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Role of Vitamin D as an Anti-Proliferative Compound on Prostate Cancer

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SUMMARY

The reason of prostate cancer is the mutation in the prostate cell. The present study was aimed at determining the effects of different concentrations of vitamin D on the anti-proliferative action of 24,25(OH)$_2$D$_3$ applied on LNCaP (cell line of human prostate cancer) cells. It was evident from the results that vitamin D having concentrations of 10^{-9}nM showed the best anti-proliferative action on prostate cancer cells (LNCaP cells) compared to other concentrations used. Most of the receptors were expressed at 10^{-9}nM resulting clear band in agarose gel image. Results also revealed that both nVDR and AR receptors (androgen receptors) were expressed well when treated with 10^{-9}nM and 10^{-10}nM concentrations of 24,25(OH)$_2$D$_3$. These findings confirm the potential use of vitamin D as an anti-proliferative compound against prostate cancer cells.

Key words: **anti-proliferative effect, prostate cancer, receptor, LNCaP cells, vitamin D**

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INTRODUCTION

Prostate cancer (CaP) is a common disease in males in the western world, including Sweden. It is the most common cause of death from cancer in men over 75 years of age (1). Prostate cancers are categorized according to the strategy on how they spread and differ from the adjacent tissues. Studies have suggested that prostate cancer is due to both genetic and environmental risk factors that include age, geography, family history, diet (2, 3). Prostate cancer is commonly diagnosed by prostate specific antigen screening or by symptoms. Prostate cancer is typically confirmed by biopsy test. Additional tests like CT scans and bone scans may be conducted to determine the mode of spread of prostate cancer (4). There are some common symptoms of prostate cancer that include urinary retention, urinary hesitancy, increased urination at night, pain with ejaculation, lower back pain, etc.

There are some options to inhibit prostate cancer that includes radiation therapy, hormonal therapy, chemotherapy, proton therapy, cryosurgery, etc. These treatments may inhibit prostate cancer in initial stage, but later aggressive androgen-independent forms of CaP can arise (5). Recent investigation has suggested that vitamin D is a way to prevent prostate cancer. Epidemiological and biochemical studies have suggested that vitamin D and its analogs are associated with endocrine and autocrine in preventing prostate cancer (6). Vitamin D and its derivative are lipophilic having low solubility. These molecules are circulatited with the help of vitamin D binding protein (DBP) that prolongs their serum half-life (7). The vitamin D metabolite (1,25(OH)2D3) plays an important role in calcium homeostasis and acts to increase absorption of calcium primarily in the intestine. Synthesis of 1,25(OH)2D3 is regulated by the parathyroid hormone (PTH), phosphate, calcium and 1,25(OH)2D3 itself (2). Testosterone plays an important role in the development of prostate gland. In the prostate, it is converted into a more potent androgen dihydrotestosteron (DHT), which binds to activate androgen receptors. The vitamine D receptor (VDR) belongs to the same family as the androgen receptors (the NR superfamily). The discovery of expressed VDR in malign prostate and prostate cell lines have made vitamin D and its metabolites therapeutic candidates for prevention of prostate cancer (8, 9). VDR is expressed in normal prostate cells and malign prostate cancer cells. Evidence suggested that dihydrotestosterones are related to risk of prostate cancer that results in dramatic apoptotic response to androgen deprivation (8, 10-12). It is also evident that there is a low risk of prostate cancer among the Asian men because of the high consumption of photochemical, such as red carotenoid, polyphenols (13). Inhabitants of sunny areas (14) and those having history of exposure to high levels of sunlight have lower risk of prostate cancer (9). In 2003, Chen and Holick at Boston University School of Medicine concluded that adequate exposure to sunlight or oral supplementation might provide a simple way to increase synthesis of vitamin D in the prostate and, therefore, decrease the risk of prostate cancer (6). However, complete picture of the molecular mechanisms of these compounds on cellular processes associated with prostate cancer needs to be understood.

The aim of this study was to investigate the anti-proliferative actions of $24,25(OH)_2D_3$ on LNCaP cells and thus, inspect the secosteroid effect on the transcription of the nuclear vitamin D and androgen receptor.

MATERIALS AND METHODS

In this investigation, prostate cancer cells (LNCap) were used. The cells were cultured, harvested and reseeded. They were then treated with different concentrations (10^{-7}nM, 10^{-8}nM, 10^{-8}nM, 10^{-9}nM, 10^{-10}nM, 10^{-11} nM) of Vitamin D in order to study the anti-proliferative action.

Counting of cells:

Ten microliter cell suspensions were mixed with 10 μl trypan blue by gentle pipetting. The number of viable cells was counted as seen as bright cells in haemocytometer. Ideally, more than 100 cells were counted to increase the accuracy of the cell count. Viable cells were counted according to the formula below:

Concentration of viable cells (cells/ml)= A×C×D

A= mean number of viable cells counted (i.e. Total viable cells counted/ Number of squares)
C= dilution factor (6)
D= Correction factor: 10^4

The concentration of viable cells (cells/ml) was 3200000 cells/ml.

RNA isolation and RT-PCR:

Total RNA from the LNCaP cells was isolated by using the Qiagen RNeasy Mini Kit according to the manufacturer's manual. Purification of total RNA from animal cells was performed by using Spin Technology. RNA concentration was estimated by measuring the absorbance (A) at 260 nm and 280 nm in a Nanodrop. Furthermore, The RNA purity was estimated by the ratio A260/A280 (purity=1.8-2.0).

The cDNA was synthesized using random hexamers. The nVDR and AR were amplified from obtained cDNA in LNCaP cells by polymerase chain reaction (PCR) using gene specific primers. RT-PCR was performed as follows: initial denaturation (95°C) for 7 min, denaturation (95°C) for 1 min, annealing (59°C) for 1 min, extension (72°C) for 30 secs and final extension (72°C) for 7 mins. GADPH was used as a control in order to

compare differences in intensity after second strand synthesis of hormone - treated cells.

Primer design:

The primers were designed for each gene by primer 3 software and further blasted to check the specificity of the desired gene (Table 1).

Table 1. *Primers used in RT-PCR*

Gene name	Forward	Reverse
nVDR	CTCCTCGATGCCCACCACAAGACCTACG	GTGGGGCAGCATGGAGAGCGGAGACAG
AR	CGGAAGCTGAAGAAACTTGG	ACACTACACTTGGCTCAATGG
GADPH	CCACCCATGGCAAATTCCATG	TCTAGACGGAGGTCAGGTCCACC

Gel electrophoresis for the PCR product:

To analyze the PCR products, 0.8% agarose gel was prepared at 150mA for approximately 45 minutes. Ethidium bromide was added to the gel for successive visualization under UV light to label the DNA fragments. Loading mix was prepared by adding 5 μl tracking dye (5x) with 20 μl of PCR product before loading the PCR products to the gel. DNA band was then visualized using VersaDoc imaging system (BIO-RAD). The experiment was performed twice to confirm the results.

RESULTS

It was evident from the present investigation that low concentration of vitamin D showed anti-proliferative actions on LNCaP cells resulting apoptosis. In 10^{-9}nM concentration of Vitamin D, both receptors (nVDR and AR) were expressed well (Figure 1 and 2). It was also observed that $24,25(OH)_2D_3$ having concentration of 10^{-9}nM was the most effective in enhancing the transcription of both receptors (nVDR and AR).

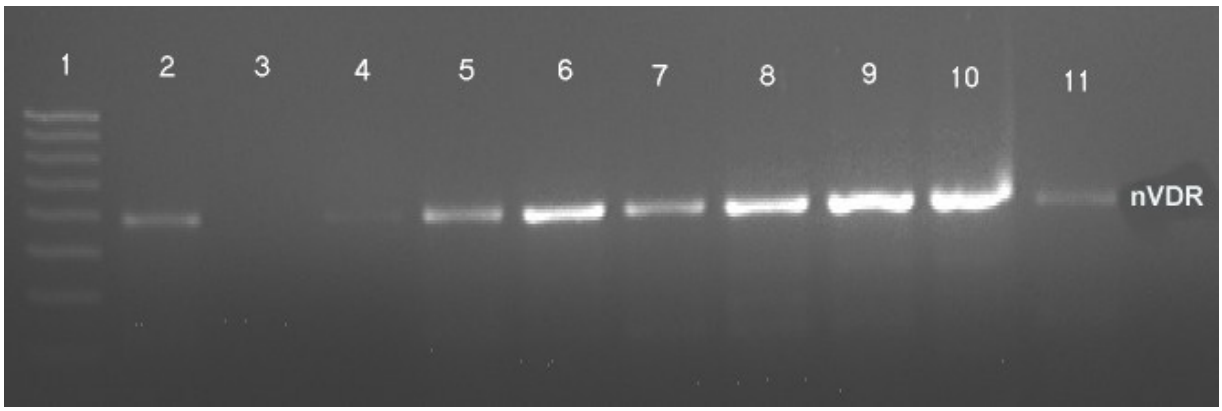

Figure 1. *Gel image showing the different bands of nVDR receptors. Here, Lane 1 - ladder, lane 2 - untreated, lane 3 - control, lane 4 - control, lane 5: 10^{-7}nM, lane 6: 10^{-8}nM, lane 7: 10^{-8}nM, lane 8: 10^{-9}nM, lane 9: 10^{-9}nM, lane 10: 10^{-10}nM, lane 11: 10^{-11}nM. Data were statistically significant ($P<0.05$).*

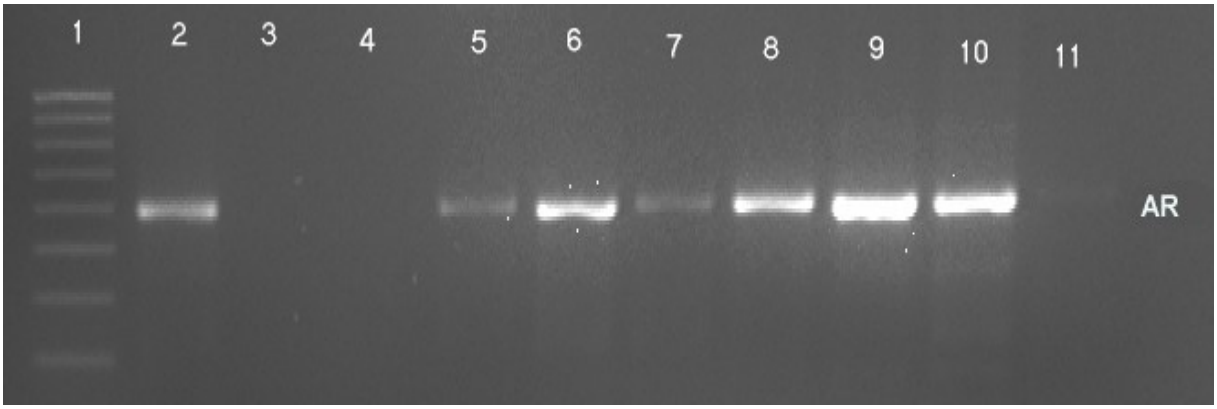

Figure 2. *Gel image showing the different bands of AR receptors. Here, Lane 1 - ladder, lane 2 - untreated, lane 3 - control, lane 4 - control, lane 5: 10^{-7}nM, lane 6: 10^{-8}nM, lane 7: 10^{-8}nM, lane 8: 10^{-9}nM, lane 9: 10^{-9}nM, lane 10: 10^{-10}nM, lane 11: 10^{-11}nM. Data were statistically significant ($P<0.05$).*

DISCUSSION

Results found in this study suggest that human prostate cancer cell lines, as well as primary cultures of non-cancerous prostatic cells, possess 1alpha-hydroxylase activity and can synthesize $1,25(OH)_2D_3$ from 25-$OH\text{-}D_3$. This indicates that $1,25(OH)_2D_3$ prevents the invasiveness of human prostate cancer cells that is supportive to other reports (1,15). It is believed that the local production of $1,25(OH)_2D$ may be responsible for the anticancer assistance of vitamin D (10). It was known that $1,25(OH)_2D_3$ was one of the most potent hormones for inhibiting both normal and cancer cell proliferation and inducing maturation (15). The biochemical evidence supports the role for vitamin D in prostate cancer which includes the demonstration of VDR and the anti-proliferative, apoptotic and pro-differentiation activities of 1a,25(OH)2D and its analogs in prostate cells *in vitro* and *in vivo* (3).

Calcitriol may affect cell cycle progression and may initiate apoptosis. The present recommended dose of vitamin D is 10μgram/day (16). It has been reported that calcitriol exerts its effects through the vitamin D receptor (VDR), a member of the nuclear receptor superfamily (17). In the recent years, it has been recognized that calcitriol exerts antiproliferative and pro-differentiating effects in many malignant cells, and retards the development and growth of tumors in animal models raising the possibility of its use as an anticancer agent (18). Potential toxic effects of vitamin D overdosage are rarely seen, only when the daily dose exceeds 10 000 IU of vitamin D on a chronic basis (19).

CONCLUSION

It was clear from the studies that intake or synthesis of vitamin D is associated with reduced incidence and death rates of prostate cancers. Our findings pinpoint that vitamin D can be used as chemoprevention to inhibit or delay prostate cancer. Further research on mechanisms of vitamin D action in prostate and identification of suitable analogs may lead to new invention in the treatment or prevention of prostate cancer.

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ULOGA VITAMINA D KAO ANTI-PROLIFERATIVNOG JEDINJENJA KOD KANCERA PROSTATE

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Sažetak

Razlog pojave kancera prostate je mutacija ćelija prostate. Cilj ove studije bio je određivanje efekata različitih koncentracija vitamina D na antiproliferativno dejstvo $24,25(OH)_2D_3$ koji se nanosi na LNCaP ćelije (ćelijska loza kancera prostate). Rezultati jasno ukazuju da je vitamin D u koncentraciji od 10^{-9}nM pokazao najbolje anti-proliferativno dejstvo na ćelije kancera prostate (LNCaP ćelije) u poređenju sa ostalim upotrebljenim koncentracijama. Većina receptora je bila eksprimirana pri koncentraciji od 10^{-9}nM, što se jasno uočava u vidu bendova na agaroznom gelu. Rezultati su takođe pokazali da su i nVDR i AR receptori (androgeni receptori) bili dobro eksprimirani kada su tretirani koncentracijama $24,25(OH)_2D_3$ od 10^{-9}nM i 10^{-10}nM. Ovi nalazi potvrđuju potencijalnu upotrebu vitamina D kao anti-proliferativnog jedinjenja protiv ćelija kancera prostate.

Ključne reči: **anti-proliferativni efekat, kancer prostate, receptor, LNCaP ćelije, vitamin D**