Combination Treatment with 1α,25-Dihydroxyvitamin D₃ and 9-cis-Retinoic Acid Directly Inhibits Human Telomerase Reverse Transcriptase Transcription in Prostate Cancer Cells

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Abstract

The vitamin D₃ receptor, which is the nuclear receptor for 1α,25-dihydroxyvitamin D₃ (VD₃), forms a heterodimer with the retinoid X receptor (RXR), which is the nuclear receptor for 9-cis-retinoic acid (9-cis-RA). The heterodimer binds to a specific response element consisting of two directly repeated pairs of motifs, AGGTGA, spaced by three nucleotides [direct repeat (DR) 3] and modulates the expression of VD₃-responsive genes. Telomerase activity, which is seen in most immortal cells and germ cells, is a complex of enzymes that maintain the length of telomeres. One of the major components of human telomerase, human telomerase reverse transcriptase (hTERT), is the catalytic subunit, and the expression of hTERT might correlate most strongly with telomerase activity. We found that the sequence of 5′-AGTTCATGGAGTTCA-3′ (DR3) is similar to that of DR3 in the promoter region of hTERT. Our results showed that the combination of VD₃ and 9-cis-RA inhibited telomerase activity through direct interaction of the heterodimer of vitamin D₃ receptor and RXR with the DR3 sequence in the hTERT promoter as well as the combination of VD₃ and selective RXR ligand did. Also, in vivo data showed that the growth of xenografts in nude mice was inhibited by VD₃ and 9-cis-RA. The results of the present study provide evidence on the molecular mechanism of the inhibition of cell growth by these agents, and they could be novel therapeutic agents for prostate cancer.

Introduction

Nuclear receptors activated by hydrophobic ligands such as steroid hormones bind to specific DNA sequences generally located in the promoter or enhancer region of those genes and are expressed as hormone response elements. Nuclear receptors positively or negatively function as transcription factors for their target genes (1). The VDR,² which is ligated by VD₃, forms a heterodimer with the RXR, which is the nuclear receptor for 9-cis-RA. The heterodimer binds to a specific response element consisting of two directly repeated pairs of motifs (AGGTGA) spaced by three nucleotides (DR3) and modulates the expression of VD₃-responsive genes (2, 3). Although many vitamin D₃ response elements have been reported, they were not the complete sequence of the theoretical DR3 (Fig. 1a).

Telomeres are specific DNA sequences at the ends of chromosomes, consisting of tandem repeats of the hexanucleotide TTAGGG in the case of humans (4). Telomere length progressively shortens with each cell division in normal somatic cells because DNA polymerase is not able to synthesize the terminal DNA segments (5). Significant reduction of telomeric sequences causes cellular senescence and death (6, 7). Although they undergo repeated cell division, malignant, embryonic, and germ cells do not entirely lose the hexanucleotide sequences because of the activation of telomerase, which is a complex of enzymes that maintain telomeres (8, 9).

One of the major components of human telomerase, hTERT, is the catalytic subunit, and the expression of hTERT might correlate most strongly with telomerase activity (10). Success in cloning the promoter region of the hTERT gene has elucidated the regulatory mechanism of telomerase. Recent studies showed that c-Myc/Max and estrogen up-regulated the transcriptional activity of hTERT (11, 12). We found a sequence (5′-AGTTCATGGAGTTCA-3′, DR3’) resembling the DR3 sequence, which is the vitamin D response element, in the promoter region of the hTERT gene. In this report, we demonstrated the direct effects of DR3’ after treatment with VD₃ alone or with both VD₃ and 9-cis-RA on

² The abbreviations used are: VDR, vitamin D₃ receptor; hTERT, human telomerase reverse transcriptase; DR, direct repeat; VD₃, 1α,25-dihydroxyvitamin D₃; 9-cis-RA, 9-cis-retinoic acid; RXR, retinoid X receptor; nVDRE, negative vitamin D response element; hVDR, human VDR; hRXR, human RXR; PTH, parathyroid hormone.
cis-RA inhibits hTERT transcription as well as telomerase activity in prostate cancer cell lines PC3 and LNCaP.

Materials and Methods

Cell Culture. LNCaP and PC3 cells, which were derived from human prostate cancers, were grown in Ham’s F-12 medium (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS in the presence of 3.5% CO2 at 37°C. Cell Culture.

Materials and Methods

Cell Culture. LNCaP and PC3 cells, which were derived from human prostate cancers, were grown in Ham’s F-12 medium (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS in the presence of 3.5% CO2 at 37°C. The cells were grown in phenol red-free Ham’s F-12 medium (Life Technologies, Inc.) containing 10% dextran-coated charcoal-treated fetal bovine serum for 48 h before each experiment. For telomerase activity assay, quantitative real-time PCR, and Western blotting assay, cells were harvested after 0, 6, 12, 24, and 48 h of treatment with VD3 and 9-cis-RA. PC3 cells were harvested after 48 h of treatment with VD3 and/or PA204, a selective RXR ligand that was kindly provided by Dr. Hiroyuki Kagechika (Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan), at 0, 1, 10, and 100 nM for telomerase activity assay and transient reporter assay.

Cell Growth Curve. PC3 cells were seeded into 24-well plates at a density of 2.0 × 103 cells/well in cell culture medium containing VD3 and/or PA204. At 0, 1, 10, and 100 nM for telomerase activity assay and transient reporter assay.

Telomerase Activity Assay. To quantitate the relative telomerase activity, stretch PCR was carried out using the Telomerase Assay System (Teyobyo, Osaka, Japan) according to the manufacturer’s instructions. After telomerase reaction at 30°C for 45 min, 26 cycles of PCR consisting of denaturation at 95°C for 30 s, annealing at 68°C for 30 s, and extension at 72°C for 45 s were performed. After electrophoresis on 10% polyacrylamide gels, the gels were stained with SYBR Green I nucleic acid gel stain (Molecular Probes, Eugene, OR). The gels were read by a FLA-3000 (Fuji Photo Film, Tokyo, Japan) and analyzed by Image Gauge (Fuji Photo Film).

Quantitative Real-Time PCR. Total RNA was isolated using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer’s protocol. cDNA was synthesized from 1 μg of RNA with oligo-deoxythymidylic acid primer and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). Real-time PCR (TaqMan) analysis was performed on an ABI PRISM 7700 (Applied Biosystems, Foster City, CA). Matching primers and a fluorescence probe were designed for the hTERT gene according to the Primer Express program (Applied Biosystems): 5’–TCGAGCAGGATCTGTGTTAAG–3’ (exon 13; forward primer); 5’–CATGGTCTGAACACCTTGGT3–3’ (exon 12; reverse primer); and 5’–CTGGAGCTGTGCACCTGC–3’ (exon 10 to exon 13; TaqMan probe). Then, 4 μl of the reverse-transcribed cDNA was used in the PCR reaction in 50 μl of 1 × TaqMan buffer; 8 mM MgCl2; 0.2 mM dATP, dCTP, and dGTP; 0.4 mM dUTP; 0.25 unit of AmpliTaq Gold; 0.125 unit of Amperase uracil-N-glycosylase; 0.02% gelatin; 100 nM forward primer; 300 nM reverse primer; and 150 nM TaqMan probe.

Western Blotting. PC3 and LNCaP cell extracts containing 50 μg of protein were lysed in lysis buffer and boiled at 70°C for 10 min. The protein samples were separated by 12% SDS-PAGE and transferred onto membranes. After blocking with skim milk in PBS, the membranes were incubated with anti-VDR rat monoclonal antibody (9A7; NeoMarkers, Fremont, CA), anti-c-Myc mouse monoclonal antibody (9E10.3; NeoMarkers), or antihuman p21 mouse monoclonal antibody (6B6; BD PharMingen, San Diego, CA) at a 1:500 dilution in 1 h at room temperature. After washing with TBS-T (Tris-buffered sodium chloride Tween 20), the membranes were treated with antimouse IgG or antirat IgG horseradish peroxidase-linked whole antibody from sheep (Amersham Pharmacia Biotech) at a 1:5000 dilution for 1 h at room temperature. After further washing with TBS-T, bands were detected with an enhanced chemiluminescence plus kit (Amersham) according to the manufacturer’s instructions.

Transient Reporter Assay. To make the deletion mutant, various lengths of DNA fragments upstream of the initiating ATG codon of the hTERT gene were PCR amplified. These PCR products were inserted into the pGL3-Basic vector (Promega, Madison, WI), which is the luciferase reporter vector, in the sense orientation relative to the luciferase coding sequence at the MluI and BglII sites (11, 12). Transient reporter assay was performed using the Dual Luciferase Reporter Assay System (Promega). Briefly, cells (1.0–2.5 × 104) were seeded on 24-well plates, cultured overnight, and transfected with Transfast (Promega) with mixtures containing 0.5 μg/well luciferase reporter plasmid as described above and 0.5 ng/well pRL-SV40 (Promega), which contains the Renilla reniformis luciferase gene, as the control vector. Then, the cell culture medium containing VD3 and/or 9-cis-RA was added to the cells at a final concentration of 10 nM for each agent. The cells were cultured for 48 h after transfection, and then luciferase assays were performed according to the manufacturer’s protocol. The level of firefly luciferase activity was standardized by R. reniformis luciferase for each transfection. All experiments were performed at least three times with each plasmid, and the results represent the average relative luciferase activity.

Gel Supershift Assay. cDNAs of hVDR and hRXRα were subcloned into the pSG-5 expression vector (Stratagene, La Jolla, CA) and expressed in LNCaP cells. After 48 h of treatment with VD3 and 9-cis-RA, the cell culture medium was collected and centrifuged at 16,000 g for 15 min. The supernatant was used to perform the gel supershift assay using anti-VDR rat monoclonal antibody (9A7; NeoMarkers, Fremont, CA), anti-c-Myc mouse monoclonal antibody (9E10.3; NeoMarkers), or antihuman p21 mouse monoclonal antibody (6B6; BD PharMingen, San Diego, CA) at a 1:500 dilution in 1 h at room temperature. After washing with TBS-T, bands were detected with an enhanced chemiluminescence plus kit (Amersham) according to the manufacturer’s instructions.

The results presented in this study indicate that the treatment of prostate cancer cells with VD3 or 9-cis-RA inhibits hTERT transcription as well as telomerase activity. This finding may have implications for the development of novel therapeutic strategies for prostate cancer.
Recombinant hVDR protein and hRXRα protein were generated using the TNT wheat germ expression system (Promega). Two pmol of each individual recombinant receptor alone or 1 pmol of each in a mixture of the two were incubated with 4 μg of anti-VDR monoclonal antibody (BA7γ) or 4 μg of anti-RXRα polyclonal antibody (D-20; Santa Cruz Biotechnology, Santa Cruz, CA) on ice for 60 min. [32P]ATP end-labeled double-stranded oligonucleotides were used as probes. The sequences of the probes were 5′-ACTGGTAAAGGGTCAATGTTCAATTTCCTC-3′ (−2540 to −2506) containing the DR3' sequence in the hTERT promoter, 5′-ACTGGTAAAGGGTTGAAGCGGGGCAATTTCCCT-3′ containing the vitamin D response element of human osteocalcin as a positive control, and 5′-ACTGGTAAAGGAACATGGTGTTCTATTTCCCTT-3′ containing the androgen response element as a negative control. The probes and recombinant receptors with or without their antibody were incubated for 10 min at room temperature in a total volume of 20 μl of binding buffer containing 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.5 mM DTT, 10% glycerol, 0.05% NP40, and 1 μg of sonicated calf thymus DNA. DNA-protein complexes were resolved by electrophoresis on 5% nondenaturing polyacrylamide gel at 4°C in 0.5× Tris-borate EDTA, and then the gel was dried and exposed at −80°C to Kodak XAR film for 48 h.

**In Vivo Effect of VD3 and 9-cis-RA.** PC3 cells were trypsinized, washed with Ham’s F-12 medium, and suspended in saline solution at 1 × 10⁷ cells/0.1 ml. Twenty nude mice (BALB/c, male) were divided into four groups, and a 0.1-ml cell suspension was s.c. injected into each nude mouse in the left flank area. Formation of a tumor nodule of 5 mm in diameter was observed about 5 days after cell inoculation. Each group was given i.p. injections of saline, VD3 (5 ng/mouse), 9-cis-RA (5 ng/mouse), or a combination of VD3 and 9-cis-RA. These injections were performed a total of 12 times at 3-day intervals. Tumor volume was measured every 3 days and was determined by measuring the largest (L) and smallest (S) diameters of the tumor and calculated as V = (L × S²)/2. Dispersion analysis was used for statistical analysis, with Dunn’s procedure as a multiple comparison procedure.

**Results**

Stretch PCR was carried out to examine whether telomerase activity in PC3 cells was decreased by treatment with VD3 and/or 9-cis-RA. Although telomerase activity in PC3 cells was not inhibited by treatment with VD3 alone or 9-cis-RA alone within 48 h, the combination of VD3 and 9-cis-RA suppressed telomerase activity (Fig. 2a). Cell growth of PC3 cells was monitored during treatment with various concentrations of VD3 and 9-cis-RA. Cell growth curve was similar at each concentration of VD3 and 9-cis-RA within 2 days, and then cell growth was suppressed in a dose-dependent manner by VD3 and 9-cis-RA.

Telomerase activity assay was examined in PC3 and LNCaP cells at different times. Telomerase activity could be influenced by the cell cycle (16, 17); hence, we suggest that this stretch PCR appears to increase telomerase activity between 12 and 24 h in PC3 cells. However, the combination of VD3 with 9-cis-RA suppressed telomerase activity within 48 h in both PC3 and LNCaP cells (Fig. 3a). To evaluate whether the combination of VD3 with 9-cis-RA inhibits expression of hTERT mRNA, quantitative real-time PCR was performed using PC3 and LNCaP cells (Fig. 3b). Marked suppression of hTERT mRNA expression was observed within 6 h after the start of treatment with VD3 and 9-cis-RA in PC3 cells. The relative quantity of hTERT mRNA after 6 h of treatment with VD3 and 9-cis-RA in PC3 cells was decreased by 30% compared with control cells. Also, combined treatment with VD3 and 9-cis-RA showed gradual suppression of hTERT mRNA expression in LNCaP cells. Some investigators suggested that suppression of telomerase activity after treatment with VD3 might cause an increase in p21WAF1/CIP1 expression (18) because the vitamin D response element is contained within the promoter region of p21WAF1/CIP1 (19). Recent studies have shown that up-regulation of c-Myc activates the transcriptional activity of hTERT (11). We examined the protein expression of p21WAF1/CIP1.
c-Myc, and VDR after treatment with both VD₃ and 9-cis-RA for different time periods in PC3 and LNCaP cells (Fig. 3c). We could not detect a significant change in VDR and p21WAF1/CIP1 protein expression. Also, there was a trend toward up-regulation of c-Myc protein expression, although hTERT mRNA was suppressed after treatment with VD₃ and 9-cis-RA.

To examine the effect of VD₃ alone or of both VD₃ and 9-cis-RA on the transcriptional activity of the hTERT promoter, luciferase assay was carried out after transcription of the 3.3-kb hTERT promoter reporter plasmid containing the DR3' sequence (pGL3-3328). When PC3 and LNCaP cells were transfected with the pGL3-3328 plasmid and treated with VD₃ or 9-cis-RA, luciferase activity did not change in comparison with that of nontreated cells. However, in cells treated with both VD₃ and 9-cis-RA, after transfection, luciferase activity was markedly decreased to ~25% in LNCaP cells and ~40% in PC3 cells (Fig. 4a). To confirm that the DR3' sequence in the hTERT promoter affects transcriptional activity, various deletion mutants of reporter plasmids were constructed by PCR and examined by luciferase assay in PC3 and LNCaP cells (Fig. 4b). Cells transfected with the hTERT promoter reporter plasmid containing the DR3' sequence showed a significant decrease of luciferase activity after treatment with VD₃ and 9-cis-RA for 48 h. However, almost no difference in relative luciferase activity was seen in cells transfected with hTERT promoter plasmid lacking the DR3' sequence after treatment with VD₃ and 9-cis-RA. These results suggested that the transcriptional activity of hTERT was down-regulated through the DR3' sequence after treatment with VD₃ and 9-cis-RA.

To determine whether the DR3' sequence is directly bound to the homodimer of VDR or the heterodimer of VDR and RXR, gel supershift assay was performed. A probe containing the androgen response element as a negative control did not bind to either the hVDR homodimer or the hVDR-RXRα heterodimer (Fig. 5a). A probe containing the vitamin D response element of human osteocalcin as a positive control could bind to the heterodimer (Fig. 5b). Although the DR3' probe could not bind to the hVDR homodimer or the hRXRα homodimer, the hVDR-RXRα heterodimer bound to the probe, and a bandshift was visualized in Lane 6 (Fig. 5c).

After the heterodimer reacted with monoclonal 9A7 anti-VDR antibody, the bandshift disappeared in Lane 7 because the antibody recognizes a 17-amino acid epitope near the target sequence.

Fig. 3. Telomerase activity, expression of hTERT mRNA, and protein expression of p21, c-Myc, and VDR at different time points after the start of treatment with 10 nM VD₃ and 10 nM 9-cis-RA in PC3 and LNCaP cells. a, quantitative stretch PCR assay. IC, internal standard to verify the amplification efficiency of PCR. b, quantitative real-time PCR assays to detect hTERT mRNA. c, Western blotting of p21, c-Myc, and VDR.

Fig. 4. Transcriptional activity of hTERT promoter after treatment with VD₃ and 9-cis-RA in PC3 and LNCaP. a, reporter plasmid of the hTERT promoter (pGL3-3328) was transfected into PC3 and LNCaP cells. After cells were incubated with or without 10 nM VD₃ and 10 nM 9-cis-RA for 48 h, luciferase assay was performed. b, various deletion mutants of hTERT promoter reporter plasmids were transfected into PC3 and LNCaP cells, which were treated with 10 nM VD₃ and 10 nM 9-cis-RA for 48 h, and luciferase assay was performed. Relative luciferase activity for each reporter plasmid is shown.

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DNA-binding domain, and the probe was not able to bind hVDR. After the heterodimer reacted with polyclonal anti-RXR/H9251 antibody, the band shifted on electrophoresis in Lane 8. These results indicate that the DR3/H11032 sequence of the hTERT promoter binds specifically to the VDR-RXR heterodimer, but to neither the VDR homodimer nor the RXR homodimer.

Tumor growth of PC3 cells was investigated during treatment with VD3 and 9-cis-RA in vivo. We set up four groups of BALB/c nude mice inoculated with human prostate cancer PC3 cells, and each group was given an i.p. injection of saline, VD3, 9-cis-RA, or a combination of VD3 and 9-cis-RA. Fig. 6 shows the mean volume (±SE) for PC3 xenografts treated with each agent. A significant difference in tumor growth (mean volume ± SE) was shown between control and the combination (VD3 plus 9-cis-RA) arm (P = 0.0053). There was a statistically significant difference in the growth curve between the VD3 arm and the combination (VD3 plus 9-cis-RA) arm (P = 0.0023).

To examine the effect of pure agonist for RXR instead of 9-cis-RA on telomerase activity and transcriptional activity of hTERT promoter, stretch PCR and luciferase assay were carried out at different concentrations of VD3 and PA024 (20, 21), which is a pure RXR agonist. Within 48 h, telomerase activity in PC3 cells was inhibited with the combination of VD3 and PA024, but not by VD3 alone or PA024 alone (Fig. 7a). Furthermore, luciferase activity of the hTERT promoter was suppressed by the combination of these agents in a dose-dependent manner as shown in Fig. 7b.

Discussion
Many previous studies reported that VD3 or its analogue could inhibit the growth of various malignant cells including those of blood (22, 23), brain (24), breast (25, 26), colon (27, 28), pancreas (18), and prostate (29–31). Recent studies demonstrated that VD3 could also inhibit hTERT expression and telomerase activity in leukemic cells (32), and some investigators have reported that 9-cis-RA has chemopreventive efficacy in rat models of prostate (33) and breast cancer (34). However, the molecular mechanisms have been largely unknown. Although transcription of p21WAF1/CIP1 was activated by VD3 in a myelomonocytic cell line, U937 (19), the present study showed no significant change in p21WAF1/CIP1.
LNCaP cells were treated with VD3 alone, no change in PC3 cells were treated with 10 nM VD3, 10 nM PA024, and 1, 10 and 100 nM of a combination of VD3 and PA024 for 48 h, the reporter plasmid of the hTERT promoter (pGL3-3328) was transfected into PC3 cells. After cells were incubated with various concentrations of VD3 and PA024 for 48 h, luciferase assay was performed.

Our results showed that the VDR-RXR heterodimer bound to the DR3-like sequence after treatment with VD3 and 9-cis-RA, and then the expression of the hTERT gene was inhibited, resulting in suppression of telomerase activity in prostate cancer cells. We also performed telomere length analyses in PC3 cells after long-term culture under treatment with VD3 and 9-cis-RA. Telomere length of the original PC3 cells was about 9.0 kb, and after passage doubling level 50, its length was shortened to about 1 kb (data not shown). Therefore, the cell growth inhibition after treatment with VD3 and 9-cis-RA was probably attributable to decreasing telomerase activity accompanied by telomere shortening over the limit.

Many investigators have reported the existence of nVDREs (37–41). PTH and PTH-related protein are typically suppressed through nVDRE. Because the sequence of their nVDRE is not the complete DR3 sequence, only the homodimer of hVDR can bind to their nVDRE, but a VDR-RXR heterodimer does not bind (40). Although the promoter region of nVDRE in avian PTH or rat PTH can bind to a VDR-RXR heterodimer (41), at present there are no reports that nVDRE of the human gene binds to the hVDR-RXR heterodimer. The present data indicated that the DR3 sequence in the hTERT promoter acted as nVDRE and specifically bound to the hVDR-RXR heterodimer. The molecular mechanism of transcriptional repression by nVDRE has been poorly understood. Our results showed that hTERT mRNA was inhibited, although c-Myc protein expression was increased by treatment with VD3 and 9-cis-RA (Fig. 3c). The explanation for this is that binding of the hVDR-RXR heterodimer to the DR3 sequence might interrupt c-Myc protein binding to E-box in the hTERT promoter. We assume that binding of the hVDR-RXR heterodimer to DR3 would involve histone deacetylation in the proximal promoter region of hTERT and suppress hTERT transcription.

It is of further interest to determine whether the hTERT promoter shows a similar negative response in other cell types. We performed quantitative real-time PCR of hTERT mRNA and a transient reporter assay in DU145 cells and a cell line with alternative lengthening of telomeres, WI38-VA13/2RA cells expressing hTERT (42). Suppression of hTERT mRNA expression and luciferase activity was not shown in either cell line after treatment with VD3 and 9-cis-RA (data not shown). A recent study reported that DU145 cell growth was not inhibited by VD3 (43), although DU145 cells expressed VDR (29). Growth of DU145 cells was not inhibited by treatment with VD3 and 9-cis-RA (data not shown). Therefore, we presume that inhibition of hTERT via DR3 requires the binding of cofactor proteins to nuclear receptors, such as CREB-binding protein/p300 (CBP/p300), steroid receptor coactivator-1/transcription intermediary factor 2 (SRC-1/TIF2), and vitamin D receptor-interacting protein/thyroid hormone receptor-activating protein (DRIP/TRAP), which bind to estrogen receptors (44). It is very likely that the DR3 element in the hTERT promoter region requires a particular cellular factor that is expressed in PC3 and LNCaP cells, but not in DU145 or WI38-VA13/2RA cells.

Prostate cancer is one of the most common cancers of older men, and its prevalence has increased over the last two decades (45). Previous studies have reported that polymorphism in the VDR gene might be an important determinant of...
prostate cancer risk (46). Epidemiological studies have demonstrated an inverse relationship between the circulating level of VD$_3$ and the incidence of prostate cancer (47). Recent studies have elucidated that telomerase activity is regulated by nuclear receptors. Estrogen mediates direct or indirect up-regulation of hTERT gene transcription in breast cancer cells (12), and dihydrotestosterone activates telomerase in prostate cancer cells (48). Dihydrotestosterone or c-Myc protein increased the transcriptional activity of hTERT in prostate cancer cells in our investigation (data not shown). We reported previously that telomerase activity is detected in almost all prostate cancers and that there was a relationship between telomerase activity and the pathological grade of prostate cancer (48). Clinically, antiestrogen agents are used for breast cancer and antiandrogenic agents are administered for prostate cancer. We speculate that the molecular mechanism of these chemical agents for hormone-dependent cancers is through an influence on telomerase activity. Interestingly, the heterodimer of VDR and RXR down-regulated hTERT gene expression. Combination treatment with VD$_3$ and 9-cis-RA could therefore be a promising candidate for prostate cancer treatment. More interestingly, the use of a selective RXR agonist (rexinoid) such as PA024, LG100268, or LG1069 might be effective to inhibit telomerase activity synergistically with VD$_3$.

In conclusion, we found a DR3-like sequence in the hTERT promoter region, which could act as a nVDRE. Our results showed that only the VDR-RXR heterodimer could bind to the DR3-like sequence after stimulation with VD$_3$ and 9-cis-RA, and then expression of the hTERT gene was inhibited, which led to suppression of telomerase activity in prostate cancer cells. Consequently, stimulation with these agents interfered with the maintenance of telomeres, and cell growth was inhibited in prostate cancer cells. These observations suggest that the combination of VD$_3$ and 9-cis-RA could be an effective cancer therapy through inhibition of the hTERT gene transcript and telomerase activity in cancer tissues possessing both VDR and RXR.

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References

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