

Docetaxel-induced growth inhibition and apoptosis in androgen independent prostate cancer cells are enhanced by 1 α ,25-dihydroxyvitamin D₃

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Abstract

Pre-treatment with high-dose 1 α ,25-dihydroxyvitamin D₃ (1,25-VD) enhanced the antitumor activity of docetaxel in the androgen-independent prostate cancer cell line, PC-3. The effect manifested as an increasing population of apoptotic cells and amount of pro-apoptotic protein, Bax, under combined treatment compared with single treatment of either 1,25-VD or docetaxel alone. We further demonstrated that pre-treatment with 1,25-VD reduced the expression of multidrug resistance-associated protein-1 at both the mRNA and protein levels. This suggests pre-treatment with 1,25-VD can potentiate cytotoxicity of docetaxel in PC-3 due to 1,25-VD reducing multidrug resistance-associated protein-1 expression.

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1. Introduction

Chemotherapy remains one of the major options for effective treatment for hormone refractory prostate cancer (HRPC). In clinical trials, docetaxel (DX) (Taxotere; Aventis Pharmaceuticals, Inc, Bridgewater, NJ), a semi-synthetic taxane, effectively reduced prostate specific antigen (PSA) levels and improved

symptoms in patients with HRPC [1,2]. Treatment with DX in combination with the active form of vitamin D has shown promising results in PSA response, time to progression, and survival in HRPC patients [3]. It is therefore, of interest to investigate the mechanism of these drug interactions for future therapeutic design.

It is widely believed that DX, similar to other members of the taxane family, binds to β -tubulin, inhibits microtubule depolymerization, and impairs mitosis hence retarding cell cycle progression in the G₂/M phase [4]. During the G₂/M phase arrest of cancer cells induced by DX treatment, Bcl-2 phosphorylation occurs [5]. Phosphorylation of Bcl-2 decreases its binding to the proapoptotic protein, Bax, which is released, translocates, and inserts into the mitochondrial membrane releasing cytochrome c, leading to apoptosis [6].

Abbreviations: 1,25-VD, 1 α ,25-dihydroxyvitamin D₃; DX, Docetaxel; HRPC, hormone refractory prostate cancer; PSA, prostate specific antigen; PCa, prostate cancer; VDR, vitamin D receptor; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; EtOH, ethanol; 5-CF, 5-carboxylfluorescein; MRP, multidrug resistance-associated protein; Pgp, P-glycoprotein; Q-PCR, quantitative PCR.

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The antiproliferative effect of $1\alpha,25$ -dihydroxyvitamin D_3 (1,25-VD), the active form of vitamin D, has been reported in several types of cancers including prostate cancer (PCa). Both apoptosis and G0/G1 accumulation were observed in 1,25-VD treated PCa cells. 1,25-VD triggered apoptosis in LNCaP and ALVA-31 is accompanied by decreased expression of several antiapoptotic proteins, Bcl-2, Bcl-X_L, Mcl-1, BAG1L, XIAP, cIAP1, and cIAP2 [7]. Overexpression of Bcl-2 blocked 1,25-VD induced apoptosis in LNCaP cells, indicating the importance of Bcl-2 in the antiproliferative effect of 1,25-VD [8]. Induction of p21^{WAF1/CIP1} expression, Rb hypophosphorylation, and CDK2 activity reduction result in G0/G1 accumulation in 1,25-VD treated LNCaP cells [9]. The fact that stable expression of p21^{WAF1/CIP1} antisense or loss of p21^{WAF1/CIP1} expression in certain PCa cell lines both abolished the antiproliferative effect of 1,25-VD indicates the increased expression of p21^{WAF1/CIP1} mediates 1,25-VD triggered G0/G1 cell cycle arrest [9,10]. In addition to antiapoptotic protein and p21^{WAF1/CIP1}, the molecules involved in the antiproliferative effect of 1,25-VD also include, but are not limited to, vitamin D receptor (VDR), androgen receptor (AR), and p53. Therefore, the sensitivity to 1,25-VD depends on the composition and activity of these molecules [11–13]. Among PCa cell lines tested, the androgen-responsive cell line, LNCaP, is the most responsive while androgen-independent cell lines, DU 145 and PC-3 show less response to 1,25-VD treatment. Overall, the antiproliferative effect of 1,25-VD involves multiple signals regulating cell cycle and apoptosis, and coordination of these signaling networks determines the sensitivity to 1,25-VD of various PCa cell lines.

The cooperative effect between 1,25-VD and paclitaxel, another member of the taxane family, has been reported [14]. The mechanism is that pre-treatment of 1,25-VD reduced expression of p21^{WAF1/CIP1}, which sensitizes the cytotoxic response to paclitaxel in PC-3 cells. In that study, extremely high concentrations of 1,25-VD were used (5 μ M), which is difficult to reach in the clinic. Therefore, we first titrated the antiproliferative effect of 1,25-VD and DX on PC-3 and LNCaP cells to select the optimal concentration in order to gain cooperative effect. We then investigated the cooperative effects and mechanistic actions of DX and 1,25-VD in LNCaP and PC-3 cells, which were selected as models representing the androgen-responsive and -independent PCa cells, respectively, for comparison. The sensitivity to cytotoxic chemotherapy agents in cancer cells can be modulated by drug resistance proteins, including P-glycoprotein (Pgp), the MRP family, and ABCG2

[15]. The expression level of MRP in PCa has been shown to correlate with the Gleason score [16,17]. Therefore, we further investigated whether 1,25-VD regulated the expression of drug resistance proteins to sensitize cells in response to DX.

2. Materials and methods

2.1. Cell proliferation assay

Cells were seeded in 24-well tissue culture plates in RPMI-1640 containing 10% FBS. After incubation for 24 h, the medium was replaced with fresh medium containing 10% FBS and cells were treated as indicated in figure legends. The final ethanol concentration was 0.1%. Cell proliferation was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay according to manufacturer's procedure (Sigma, St Louis, MO). Briefly, the stock solution of MTT (5 mg/ml PBS) was added into each well at a 10-fold dilution. After 2 h incubation at 37 °C, the stop solution was added to extract the formazan product and the absorbance was recorded.

2.2. Flow cytometric analysis

LNCaP and PC-3 cells were seeded in 60-mm dishes at a density of 10^5 cells and 2×10^4 cells, respectively. After treatment, both attached and floating cells were harvested and stained with Annexin V-PE according to the manufacturer's procedure (BD Bioscience, San Diego, CA). The PE positive cell population representing apoptotic cells was determined by using the FACScan flow cytometer.

2.3. Western blot analysis

Total cell lysates were prepared by lysing cells in ice-cold RIPA buffer (1% Igepal CA-630, 0.5% sodium deoxycholate, and 0.1% SDS in PBS). The protein concentration was evaluated with the Bio-Rad reagent kit. For analyses, proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in TBST (10 mM Tris-Cl/pH 7.4, 150 mM NaCl, 0.05% Tween20) containing 5% nonfat dry milk for 1 h at room temperature. Membranes were probed with primary antibodies against Bax (Santa Cruz), Bcl-2 (DAKO), multidrug resistance-associated protein-1 (MRP-1) (Santa Cruz, Santa Cruz, CA), and β -actin (Santa Cruz), and then the secondary antibodies (Santa Cruz) in TBS. The immunoreactive bands were visualized by chemiluminescence (Amersham, Piscataway, NJ), or by the 5-bromo-4-chloro-3-indolylphosphate-nitro blue tetrazolium phosphatase substrate (Bio-Rad Laboratories, Hercules, CA). Protein expressions were quantified using a Versa-Doc gel documentation system (Bio-Rad).

2.4. RT-PCR and quantitative PCR(Q-PCR) assay

Total RNA was extracted from cells using Trizol (Invitrogen, Carlsbad, CA). RT-PCR was carried out by reverse transcription with the Super Script II kit (Invitrogen). Q-PCR was performed with SYBR Green PCR Master Mix on an iCycler IQ multi-color Q-PCR detection system (Bio-Rad). Primer sequences were β -actin: sense 5'-TGTGCCCATCTACGAGGGGTATGC-3' and anti-sense 5'-GGTACATGGTGGTGCCGCCAGACA-3'; MRP-1: sense 5'-GCTGAGTTCCTGCGTACCTATGC-3' and anti-sense 5'-TGTTGTGGTGCCTGCTGATGTC-3'. The PCR was performed as follows: initial denaturation at 95 °C for 10 min, and 45 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Δ CT values were calculated by subtracting the cycle threshold (CT) value from the corresponding β -actin (internal control) CT value from each time point. Then relative amounts were calculated by comparing the Δ CT value of the treated group to the Δ CT value of the control group.

2.5. 5-Carboxyfluorescein (5-CF) accumulation assay

MRP function was measured by modified 5-CF accumulation assay as described previously [18]. Briefly, cultured cells were incubated with DMSO or 200 μ M indomethacin for 30 min then exposed to 2 μ M 5-CF diacetate for another 30 min. After rinsing three times with ice cold phosphate-buffered saline, cells were lysed using Triton X-100. 5-CF cellular accumulation was then evaluated using a spectrofluorimeter (Molecular Devices, Sunnyvale, CA). The excitation and emission wavelengths were 492 and 518 nm, respectively.

3. Results

3.1. Dosage titration of 1,25-VD and DX in PC-3 and LNCaP cells

In order to optimize the dose for 1,25-VD and DX combination treatment, we first carried out dosage titration of each compound to demonstrate their independent effect on PCa cells. PC-3 and LNCaP cells were treated with five different concentrations of 1,25-VD from 1 to 100 nM for 6 days. On day 6, the growth inhibitory effect of 1,25-VD on LNCaP was around 10–20% higher than PC-3 (Fig. 1a). Although 100 nM 1,25-VD yields less than 40% growth inhibitory effect in treated cells, we did not pursue higher dosage considering the potential toxicity *in vivo*. Five different concentrations of DX ranging from 0.1 to 10 nM were used to treat PC-3 and LNCaP for 6 days. PC-3 cells are more sensitive to DX than LNCaP with

an IC₅₀ at 1.5 nM for PC-3 and 8 nM for LNCaP (Fig. 1b).

3.2. 1,25-VD pre-treatment promoted growth inhibitory effect of DX on PC-3

To test the growth inhibitory effect of the 1,25-VD and DX combined treatment on PCa cells, we first performed growth assays 6 days after simultaneously treating cells with both compounds. The co-treatment of 1,25-VD and DX on PCa cells did not yield a better growth inhibitory effect than their independent treatments (data not shown). This is similar to a previous report where no greater antitumor effect was observed under co-treatment of 1,25-VD and paclitaxel, which is another compound of the taxane family [14]. In another treatment design, cells were pre-treated with 1,25-VD for 2 days only and then treated with DX for another 6 days. Two days pre-treatment of 1,25-VD yielded less antiproliferative effect, compared to Fig. 1a, where

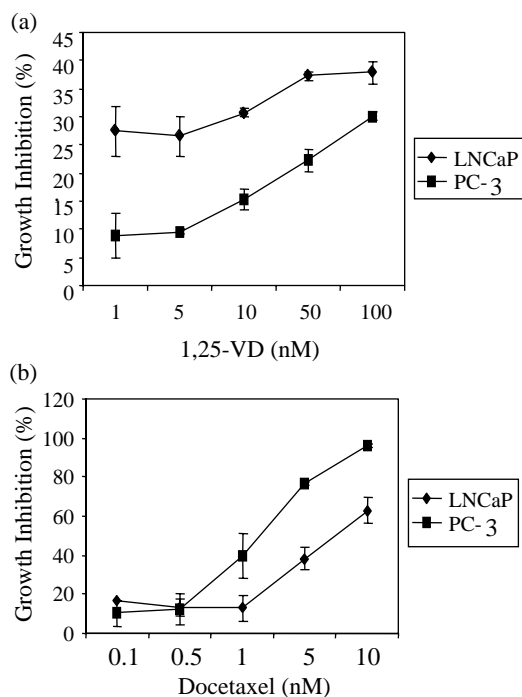


Fig. 1. Dose-dependent growth inhibitory effect of 1,25-VD and DX in PCa cell lines. (a) PC-3 and LNCaP cells were seeded at a density of 2×10^3 cells/well and 10^4 cells/well, respectively, in 24-well plates and cultured in 10% FBS supplemented RPMI. After 24 h, cells were treated with different concentrations of 1,25-VD (panel a), DX (panel b) or their respective vehicles for 6 days. On day 6, the MTT assay was performed to measure the viable cells. The data were expressed as percent of control (in the presence of vehicle only). Each point represents the mean \pm standard deviation (SD) of triplicate determinations.

6 days continuous treatment of 1,26VD was performed. Also, the antiproliferative effect of DX was lower compared to the same dosage in Fig. 1b. This is probably because after pre-treatment with EtOH for 2 days, the cell density was higher when the treatment of DX started. Nonetheless, we did see a greater growth inhibitory effect from the combined treatment compared to the single treatment of either compound in PC-3 cells, but not in LNCaP cells (Fig. 2a). Furthermore, we calculated and compared the IC_{50} of DX when treated alone or combined with 1,25-VD pre-treatment in PC-3 cells. DX treatment alone inhibited PC-3 cells growth with an IC_{50} of 4 nM. Antiproliferative effect of DX was promoted when pre-treatment of 1,25-VD was combined with DX where the IC_{50} was reduced to 2.7 nM (Fig. 2b).

3.3. 1,25-VD pre-treatment increased DX induced apoptosis in PC-3

DX inhibits microtubular polymerization, arrests cells in the G2/M phase of the cell cycle and induces apoptosis by Bcl-2 phosphorylation [5]. 1,25-VD is known to arrest cells in G1 phase and also triggers apoptosis by decreasing Bcl-2 expression [7]. Therefore, we examined whether the apoptosis population was increased in combined treatment with 1,25-VD and DX. PC-3 cells were treated with 100 nM 1,25-VD for 2 days followed by 0.5 nM DX treatment for 3 days. High concentrations of 1,25-VD alone increased apoptosis while low concentrations of DX alone had minor effects on apoptosis. Combined treatment of these two compounds yields additive effects in apoptosis induction compared with single treatment (Fig. 3a). In LNCaP, 1 day pre-treatment with 1,25-VD followed by 3 days in normal medium did not cause increased apoptosis compared to EtOH treatment, while DX alone did increase the apoptotic population from 10 to 15% (Fig. 3b). However, combined treatment did not yield a greater apoptotic population compared with DX alone in LNCaP. This result suggests pre-treatment of 1,25-VD did promote the growth inhibition effect of DX in PC-3, but not LNCaP, through increasing apoptosis.

3.4. Bax expression is increased in PC-3 under 1,25-VD and DX combined treatment

Several apoptosis-regulating proteins are known to be involved in 1,25-VD and DX stimulated apoptosis. Here we examined the expression and phosphorylation of Bcl-2, the anti-apoptotic protein, and the expression

of Bax, the pro-apoptotic protein. Although the expression and phosphorylation of Bcl-2 did not change in PC-3 under either single or combined treatment, Bax expression was increased under single

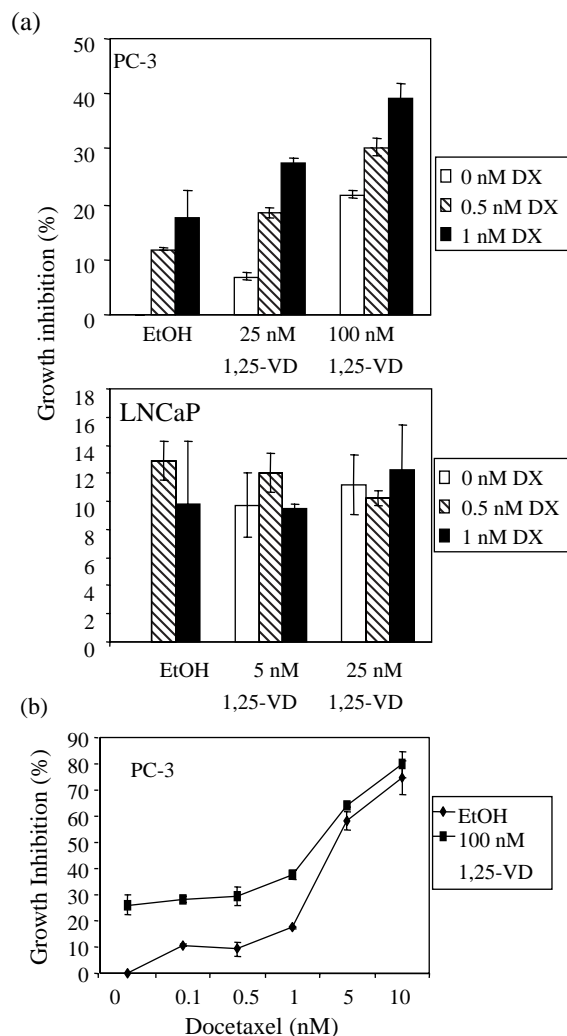


Fig. 2. Pre-treatment with 1,25-VD promotes the growth inhibitory effect of DX in PC-3 but not LNCaP cells. (a) PC-3 and LNCaP cells were seeded at a density of 2×10^3 cells/well and 10^4 cells/well, respectively, in 24-well plates and cultured in 10% FBS supplemented RPMI. After 24 h, cells were treated with EtOH or different concentrations of 1,25-VD for 2 days. Medium was replaced and cells were treated with different concentrations of DX as indicated. After 6 days, the MTT assay was performed to measure the viable cells. Each treatment condition and assay was performed in triplicate, and the percentage of growth inhibition attained by comparing with EtOH treatment was calculated. The mean \pm SD was plotted. (b) PC-3 cells were seeded and treated for growth assay as described in (a). The mean \pm SD from three independent experiments was plotted against the concentration of DX. IC_{50} of DX with pre-treatments of EtOH or 100 nM 1,25-VD were calculated.

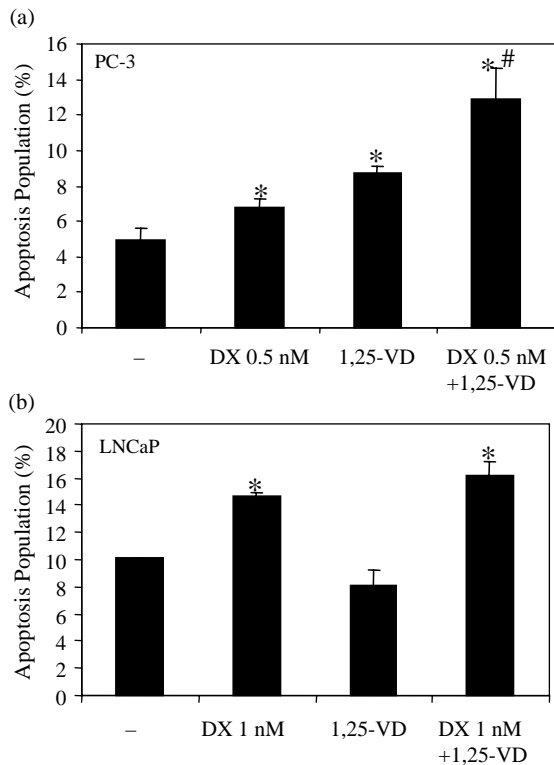


Fig. 3. Pre-treatment with 1,25-VD increases the apoptosis population induced by DX treatment in PC-3 but not LNCaP cells. (a) PC-3 cells were seeded at a density of 2×10^4 in 60-mm dishes and cultured in 10% FBS supplemented RPMI. After 24 h, cells were treated with EtOH or 100 nM 1,25-VD for 2 days. Medium was replaced and cells were treated with or without 0.5 nM DX. After 3 days, the apoptosis populations were assayed and plotted. * $P < 0.05$ compared with EtOH treated group by using Student's *t*-test. (b) LNCaP cells were seeded at a density of 10^5 cells in 60-mm dishes and cultured in 10% FBS supplemented RPMI. After 48 h, cells were treated with EtOH or 100 nM 1,25-VD for 1 day. Medium was replaced and cells were treated with or without 1 nM DX. After 3 days, the apoptosis populations were assayed and plotted. * $P < 0.05$ compared with EtOH treated group, # $P < 0.05$ compared with DX alone treated group by using Student's *t*-test.

treatment of both compound and further increased under combined treatment (Fig. 4, left panel). Since the expression of Bax was further increased while Bcl-2 remained the same in combined treatment compared with single treatment, pro-apoptotic events were further promoted in PC-3 with the combination treatment. On the other hand, the phosphorylation status of Bcl-2 changed dramatically in LNCaP cells with 1,25-VD and DX alone treatment with no further changes in combination treatment (Fig. 4, right panel). No change of Bax expression was observed in LNCaP. Although Bcl-2 phosphorylation increased 2 days after 1,25-VD pre-treatment, apoptosis population did not increase at day three as shown in Fig. 3. This suggests other apoptosis regulating signals are involved. Overall, the correlation between the Bax expression and the additive effect of apoptosis stimulation of 1,25-VD and DX indicates that the pro-apoptotic pathway is involved in the additive effect of combination treatment in PC-3 cells.

3.5. The expression and function of MRP-1 are reduced in 1,25-VD treated PC-3 cells

The cytotoxicity of docetaxel is significantly reduced by the expression of MRP in cancer cells [19]. To test whether 1,25-VD promotes the anti-proliferative effect of DX by modulating the function of MRP, we examined their expression by Q-PCR. The treatment with 1,25-VD alone down-regulated MRP-1 mRNA expression in PC-3 cells (left panel), but not in LNCaP cells (right panel) (Fig. 5a). This reduction also occurred in combined treatment. We then examined the protein level of MRP-1 1 day after changes in mRNA level were observed. The protein expression of MRP-1 in 1,25-VD treated PC-3 cells decreased, and was further decreased after combined treatment (Fig. 5b).

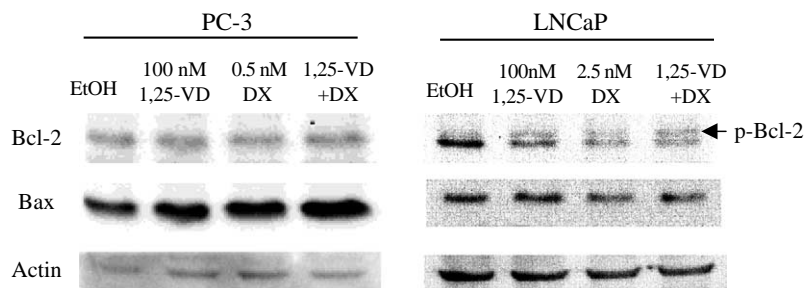


Fig. 4. Pre-treatment with 1,25-VD further increases the protein expression of Bax in DX treated PC-3, but not LNCaP cells. PC-3 and LNCaP cells were seeded at a density of 4×10^4 and 2×10^5 cells, respectively, in 60-mm dishes and cultured in 10% FBS supplemented RPMI. After 24 h, cells were treated with EtOH or 100 nM 1,25-VD for 1 day. Medium was replaced and cells were treated with 0.5 nM or 2.5 nM DX for another 2 days. Cells were then lysed and proteins were harvested for detection of Bcl-2, Bax, and actin by the Western blotting assay.

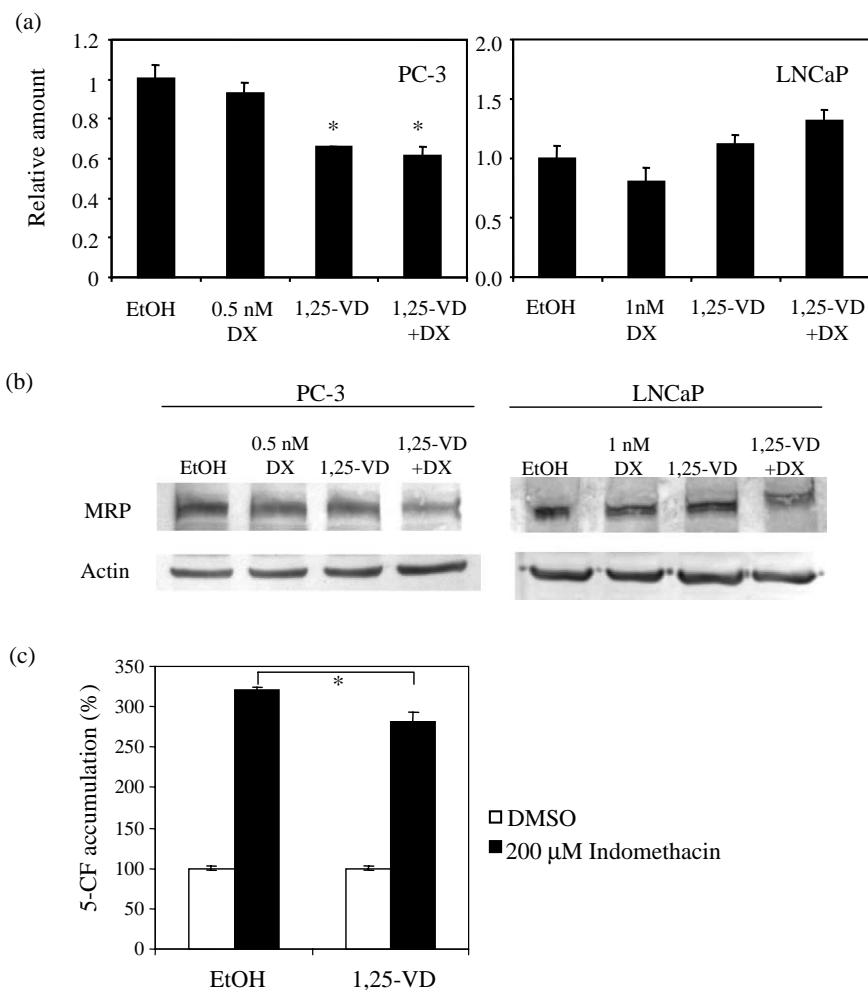


Fig. 5. The expression and function of MRP-1 in PC-3 after DX, or 1,25-VD alone, or combined treatment. (a) PC-3 and LNCaP cells were seeded at a density of 4×10^4 and 2×10^5 cells, respectively, in 60-mm dishes, then cultured in 10% FBS supplemented RPMI. After 24 h, PC-3 cells were treated with EtOH or 100 nM 1,25-VD for 2 days. Forty-eight hours after seeding, LNCaP cells were treated with EtOH or 100 nM 1,25-VD for 1 day. Medium was replaced and cells were treated with or without DX for 1 day. RNA was then harvested for detection of MRP-1 by Q-PCR. Each treatment condition and assay was performed in triplicate. The relative amount was measured by comparing with no treatment after being normalized by actin expression. The mean \pm SD was plotted. * $P < 0.01$ compared to EtOH treatment by using Student's *t*-test. (b) PC-3 and LNCaP cells were seeded and treated as described in (a), except that cells were treated with or without DX for 2 days before harvesting protein. 100 μ g protein from total lysate of PC-3 cells and 60 μ g of LNCaP cells were loaded on 6% SDS-PAGE. The expression of MRP-1 and actin were detected by Western blotting. (c) PC-3 cells were seeded at a density of 2×10^4 cells/well of 24-well plate. After 24 h, cells were treated with EtOH or 100 nM 1,25-VD for 48 h and the MRP function assay was performed. The accumulated 5-CF in cells was extracted and measured by fluorescence reader. The 5-CF amount in DMSO treated cells was set as 100%. The relative 5-CF amount was calculated and plotted. * $P < 0.01$ compared to EtOH treatment in the presence of indomethacin by using Student's *t*-test.

We next determined whether this decreased expression reflected a reduced function of MRP-1. A previously described 5-CF accumulation assay was used to study the functional activity of MRP [18]. In this assay, the nonfluorescent 5-CF diacetate passively and rapidly diffuses into cells and is converted to the fluorescent anion 5-CF by intracellular esterases. 5-CF is effluxed from cells by the MRP family of transporters. The

increased accumulation of 5-CF in the presence of a specific blocker for MRP, indomethacin, represents functional MRP in cells. In Fig. 5c, 1,25-VD treated PC-3 cells had less accumulation of 5-CF (175% increase) in the presence of indomethacin compared to EtOH treated cells (208% increase) indicating the reduced function of MRP. This suggests that by pre-treatment with 1,25-VD, the MRP-1 expression and

function are decreased, which results in accumulation of DX in cells and potentiates the cytotoxicity of DX.

4. Discussion

In advanced PCa patients, the options of treatment are few and prognosis is poor in most of cases. Recently, two phase III studies using DX combined with prednisone or estramustine showed increasing survival in hormone refractory PCa patients [20,21]. In addition, preclinical and clinical phase II studies using combined treatment with 1,25-VD and DX showed promising results in inhibition of PCa cell growth [3,22]. In order to facilitate the cooperative effect of combined treatment and to benefit future therapeutic design, we investigated the mechanisms by which 1,25-VD pre-treatment can promote the cytotoxicity of DX in PCa cell lines. LNCaP and PC-3 cells were chosen for this investigation to represent the androgen-responsive PCa, and HRPC, respectively. The higher sensitivity to 1,25-VD in LNCaP than in PC-3 is well documented with studies showing the presence of AR or the loss of p53 function are parts of mechanisms that attenuate the effect of 1,25-VD [12,13]. In contrast, the higher sensitivity to DX in PC-3 cells might be due to the rapidly proliferating characteristic of PC-3 cells increasing the opportunity for DX to block microtubule function during mitosis, or due to the lower MRP-1 expression level in PC-3 (data not shown) that results in accumulation of DX in PC-3 cells.

When pre-treated with 1,25-VD, the antiproliferative effect of DX was promoted in PC-3 cells, but not in LNCaP cells. Both 1,25-VD and DX exert antiproliferative effects through interfering with the cell cycle and apoptosis. After dissecting the effect of combined treatments in cell cycle and apoptosis, we did not detect further G0/G1 or G2/M arrest (data not shown). However, we did observe enhanced apoptotic populations in PC-3 cells under combined treatment with 1,25-VD and DX. This was confirmed by the detection of increasing amounts of the pro-apoptotic protein, Bax, in cells treated with either agent, and a further increase in combined treatment. The increase of Bax protein in retinoblastoma cells by 1,25-VD treatment has been reported previously [23], but not in PCa cell lines, LNCaP or ALVA-31 [7]. We also did not observe Bax increases in 1,25-VD treated LNCaP cells, but did so in PC-3 cells. As for the anti-apoptotic protein, Bcl-2, we did observe the phosphorylation in 1,25-VD or DX treated LNCaP cells, but not in PC-3 cells. The reasons behind such

discrepancies in regulating apoptosis signals among PCa cells by 1,25-VD and DX requires further investigation.

Although taxanes are the major substrates of Pgp, MRP did promote modest desensitization toward taxanes [24,25]. In our study, no reduction of Pgp expression was observed in PC-3 cells under combination treatment (data not shown). However, we did see a reduced MRP expression in RNA and protein level in PC-3 cells under 1,25-VD and DX alone and in combined treatment. The discrepancy observed in the drug-induced reduction of MRP-1 expression between LNCaP and PC-3 cells might be contributed by the status of functional p53 in the cells. Since the promoter of MRP can be activated by mutant p53 [26], and LNCaP cells express functional, wild type p53, while PC-3 cells express non-functional, truncated p53 [27], it is therefore possible that the suppressive effect of p53 is absent in PC-3 cells so that 1,25-VD is able to regulate MRP-1.

The application of Pgp inhibitor in clinical treatment for cancer has been suggested, however, the correlation of MRP expression in cancer is not well studied. The administration of probenecid, a MRP inhibitor, increased the accumulation of methotrexate and folate analogues in tumor cells, hence enhancing their antitumor efficacy [28,29]. However, probenecid and other MRP inhibitors such as ofloxacin, erythromycin, and rifampicin also increase the expression of MRP [30]. In addition, the physiological functions of the MRP family, including the inflammatory process, hepatobiliary elimination of bilirubin glucuronide, and protecting certain tissues from toxic agents, have to be taken under consideration when applying inhibitors in the clinic [31].

In summary, we confirmed that combination treatment with 1,25-VD enhanced the antiproliferative effect of DX and identified that a potential mechanism for 1,25-VD to sensitize cell response to DX in the PC-3 cell line is by down-regulating MRP-1 expression and function. Other MRP-1 substrates, such as vinca alkaloids, methotrexate, and camptothecins, are potential candidates for combination treatment with 1,25-VD in PCa. However, the mechanism of how 1,25-VD regulates MRP and how this effect varies among different cell lines needs to be investigated in order to maximize the benefit of such combination treatment in patients. Whether 1,25-VD may serve as a better option in chemosensitization compared to MRP inhibitors depends on its selectivity among cancer and normal cells.

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