibition of Protein Kinase C/Twist1 Signaling Augments Anticancer Effects of Androgen Deprivation and Enzalutamide in Prostate Cancer

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Abstract

Purpose: The progression of prostate cancer to metastatic and castration-resistant disease represents a critical step. We previously showed that the transcription factor Twist1, which promotes epithelial-mesenchymal transition, was involved in castration-resistant progression. Similarly, protein kinase C (PKC) has been implicated in both metastatic progression and castration resistance in prostate cancer.

Experimental Design: In this study, we aimed to elucidate the role of PKC/Twist1 signaling in castration resistance, and to apply this information to the development of a novel therapeutic concept using PKC inhibitor Ro31-8220 against prostate cancer using various prostate cancer cell lines.

Results: Androgen deprivation and the next-generation antiandrogen enzalutamide induced PKC activation and Twist1 expression, which were reversed by the PKC inhibitor Ro31-8220. Ro31-8220 suppressed cell proliferation in androgen-dependent prostate cancer LNCaP cells, which was augmented by its combination with androgen deprivation or enzalutamide. The favorable anticancer effects of the combination of Ro31-8220 and enzalutamide were also observed in castration-resistant C4-2 and 22Rv1 cells. Furthermore, PKC phosphorylation was elevated in castration-resistant and enzalutamide-resistant cells compared with their parental cells, leading to persistent sensitivity to Ro-31-8220 in castration- and enzalutamide-resistant cells.

Conclusions: Taken together, these findings indicate that PKC/Twist1 signaling contributes to castration resistance as well as enzalutamide resistance in prostate cancer, and suggest that therapeutics targeting PKC/Twist1 signaling, such as PKC inhibitors, represent a promising novel therapeutic strategy for prostate cancer, especially castration-resistant prostate cancer, when combined with enzalutamide.

Introduction

Prostate cancer is the most common noncutaneous cancer and the second leading cause of cancer-related mortality among men in developed countries. Androgen-deprivation therapy is the gold standard treatment for recurrent or advanced prostate cancer (1). Although most prostate cancers are initially dependent on androgen receptor (AR) signaling for cell proliferation and cellular survival and respond well to androgen-deprivation therapy, most eventually relapse in a castration-resistant manner during such therapy, and are defined as castration-resistant prostate cancer (CRPC; ref. 2). Several novel AR-targeting agents against CRPC have recently appeared. For example, the cytochrome P17 inhibitor abiraterone acetate has been accepted by the U.S. Food and Drug Administration (FDA) for use in a postchemotherapy setting in 2011 (3) and in a prechemotherapy setting in 2013 (4). More recently, the next-generation antiandrogen enzalutamide has been approved by the FDA (5). Enzalutamide (MDV3100) is one of the most anticipated agents for CRPC treatment; however, efficacy, represented by decline of prostate-specific antigen, is seen in only around 60% of patients with CRPC (5). Novel therapeutic strategies are therefore required to improve the therapeutic effects of enzalutamide.

Aberrant activation of AR under low levels of circulating androgens is critical to the development of castration resistance (6). The mechanisms responsible for this include AR overexpression (7, 8), AR mutations (7), AR coregulators (9), AR activation by intracellular signal-transduction pathways (10), de novo androgen synthesis (11), and AR splice variants (7). The transcription factor Twist1 is known to promote epithelial-mesenchymal transition (EMT) and...
binds to the E-box (5'-CANNTG-3') sequence to upregulate the expression of its target genes (12). We previously suggested that Twist1 promoted castration resistance through upregulation of AR expression (13). Twist1 was upregulated following androgen ablation in a mouse xenograft model (14), as well as in human prostate cancer tissues (15). Targeting Twist1 in prostate cancer cells may represent a promising strategy against CRPC by increasing its vulnerability to androgen-deprivation therapy. However, to the best of our knowledge, no agents directly targeting Twist1 have been identified to date.

Protein kinase C (PKC) has also been suggested to be involved in both prostate cancer progression and castration resistance. The PKC isozymes comprise a large family, which is divided into 3 subfamilies of conventional, novel, and atypical isoforms, according to their structures and regulation mechanisms. The conventional PKCs, including PKC-α, PKC-βI, PKC-βII and PKC-γ, are activated by calcium, phosphatidylycerine, and diacylglycerol (DAG). The novel PKCs, including PKC-δ, PKC-ε, PKC-η, and PKC-θ, only require phosphatidylyserine and DAG for activation, whereas the atypical PKCs, including PKC-λ/ι and PKC-ζ, only require phosphatidylyserine for activation (16). PKC serine/threonine kinases are involved in signal transduction regulating cell proliferation, differentiation, tumorigenesis, apoptosis, EMT, and cytoskeletal remodeling (16). Several studies have demonstrated the involvement of PKCs in prostate cancer development and progression. Overexpression of PKC-ε in the prostate in a transgenic mouse model was associated with the occurrence of prostatic intraepithelial neoplasia (17). Conversely, deletion of PKC-ε in TRAMP mice inhibited prostate cancer development and metastasis (18). Similarly, PKC-β is also shown to be augmented in prostate cancer and implicated in prostate cancer development (19–21), as supported by numerous in vitro studies showing that inhibition of PKC signaling attenuated prostate cancer proliferation (21–23). Although, PKCs have also been implicated in castration resistance, and constitutively active PKC signaling promoted androgen-dependent, as well as castration-resistant proliferation of prostate cancer cells (24). As well, in human prostate cancer tissues, PKC signaling was highly activated in CRPC specimens compared with hormone-naïve cancers (10). Similarly, PKC expression correlated with poor clinical parameters such as biochemical failure after radical prostatectomy; Gleason score, and clinical stage (22, 23). These observations collectively implicate PKCs in the pathogenesis of prostate cancer, and suggest that the PKC signaling pathway, similar to Twist1, may be a promising therapeutic target in prostate cancer.

Considering such common features of PKC and Twist1 in prostate cancer pathogenesis, especially such as EMT and castration resistance, a close link between PKC and Twist1 has been suggested. We therefore aimed to elucidate the link of PKC/Twist1 signaling as well as their roles in castration- and enzalutamide-resistant prostate cancer, and examine the relevance of therapeutics targeting PKC signaling, combined with androgen-deprivation and enzalutamide treatment.

Materials and Methods

Cell culture

Human prostate cancer LNCaP, C4-2, and 22Rv1 cells were cultured in RPMI 1640 (Invitrogen) containing 10% FBS. LNCaP and 22Rv1 cells were obtained from the American Type Culture Collection and LNCaP cells were used after 10 to 40 rounds of propagation. C4-2 cells were kindly provided by Dr. M. Gleave (Vancouver Prostate Centre, Vancouver, BC, Canada). Enzalutamide-resistant derivatives of 22Rv1 cells (22Rv1/MDV cells) were established by long-term culture in the appropriate medium containing gradually increasing concentrations of enzalutamide, and maintained in media containing 50 μmol/L enzalutamide. The cell lines were maintained in a 5% CO₂ atmosphere at 37°C.

Antibodies

Antibodies against cyclin D1 (sc-718), AR (N-20, sc-816), and Twist1 (sc-81417) were purchased from Santa Cruz Biotechnology. Antibodies against retinoblastoma protein (pRB; #9309), phosphorylated pRB(Ser807/Ser811) (p-pRB, #9308), cleaved caspase3 (#9664), caspase3 (#9662), cleaved PARP (#9541), PARP (#9542), and phosphorylated PKCε(#9371) (p-PKC; #9371) were purchased from Cell Signaling Technology. Antibodies against E-cadherin (#610181) and fibronectin (#610077) were purchased from BD Biosciences. Anti-PKC (SAB4502356) and anti-β-actin (A3854) antibodies were obtained from Sigma. Anti-PSA antibody (#1984) was obtained from Epitomics.

Transfection with siRNAs and plasmids

The double-stranded RNA 25-base-pair oligonucleotides were commercially generated (Invitrogen). The target sequence was listed in Supplementary Table S1.
pCMV-AR plasmid expressing wild-type AR was kindly provided by Dr. C. Chang (University of Rochester, Rochester, NY). Prostate cancer cells were transfected with siRNA or plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol.

RNA isolation, reverse transcription, and quantitative real-time PCR

RNA isolation and reverse transcription were performed as described previously (25, 26). Quantitative real-time PCR was performed using TaqMan Gene Expression Assays for Twist1 (Hs00361186_m1), full-length AR (Hs00171172_m1), AR V7 (made to order), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Hs02758991_g1; Applied Biosystems) and TaqMan Gene Expression Master Mix (Applied Biosystems) with a 7900HT PCR system (Applied Biosystems). The transcript levels of the target genes were corrected according to the corresponding GAPDH transcript levels. All values represent the results of at least 3 independent experiments. Representative Ct values are listed in Supplementary Table S2.

Western blotting analysis

Whole-cell, nuclear, and cytoplasmic extracts were prepared as described previously (25). Briefly, the concentrations of the prepared protein extracts were quantified using a protein assay (Bio-Rad) based on the Bradford method. Aliquots (30 μg protein) were separated by 4% to 20% SDS-PAGE and transferred to polyvinylidene difluoride microporous membranes (GE Healthcare Bio-Science) using a semi-dry blower. The membranes were then incubated with the primary antibodies described above for 1 hour at room temperature, followed by incubation with peroxidase-conjugated secondary antibodies for 40 minutes at room temperature. The bound antibodies were visualized using an ECL Kit (GE Healthcare Bio-Science), and images were obtained using an image analyzer (LAS-3000 mini; Fujifilm).
PKC inhibitors suppress induction of Twist1 and AR. A (left), LNCaP cells were incubated in FBS-supplemented medium with 2.5 μmol/L Ro31-8220 for the indicated durations. Quantitative real-time PCR was performed using the indicated primers and probes for Twist1, AR, and GAPDH. Twist1 and AR transcript levels were corrected according to the corresponding GAPDH transcript level. All values represent the results of at least 3 independent experiments. The level of each transcript in untreated cells was defined as 1. Boxes, mean; bars, ± SD. *, P < 0.05 (compared with no treatment); (right) LNCaP cells were incubated with 2.5 μmol/L Ro31-8220 for the indicated durations. Whole-cell extracts were subjected to SDS-PAGE, followed by Western blotting analyses of the indicated proteins. B (left), LNCaP cells were incubated in CSS-supplemented medium with or without 2.5 μmol/L Ro31-8220 for 0, 3 (Twist1), and 12 hours (AR). Quantitative real-time PCR was performed using the primers and probes for Twist1, AR, and GAPDH. Twist1 and AR transcript levels were corrected according to the corresponding GAPDH transcript level. All values represent the results of at least 3 independent experiments. The level of each transcript in untreated cells without Ro31-8220 was defined as 1. Boxes, mean; bars, ± SD. *, P < 0.05; (right) LNCaP cells were incubated in CSS-supplemented medium with or without 2.5 μmol/L Ro31-8220 for the indicated durations. Whole-cell extracts were subjected to SDS-PAGE, followed by Western blotting analyses of the indicated proteins. C (left), LNCaP cells were incubated with 10 μmol/L enzalutamide and/or 2.5 μmol/L Ro31-8220 for 72 hours. Quantitative real-time PCR was performed using the indicated primers and probes for Twist1, AR, and GAPDH. Twist1 and AR transcript levels were corrected according to the corresponding GAPDH transcript level. All values represent the results of at least 3 independent experiments. (Continued on the following page.)

Figure 2. PKC inhibitor suppresses induction of Twist1 and AR.
Cell-proliferation assay
The cell-proliferation assay was performed as described previously (25). Briefly, 2.5 × 10^4 LNCaP cells seeded into 12-well plates were transected with 1.0 μg/mL of the indicated plasmid and incubated for 24 hours, followed by treatment with or without 5 μmol/L Ro31-8220 for 48 hours. Cells were harvested with trypsin and counted daily using a cell counter (Invitrogen). The results presented are representative of at least 3 independent experiments.

Cytotoxicity analysis
Cytotoxicity analyses were performed as described previously (26). Briefly, prostate cancer cells (2.5 × 10^4) were seeded in 96-well plates. Various concentrations of enzalutamide with or without Ro31-8220 were applied the following day. After 48 hours, surviving cells were stained using the alamarBlue assay (TREK Diagnostic Systems) at 37°C for 180 minutes. The absorbance of each well was measured using the ARVO MX (Perkin Elmer Inc.) plate reader. The results presented are representative of at least 3 independent experiments. Synergistic effects of enzalutamide and Ro31-8220 were calculated by analyzing the data using CalcuSyn software (BIOSOFT). A dose-effect curve was drawn for each treatment and the combination index was calculated at several effective doses (combination index = 1; additive effect, combination index < 1; synergy effect, combination index > 1; antagonistic effect).

Statistical analysis
All data were assessed using the Student t test. Levels of statistical significance were set at P < 0.05.

Results
Blocking AR signaling induces PKC phosphorylation and Twist1 expression
The responses of PKC and Twist1 to androgen-deprivation therapy by castration and/or an antiandrogen agent were examined by measuring Twist1 transcription levels after androgen depletion. As shown in Fig. 1A, androgen depletion increased Twist1 transcription in androgen-dependent LNCaP cells, although it was unaffected when the cells were incubated with dihydrotestosterone (DHT). PKC phosphorylation and Twist1 protein were also induced by androgen depletion in LNCaP cells, which effects were abolished by incubation with androgen (Fig. 1B). Similarly, blocking AR signaling using the novel antiandrogen enzalutamide upregulated Twist1 transcription (Fig. 1C). PKC phosphorylation, and Twist1 protein expression, which was accompanied by a decreased E-cadherin and an increased fibronectin expression (Fig. 1D), consistently with the previous reports on EMT promotion by enzalutamide (27, 28).

PKC inhibitor suppresses Twist1 and AR induction
We used the small molecule inhibitor of PKC, Ro31-8220, to elucidate the relationship between PKC and the Twist1/AR pathway. Ro31-8220 reduced Twist1 and AR transcript levels (Fig. 2A) and Twist1 and AR protein levels in LNCaP cells (Fig. 2A). Accordingly, we investigated the effects of Ro31-8220 on Twist1 and AR inductions by blockade of AR signaling. As shown in Fig. 2B, Ro31-8220 suppressed the induction of Twist1 and AR transcript and Twist1 and AR protein expression under androgen-depleted conditions. Similarly, Ro31-8220 abolished the induction of Twist1 and AR mRNA by enzalutamide, accompanied by similar effects on Twist1 and AR protein levels (Fig. 2C), although AR protein expression was decreased at 72 hours possibly because of an increased AR instability (29).

To further elucidate what PKC isoforms mediate the effect of Ro31-8220, we examined Twist1 and AR transcript levels after knockdown of PKC isoforms. As shown in Fig. 2D, among various PKC isoforms, knockdown of PKC-β or PKC-ε reduced Twist1 and AR transcript levels. Consistently, PKC-β or PKC-ε shutdown suppressed Twist1 and AR protein expression (Fig. 2D).

PKC inhibitor Ro31-8220 augments therapeutic effects of androgen depletion and enzalutamide in androgen-dependent LNCaP cells
Given the above results, we examined the therapeutic potential of Ro31-8220 for prostate cancer. Cellular sensitivities of LNCaP cells to Ro31-8220 were examined with or without androgen. As shown in Fig. 3A, androgen depletion augmented the anticancer effect of Ro31-8220. This enhancing effect of androgen depletion on Ro31-8220 cytotoxicity was supported by reduced phosphorylation of pRB and cyclin D expression, which regulate cell-cycle transition from G1 to S phase (Fig. 3B).

We also revealed that Ro31-8220 augmented the therapeutic effect of enzalutamide. The combination index demonstrated synergism between enzalutamide and Ro31-8220 (Ed50 = 0.43498, Ed75 = 0.30484, and Ed95 = 0.22206; Fig. 3C). This effect was confirmed by decreases in pRB phosphorylation and cyclin D1 expression (Fig. 3D). Inversely, AR overexpression partially rescued the suppression of cell proliferation by Ro31-8220 (Fig. 3E).

(Continued.) The level of each transcript in untreated cells without Ro31–8220 was defined as 1. Boxes, mean; bars, ± SD. *: P < 0.05; (right) LNCaP cells were incubated with 10 μmol/L enzalutamide and/or 2.5 μmol/L Ro31-8220 for the indicated durations. Whole-cell extracts were subjected to SDS-PAGE, followed by Western blotting analyses of the indicated proteins. D (left), LNCaP cells were transfected with 40 nmol/L of the indicated siRNA and incubated for 48 hours. Quantitative real-time PCR was performed using the indicated primers and probes. The transcript level of the target transcript was corrected according to the corresponding GAPDH transcript level. All values represent the results of at least 3 independent experiments. The level of each transcript in untreated cells without Ro31–8220 was defined as 1. Boxes, mean; bars, ± SD. *: P < 0.05 (compared with control siRNA); (right) LNCaP cells were transfected with 40 nmol/L of the indicated siRNA and incubated for 48 hours. Whole-cell extracts were subjected to SDS-PAGE, followed by Western blotting analyses of the indicated proteins.
PKC phosphorylation is upregulated, and Ro31-8220 augments the therapeutic effect of enzalutamide in castration-resistant C4-2 cells

We compared the PKC activation statuses of androgen-dependent prostate cancer and CRPC. C4-2 cells are well-known castration-resistant derivatives of androgen-dependent LNCaP cells (30). PKC phosphorylation level was increased in C4-2 cells compared with LNCaP cells (Fig. 4A). Similarly, in another castration-resistant CxR cell line, which we established previously (13), PKC phosphorylation was also increased (Fig. 4A). Because enzalutamide has shown efficacy even in CRPC (5), we investigated the combined effect of Ro31-8220 and enzalutamide in C4-2 cells. As shown in Fig. 5B, Ro31-8220 exerted a modest suppressive effect on cellular viability in C4-2 cells, indicated by combination index values ($ED_{50} = 1.72601$, $ED_{75} = 0.99113$, and $ED_{90} = 0.65574$; Fig. 4B). This synergistic effect of enzalutamide and Ro31-8220 was confirmed by decreased pRB phosphorylation and cyclin D1 expression (Fig. 4C), as in LNCaP cells.

Ro31-8220 downregulates full-length AR and AR splice variant, and augments the therapeutic effect of enzalutamide in 22Rv1 cells

We subsequently examined the effects of Ro31-8220 on Twist1 and AR expression in another castration-resistant 22Rv1 cell line, which expresses the usual full-length AR, as...
Ro31-8220 reduced expression levels of Twist1, as well as full-length AR and AR V7 at both the transcript and protein levels (Fig. 5A). Furthermore, Ro31-8220 augmented the suppressive effect of enzalutamide similarly to Ro31-8220 (Fig. 5E).

PKC phosphorylation is upregulated, and Ro31-8220 exerts enhanced cytotoxic effect in enzalutamide-resistant 22Rv1 cells

We finally aimed to clarify the effects of Ro31-8220 in enzalutamide-resistant cells. As noted above, enzalutamide is effective even in CRPC. Indeed, enzalutamide was also effective in 22Rv1 cells at a relatively high concentration, compared with that in LNCaP and C4-2 cells (Figs. 4 and 5). We established an enzalutamide-resistant 22Rv1 cell line (22Rv1/MDV cells) by long-term culture in gradually increasing concentrations of enzalutamide. As shown in Fig. 6A, 22Rv1/MDV cells were about twice as resistant to enzalutamide as their parental 22Rv1 cells, although no mutation was detected in the AR-coding region (data not shown). PKC phosphorylation was increased in 22Rv1/MDV cells, accompanied by increased transcript and protein expression of Twist1, full-length AR, and AR V7 compared with parental cells (Fig. 6B). The PKC inhibitor Ro31-8220 was therefore more cytotoxic to 22Rv1/MDV cells than to 22Rv1 cells (Fig. 6C).

Discussion

The mechanism responsible for resistance to androgen-deprivation therapy is thought to involve activation of the prosurvival and antiapoptotic pathways, including AR signaling (32). PKC and Twist1 signaling have been considered to promote castration resistance, possibly through AR signaling (13, 14, 22). Both PKC and Twist1 are consistently activated by blocking AR function, suggesting that androgen-deprivation therapy itself evokes treatment resistance, while exerting an excellent therapeutic effect on androgen-dependent prostate cancer. The rationale of this study was therefore that blocking activation of prosurvival and antiapoptotic pathways might augment the therapeutic effects of androgen-deprivation therapy.

Because no direct Twist1 inhibitor is available, we used the small molecule PKC inhibitor Ro31-8220 to suppress the unfavorable effects of androgen-deprivation therapy. Indeed, blocking AR signaling induced Twist1 expression, resulting in activation of the AR pathway and contributing to prosurvival and antiapoptotic effects in prostate cancer. The PKC inhibitor Ro31-8220 thus blunted the induction of Twist1 and AR by shutdown of AR signaling, indicating direct or indirect regulation of Twist1 by PKC, especially

![Figure 4](https://www.aacrjournals.org/clin-cancer-research/article-pdf/20/4/957/26687196/957.pdf)
Figure 5. Ro31-8220 downregulates full-length AR and AR splice variant, and augments the therapeutic effect of enzalutamide. A (left), 22Rv1 cells were incubated with 1 μmol/L Ro31-8220 for the indicated durations. Quantitative real-time PCR was performed using the indicated primers and probes. The transcript level of the target transcript was corrected according to the corresponding GAPDH transcript level. All values represent the results of at least 3 independent experiments. The level of each transcript from mock-transfected cells was defined as 1. Boxes, mean; bars, ± SD. B, 22Rv1 cells were incubated with 1 μmol/L Ro31-8220 for the indicated durations. Whole-cell extracts were subjected to SDS-PAGE, followed by Western blotting analyses of the indicated proteins. C, 22Rv1 cells were treated with or without 50 μmol/L enzalutamide and 2.5 μmol/L Ro31-8220. After incubation for 48 hours, whole-cell extracts were subjected to SDS-PAGE, followed by Western blotting analyses of the indicated proteins. D (left), 22Rv1 cells were transfected with 40 nmol/L of the indicated siRNA and incubated for 48 hours. Quantitative real-time PCR was performed using the indicated primers and probes. The transcript level of the target transcript was corrected according to the corresponding GAPDH transcript level. All values represent the results of at least 3 independent experiments. The level of each transcript in control siRNA-transfected cells was defined as 1. Boxes, mean; bars, ± SD. E, 22Rv1 cells transfected with 40 nmol/L of the indicated siRNA were seeded in 96-well plates. On the following day, vehicle or 50 μmol/L enzalutamide were applied. After 48 hours, the cell survival rates were analyzed by cytotoxicity analyses. Boxes, mean; bars, ± SD.
PKC-β and PKC-ε. To the best of our knowledge, no previous studies have reported on the regulation of Twist1 signaling by PKC, although PKC is known to be involved in the regulation of EMT (33), suggesting that PKC may regulate EMT through Twist1 signaling. In contrast, Twist1 has been shown to regulate AR expression as a transcription factor in prostate cancer cells (13). In line with these findings, PKC inhibition suppressed Twist1 activity, resulting in suppression of AR induction by androgen-deprivation therapy. Taken together, PKC seemed to regulate Twist1/AR signaling in response to androgen-deprivation therapy, functioning as an inhibitor of castration resistance, at least in an in vitro.

When the PKC inhibitor Ro31-8220 was used in combination with androgen deprivation therapy, it augmented the therapeutic effects of androgen depletion, as well as the effects of the novel antiandrogen enzalutamide in androgen-dependent prostate cancer cells. Androgen-deprivation therapy is known to induce cell-cycle arrest at G1–S phase (13, 15). The addition of a PKC inhibitor enhanced the decreases in phosphorylated pRB and cyclin D1 expression induced by androgen-deprivation therapy, suggesting augmentation of cell-cycle arrest at G1–S phase by PKC inhibition. Ro31-8220 also demonstrated an excellent synergistic therapeutic effect with enzalutamide in 22Rv1 cells, whereas the synergistic effect was modest in C4-2 cells; this difference may be related to the lower Twist1 expression in C4-2 cells compared with 22Rv1 cells (data not shown). Enzalutamide has recently been shown to have a remarkable therapeutic effect on CRPC, even after chemotherapy (5). Based on the results of a phase III clinical trial, the FDA has approved enzalutamide for CRPC in a post-docetaxel setting. PKC inhibitors could thus be applied to prostate cancer from the androgen-dependent to the castration-resistant stages.

So far, several inhibitors targeting PKC have shown significant antitumor effects in various cancers in preclinical and clinical studies. Midostaurin (34) was the first PKC inhibitor evaluated in clinical trials; however, it has failed to demonstrate significant activity in clinical trials to date (35). ISIS 3521, a phosphorothioate antisense oligonucleotide targeting the 3'-untranslated region of PKC-α mRNA (36, 37), demonstrated anticancer activity in various types of cancers, including tumors refractory to conventional chemotherapy (38, 39), but has so far failed to exert clinically significant efficacy when combined with chemotherapy against advanced lung cancer in phase III trials (40). In contrast to these disappointing results, PKC-β inhibitor enzastaurin as a single agent showed promising results in phase II trials against diffuse large B-cell lymphoma (41), although one phase III trial in patients with recurrent glioblastoma found no superior effect to conventional therapy (42). A further phase III study in patients with diffuse large B-cell lymphoma is currently underway (40). Moreover, a phase I study combining enzastaurin with gemcitabine and cisplatin has suggested a promising effect in combination with chemotherapy (43). Several PKC inhibitors are now...
under development for clinical use, and are expected to be used in the future, although the route to clinical use is not easy. About prostate cancer, enzastaurin has not yet shown promising results as a single agent, although the possibility of combining enzastaurin with docetaxel has been suggested (44). The results of this study indicated that PKC inhibition, combined with blocking AR signaling, augmented the therapeutic effect of each individual component, suggesting the possible therapeutic value of a PKC inhibitor administered concurrently with androgen-deprivation therapy. The limited therapeutic options for CRPC suggest that this option should be explored further.

In conclusion, PKC/Twist1 signaling upregulated AR transcription in response to AR inhibition. In addition, inhibition of PKC augmented the anticancer effect of androgen-deprivation therapy, including that achieved with enzalutamide. These results suggest that PKC/Twist1 signaling may contribute to cellular survival during androgen-deprivation therapy through regulation of AR transcription. PKC seems to represent a promising target for prostate cancer treatment, especially in combination with androgen-deprivation therapy, although additional experiments using an in vivo model would be required before clinical application.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References

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