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Calcidiol and prostate cancer☆

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Abstract

Epidemiological studies suggest that serum calcidiol (25(OH)-Vitamin D_3) seems to be associated with several cancers including prostate cancer. We have made several experimental studies in order to clarify the mechanism(s) involved in the association. Calcidiol has been regarded as an inactive prohormone for calcitriol, which possesses the highest biological activity of the Vitamin D metabolites, when it is evaluated on the basis of bioactivity/nmol. However, we found recently that at the physiological concentration calcidiol (100–200 nM) is an active hormone, whereas calcitriol (1α,25$(OH)_2$-Vitamin D_3) (100 pM) is inactive in human primary prostate stromal cells. Calcidiol is able to inhibit cell growth and to induce or inhibit several genes including 1α-hydroxylase and 24-hydroxylase genes. This suggests that calcidiol might be an independent endocrine system involved in the control of cell differentiation and proliferation, whereas calcitriol might be mainly involved in the regulation of calcium and phosphorous balance.

Several mechanisms may mediate the action of Vitamin D in the prostate. This is a review of some recent studies on the role of (1) Vitamin D metabolism, (2) growth factors and (3) fatty acid metabolism.

Keywords: Prostate cancer; Vitamin D_3; Growth factors; 24-Hydroxylase; 1α-Hydroxylase; Fatty acid metabolism

1. Introduction

Epidemiologic studies have suggested that the cause of prostate cancer (PCa) is multifactorial, involving both genetic and environmental risk factors (age, race, geography, diet and hereditary factors) [1,2]. Some of these risk factors may be related to conditions that can alter Vitamin D status. It has been estimated that approximately 10% of prostate cancer cases are hereditary, the majority of PCa (90%) being sporadic. A number of genes have been suggested to be associated with PCa risk, including androgen (AR) and Vitamin D receptors (VDR), 5α-reductase, 1α-hydroxylase, 24-hydroxylase, cell cycle regulators and several growth factors [3]. Many of these genes are regulated by androgens and/or Vitamin D. It has been estimated that approximately 6% of prostate cancer mortality is caused by Vitamin D insufficiency [4,5]. However, the estimate may be influenced by the geographic location. The estimation based on our study in Finland [6] is significantly higher with the possibility of 26% of PCa cases in Finland being related to Vitamin D insufficiency due to low UV radiation during the 7 months of the winter season. This suggests that Vitamin D is a significant risk factor for prostate cancer, but the causal relationship remains to be studied.

Alongside the cloning of the nuclear receptor for Vitamin D, it became obvious that this receptor, although in low concentration, could be found in most tissues and cells, including malignant cells not previously appreciated as targets of Vitamin D action [7]. The results suggested that the functions of Vitamin D might be more prevalent than previously anticipated. Moreover, the role of Vitamin D as a hormone and para- and autocrine factor became evident. It has been shown that Vitamin D is an important regulator of growth, differentiation and apoptosis in many tissues [8–12]. In most normal and cancer cells, Vitamin D acts as an antiproliferative factor, but there are some exceptions where Vitamin D at certain concentrations may stimulate cell proliferation. These effects are believed to be mediated mainly by the

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nuclear VDR, which is also expressed in cancer cells, wherefore there has been increasing interest in the role of Vitamin D in carcinogenesis [13], and the exact mechanisms whereby Vitamin D exerts its antiproliferative action are under an intense investigation. This article is a general review of the role of Vitamin D in prostate cancer/cells with a focus on local Vitamin D metabolism and other mechanisms, which might be important in the control of cell proliferation by Vitamin D.

2. Epidemiological studies

Early epidemiological studies based on indirect evidence suggested a relationship between Vitamin D (sunlight) and colon cancer as well as breast cancer [1,14,15]. The protective action of sunlight and Vitamin D against several cancer types became evident on the basis of several epidemiological studies [5,14–17]. Schwartz and Hulka [4] put forward the hypothesis that Vitamin D deficiency also increases the risk of prostate cancer (PCa). The hypothesis was supported by the findings that the rate of PCa is inversely proportional to incident ultraviolet radiation [5,16] and that serum calcitriol is a predictor for the development of PCa [17]. However, other reports have questioned these findings [18–21]. It also seems evident that prostate cancer by itself can have an adverse effect on Vitamin D homeostasis, and that various treatments for PCa may alter circulating calcitriol levels [22].

During the past two decades Vitamin D deficiency has become more common in Finland and, therefore, it was reasonable to study the association between Vitamin D insufficiency and cancer in Finland. During the same period, the age-adjusted incidence of prostate cancer in Finland is continuously increasing and PCa has become the most common cancer among males. We performed a pre-diagnostic nested case-control study on the 19,000 Finnish men who attended the first screening visit within the Helsinki Heart Study [23]. We decided to assay only the major serum metabolite, calcidiol, which reflects Vitamin D deficiency better than calcitriol and is an important precursor of calcitriol in the prostate [24,25]. Our study [6] brought out the following interesting aspects concerning prostate cancer: (1) Vitamin D deficiency may be an important cause of cancer in countries having seasonally low UV irradiation. (2) The prostate cancer risk attributable to Vitamin D deficiency is higher among pre-andropausal (3.5-fold) than post-andropausal men (1.2-fold). (3) Vitamin D probably plays a role in the initiation, promotion and progression of cancer. (4) The protective action of Vitamin D seems to be dependent on androgens. (5) High serum Vitamin D levels delay the development of prostate cancer. (6) There was a 6.3-fold increase of non-localized PCa in men with low calcidiol serum levels.

Recently, we have performed a more extensive study among 200,000 Nordic men in Finland, Sweden and Norway [26], analyzing serum calcidiol levels of 622 prostate cancer cases and 1451 matched controls. In summary, we found

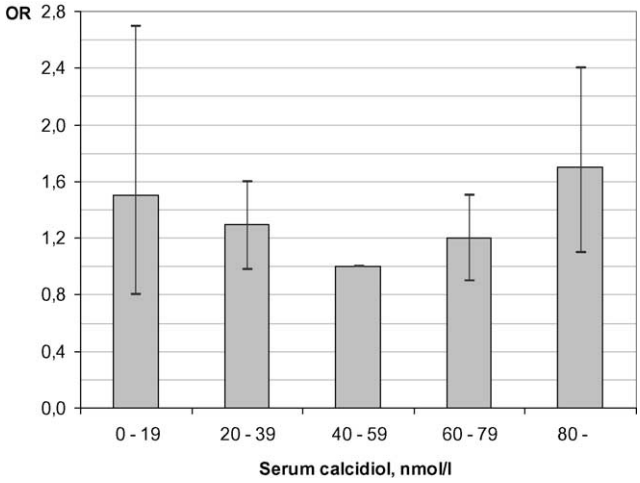

Fig. 1. Relative risk of prostate cancer according to different calcidiol serum levels among Nordic men (Finland, Sweden and Norway) [26]. OR = odd's ratio.

that both low (< 20 nmol/l) and high (> 80 nmol/l) calcidiol serum concentrations are associated with higher prostate cancer risk (Fig. 1). The normal average serum concentration of calcidiol (40–60 nmol/l) comprises the lowest risk of prostate cancer. The U-shaped risk curve of prostate cancer might be due to similar calcidiol availability within the prostate: low Vitamin D serum concentration apparently leads to a low tissue concentration and to weakened mitotic control of target cells, whereas a high Vitamin D level might lead to Vitamin D resistance through increased inactivation by enhanced expression of 24-hydroxylase [27]. This finding might explain the variable epidemiological results on the prostate cancer and Vitamin D levels [28].

3. Vitamin D and control of cell proliferation in prostate

The epidemiological results can be explained on the basis of experimental data. Proliferation of normal and malignant human prostate cells can be inhibited by Vitamin D [6,29,30]. The Vitamin D-induced anti-proliferative action is mediated predominantly through a G1/S phase block of the cell cycle (for review see [3]). The blocking of the G1/S phase by Vitamin D is known to be associated with alteration of protein levels or kinase activity of CDK2, CDK4 and CDK6 ultimately leading to inhibition of phophorylation of the retinoblastoma protein. In the LNCaP prostate cancer cell line, CDK2 kinase activity has been shown to decrease by Vitamin D [31].

The anti-proliferation action of Vitamin D is not only due to its direct effects on cell cycle, but it also includes differentiation of cancer cells and an increased apoptosis [3]. LNCaP prostate cancer cells have been shown to undergo apoptosis after calcitriol treatment although there are varying reports concerning the extent of the induced cell death [32–34]. In the study by Blutt et al. $1,25(OH)_2D_3$ was shown to induce

a 5-fold increase in the apoptosis of LNCaP cells accompanied by down-regulation of Bcl-2 and Bcl-X_L anti-apoptotic proteins [34]. The association of Bcl-2 down-regulation with apoptosis was assessed with cells over-expressing Bcl-2 gene. In these cells, although failing to induce apoptosis, calcitriol was still able to cause marked growth inhibition. This indicates that in LNCaP cells both cell cycle arrest and apoptosis are involved in the action of calcitriol. Likewise, neither the capability of calcitriol to trigger apoptosis nor the down-regulation of Bcl-2 in the apoptotic process is straightforward but displays cell-specificity. Furthermore, VD inhibits the invasion of prostate cancer cells [35]. Metastasis of rat Dunning tumour has been largely inhibited by Vitamin D and by its less calcemic analogue EB1089 [36]. Finally, Vitamin D may also exert its anti-tumour effects through anti-angiogenic activity [37]. In summary, these findings suggest that Vitamin D can influence all the central mechanisms involved in the control of cell proliferation, apoptosis and tumour progression.

4. The role of Vitamin D metabolism

1α,25-Dihydroxyvitamin D_3 (calcitriol) is produced from the major circulating metabolite, 25OH-Vitamin D_3 (calcidiol) catalyzed by 25-hydroxyvitamin D_3 1α-hydroxylase (1α-hydroxylase, CYP27B1) mainly in the kidney, as well as in some extrarenal tissues. Both calcitriol and calcidiol are inactivated by 25-hydroxyvitamin D_3 24-hydroxylase (24-hydroxylase, CYP24) in the kidney and in the other Vitamin D_3 target tissues [38]. 1α-Hydroxylase has been found in the prostate epithelial cells [39] and its expression is not regulated by parathyroid hormone and calcium [40]. We found 1α-hydroxylase expressed in human prostate stromal cells and that its expression is upregulated by calcidiol [27]. In addition, the expression of 24-hydroxylase is highly inducible by calcidiol and calcitriol in prostate epithelial and stromal cells [27] (Fig. 2).

Calcidiol was earlier believed to be a prohormone mostly because of its significantly lower affinity to VDR (1/600–1/700) than calcitriol. But its serum concentration is 1000-fold higher than that of calcitriol. The growth inhibitory effect of Vitamin D on the prostatic epithelial cells has thought to be mediated only by calcitriol. By using the inhibitor of 1α-hydroxylase, we recently demonstrated that calcidiol at 250 nM is an active hormone in human prostate stromal cells [27]. Based on these results we proposed a new Vitamin D endocrine system mediated by calcidiol. The results fit well with those epidemiological studies suggesting that an increased prostate cancer risk is associated with low serum level of calcidiol but not with calcitriol [6,18,19,28,41]. Calcidiol better reflects Vitamin D insufficiency than calcitriol. Barger-Lux and Heaney have shown that the large seasonal fluctuation in the serum levels of calcidiol is associated with summer sun exposure, but this does not produce significant changes in calcium absorption fraction [42]. However, the serum levels of calcitriol is not affected by the season [43]. Therefore, it can be assumed that calcidiol might be the most important factor in controlling cell proliferation in the prostate. When the serum concentration of calcidiol is low, the control of cell proliferation is weak. It is also possible that a low calcidiol concentration can stimulate mitotic activity [44].

The reason for the increased risk of prostate cancer among the men having a high calcidiol concentration [26] is not known. Our recent study suggest an explanation for the epidemiological finding: Since calcidiol appears to induce 24-hydroxylase expression at the high physiological concentrations [27] leading, in turn, to partial or complete Vitamin D resistance, both low and high calcidiol concentration may exert similarly weak Vitamin D action and a weak mitotic control. The circulating calcidiol above 200 nM is not rare among outdoor persons with plentiful sun exposure [45].

Our first epidemiological study suggested androgen-dependency of Vitamin D action on prostate cancer [6]. There is a cross-talk between Vitamin D and androgen signalling systems. The expression of Vitamin D receptor is regulated by androgen, and that of androgen receptor by Vitamin D [46–48]. We recently found that androgen can inhibit the ability of Vitamin D to induce the expression of 24-hydroxylase in

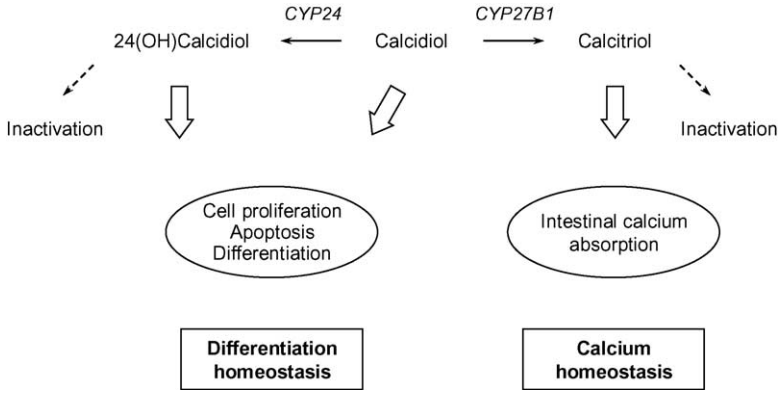

Fig. 2. Scheme of the Vitamin D endocrinology and the metabolic pathways of calcidiol. Calcidiol, 24-calcidiol (24,25-dihydroxy-Vitamin D_3) and calcitriol are active hormones. At the physiological concentration, calcitriol can regulate calcium balance, but not general cell differentiation, whereas calcidiol and 24-calcidiol are less calcemic and more potent in regulation of differentiation than calcitriol [27]. CYP24 = 24-hydroxylase; CYP27B1 = 1α-hydroxylase.

LNCaP cells [49], suggesting that the Vitamin D catabolism can be significantly inhibited by androgen. Therefore, a combined treatment of Vitamin D and androgens might be a promising therapy in prostate cancer.

5. Interaction with growth factor signaling

The regulation of prostate cell differentiation and growth by Vitamin D seems to be mediated by its interaction with growth factors. Vitamin D regulates the expression of several growth factors, their receptors and the availability of the growth factors in prostate. Specific paracrine-autocrine signaling pathways of main growth factor families such as insulin-like growth factor (IGF), transforming growth factor β (TGF-β), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) can be regulated by Vitamin D at different levels (Table 1.).

In the prostate, IGF-I is important mitogenic and anti-apoptotic factor, which regulates the proliferation of prostate epithelial cells and is involved in the transformation process of epithelium. Numerous studies demonstrate that Vitamin D reduces the availability of active IGF-I in prostate cells primary by up-regulation of the expression of IGF binding proteins. Nickerson and Huynh showed that the regression of ventral prostate induced by Vitamin D analogue EB1089 was associated with 15–25-fold increases in gene expression of IGFBPs-2, -3, -4 and -5 [50]. In different normal, BPH and cancer prostate epithelial cell lines the induction of IGFBP-3 expression by Vitamin D was shown to play the crucial role in basal and IGF-induced cell growth [51,52]. In LNCaP human prostate cancer cell line, the anti-proliferation actions of Vitamin D are mediated partially by induction of IGFBP-3, which, in turn, increased the levels of the cell cycle inhibitor p21 leading to growth arrest [53]. A putative VDRE was identified in the promoter region of IGFBP-3, which strongly binds VDR [54].

Cross-talk between Vitamin D and TGF-β, a super-family of multifunctional cytokines playing an important role in prostate epithelial cell transformation and cancer progression, is not well understood. In PC-3 prostate cancer cells calcitriol elevates TGF-β1 expression and signalling, as well as receptor levels. Blocking antibody against TGF-β1 reduces calcitriol mediated growth inhibition [55]. In NRP-152 cells, a non-tumourigenic epithelial line derived from rat dorsal–lateral prostate, calcitriol induces TGF-β2 and 3 but not TGF-β1. It also induces fibronectin and thrombospondin through induction of TGF-β, thus providing a mechanism for Vitamin D induced prostate cell differentiation [56]. We found that calcitriol induces the expression of newly characterized member of TGF-β family, prostate-derived factor (PDF, also known as MIC-1, PLAB, NAG-1, GDF-15, PTGF-β) [57]. The role of PDF in prostate cell growth is poorly understood, but it is known pro-apoptotic and pro-differentiation properties suggest a role in the inhibition of prostate cell growth by Vitamin D.

KGF (FGF7) is one of the intraprostatic growth factors and a potent growth factor for BPH cells. It is shown that calcitriol and Vitamin D analogue, 1,25-dihydroxy-16ene-23yne D_3 counteract the mitogenic activity of KGF. Vitamin D analogue induced bcl-2 protein expression, intracellular calcium mobilization, and apoptosis in both unstimulated and KGF-stimulated BPH cells and DU145 prostate cancer cells. A short-term incubation with analogue reduced the KGF-induced tyrosine phosphorylation of a 120 kDa protein, corresponding to the KGF receptor, a rapid and direct cross-talk between these two molecules was suggested [58,59]. In addition, we found an up-regulation of KGF expression in breast cancer cells by Vitamin D [60].

Although HGF is known to stimulate the growth of normal epithelial cells, including those from prostate, Qadan et al. reported on HGF-inhibited ALVA-31 and DU 145 hormone-refractory cell lines. Moreover, HGF and Vitamin D additively inhibited growth in each androgen-unresponsive cell line, with the greatest growth inhibition in ALVA-31 cells [61]. Further studies in ALVA-31 cells revealed distinct co-operative actions of HGF and Vitamin D. Cell cycle redistribution (decrease of the fraction of cells in G1, with a corresponding increase in the later cell cycle phases instead of the accumulation of cells in G1 phase of the cell cycle seen during Vitamin D inhibition of androgen-responsive LNCaP cells) suggested that in androgen-unresponsive prostate cancer cells, HGF and Vitamin D act together to slow cell cycle progression via control at sites beyond the G1/S checkpoint, the major regulatory locus of growth control in androgen-sensitive prostate cells.

Table 1
Summary of the growth factors regulated by Vitamin D in prostate

Growth factor family	Factors regulated	Cellular function	References
IGF	IGFBP-3 expression↑	Proliferation↓	[51–53]
	IGFBP-6 expression↑	Growth inhibition	[80]
	IGF-II expression↓	Proliferation↓	[51]
TGF-β	TGF-β1, TGF-β2 and TGF-β3 expression↑	Differentiation↑, proliferation↓	[55,56]
	TGF-β receptor expression↑		[55]
	PDF expression↑	Growth inhibition	[57]
FGF	Counteraction of the mitogenic activity of KGF	Proliferation↓	[58,59]
HGF	Cooperation with the growth inhibitory effect of HGF	Proliferation↓	[61]
VEGF	PDGFR-β expression↓	Growth inhibition	[63]

PDGF, a member of VEGF family of angiogenic factors, is produced mostly by epithelial cells of human prostate, but acts on prostate stromal cells. It has been shown that cultured human prostate cells derived from patients exhibiting BPH express high affinity PDGFRβ what is activated in response to PDGF-BB isoform, which lead to the activation of mitogenesis [62]. We found that calcitriol down-regulates the expression of PDGFRβ mRNA in prostate primary stromal and LNCaP prostate cancer cells, suggesting a role for cross-talk with PDGF in anti-proliferative action of Vitamin D [63].

6. Role of fatty acid metabolism

Fatty acid synthase (FAS/FASN), a key metabolic enzyme involved in the de novo biosynthesis of fatty acids, has been found to be a potential anticancer target [64,65]. The other key enzyme in fatty acid metabolism is mitochondrial Acyl-CoA (ACS; or fatty acid CoA ligase; FACL). Fatty acid synthesis is controlled by a long and a short-term regulations. The long-term regulation of fatty acid synthesis occurs through alterations in the rate of synthesis of acetyl-CoA carboxylase (ACC), the first and rate-limiting enzyme of the fatty acid synthesis, and that of fatty acid synthase (FAS), the second and a key enzyme of the fatty acid synthesis [66–68]. The short-term regulation involves cellular fatty acyl-CoAs [69], but the mechanisms are not fully understood. Inhibition of the de novo fatty acid synthesis by free fatty acids appears to depend on the formation of long chain fatty acyl-CoAs [70]. Long chain fatty acyl-CoAs have shown to inhibit ACC [71–74] and FAS [75]. It is proposed that long chain fatty acyl-CoAs exert their effects on ACC and FAS by means of feedback inhibition [76]. It is further suggested that the inhibitory effect of long chain fatty acyl-CoA on the fatty acid synthesis may be due to its regulation of lipogenic enzymes in a feedback manner through suppression of the gene transcription [77].

We have recently found that FAS, which is over-expressed and associated with prostate cancer development, was down-regulated by Vitamin D_3 in LNCaP cells [78]. We have also found that FACL3, which utilizes preferentially myristic acid, eicosapentaenoic acid (EPA), and arachidonic acid as substrates to form long chain fatty acyl-CoAs, was upregulated by Vitamin D_3, contributing to the growth inhibitory effect of Vitamin D_3 in LNCaP cells [79]. FACL3/ACS3 is a dominant isoform of FACL/ACS expressed in LNCaP cells as indicated by measuring the relative expression of each isoform [79]. Furthermore, our study showed that calcitriol had no significant effect on the expression of FACL1 (FACL2), FACL4 and FACL6 except for its down-regulation of FACL5 at 24 and 48 h by around 2-fold. The up-regulation of FACL3/ACS3 expression by calcitriol was accompanied with increased activity of FACL/ACS as demonstrated by enzyme activity assay using a ^{14}C-labeled substrate preferential for FACL3/ACS3. The growth inhibitory effect of calcitriol on LNCaP cells was significantly attenuated by FACL3/ACS3 activity inhibitor. Androgen withdrawal, in the presence of anti-androgen casodex or in AR-negative prostate cancer cells (PC3 and DU145), Vitamin D_3 failed to regulate FACL3/ACS3 expression. The up-regulation of FACL3/ACS3 expression by Vitamin D_3 was recovered by the addition of DHT in dextran coated charcoal treated serum medium. Western blot analysis showed that the expression of androgen receptor (AR) protein was consistent with Vitamin D_3 regulation of FACL3/ACS3 expression. Taken together, our data suggest that the up-regulation of FACL3/ACS3 expression by Vitamin D_3 is through an androgen/AR-mediated pathway and may contribute to the Vitamin D_3 anti-proliferative effect in prostate cancer LNCaP cells. A schematic presentation of Vitamin D regulation of fatty acid metabolism and cell proliferation is shown in Fig. 3. In summary, Vitamin D seems to act androgen-dependently directly on the expression of FACL3 leading to an increase of Acyl-CoA, which, in turn, inhibits FAS expression. A decreased long-chain fatty acid synthesis may trough unknown regulatory processes to decreased cell proliferation and increased apoptosis.

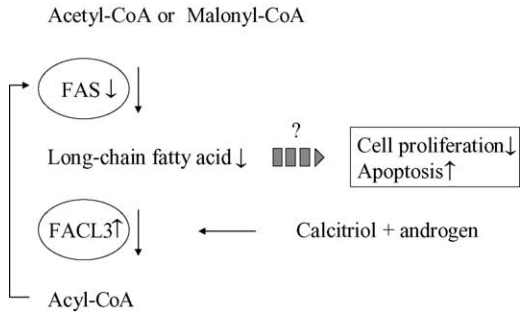

Fig. 3. A schematic presentation of Vitamin D regulation of fatty acid metabolism and cell proliferation. The primary target of Vitamin D seems to be an androgen-dependent regulation of the expression of FACL3 leading to a down-regulation of FAS expression [78,79].

7. Conclusions

Vitamin D has a potent growth inhibitory function in prostate cancer with a multitude of mechanisms. It can regulate cell cycle regulatory proteins [3], expression of growth factors and their receptors, nuclear receptor expression and its own local metabolism as well as fatty acid metabolism.

Vitamin D, especially the main metabolite, calcidiol, can regulate prostate growth as well as it can also induce apoptosis or sensitize cells for apoptosis. Thus Vitamin D has a wide range of potential therapeutic uses in treating cellular defects characterized by increased cell proliferation and decreased differentiation not only prostate cancer. Epidemiological studies have demonstrated that Vitamin D may exert a protective effect against prostate carcinogenesis by regulating cancer initiation, promotion and progression [6]. However, also a high serum calcidiol levels seems to be a risk factor

for prostate cancer [26]. In both situations, there seems to be an insufficiency of Vitamin D action.

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