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Interaction of nuclear receptor ligands with the Vitamin D signaling pathway in prostate cancer

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Abstract

A number of hormonal ligands and/or the nuclear receptors that mediate their actions have been targeted for prostate cancer therapy. Androgens, the ligands for the androgen receptor (AR), are critical for the growth of prostate cancer. Inhibition of androgen production has been the mainstay of treatment for advanced prostate cancer for decades. Other more recently tested targets include retinoid receptors (RAR and RXR), glucocorticoid receptors (GR), estrogen receptors (ER) and peroxisome proliferator-activated receptors (PPAR). Calcitriol, acting through the Vitamin D receptor (VDR), has many tumor suppressive activities in the prostate, including inhibition of proliferation, induction of apoptosis and/or differentiation, and reduction of cellular invasion. Because of these properties, calcitriol and its less hypercalcemic analogs are being evaluated as agents to prevent or treat prostate cancer. Androgens, retinoids, glucocorticoids, estrogens and agonists of PPAR directly or indirectly impact Vitamin D signaling pathways, and vice versa. In order to design the most effective strategies to use calcitriol to prevent or treat prostate cancer, the interactions of other nuclear receptors and their ligands with the Vitamin D signaling pathway need to be considered.

Keywords: Prostate cancer; Vitamin D; Nuclear receptors; Glucocorticoids; Retinoids; IGFBP-3

1. Introduction

Androgen deprivation and/or blockade of androgen receptor (AR) activity is classically used to treat prostate cancer [1]. Other nuclear receptors besides AR have been targeted for therapy by retinoids [2–5], glucocorticoids [6,7], estrogens [8] and thiazolidinedione agonists of peroxisome proliferator-activated receptor (PPAR)γ [9]. While most of these ligands show some activity against prostate cancer, efficacy is often limited by toxicity. This is true as well for the ligand of the Vitamin D receptor (VDR), 1,25-dihydroxyVitamin D_3 (calcitriol). Although calcitriol exerts a multitude of anti-tumor activities against cultured prostate cancer cells and xenografts, activity in humans is limited by hypercalcemia [10]. To improve the therapeutic index of calcitriol, current strategies focus on therapies that include intermittent dosing regimens, calcitriol in combination with other agents, including nuclear receptor ligands, or the use of less calcemic analogs of calcitriol. These strategies are based on limited, but evolving, knowledge of the interactions of other nuclear receptor signaling pathways with Vitamin D in the prostate.

2. Vitamin D

2.1. Calcitriol

Calcitriol is the physiologically active metabolite of Vitamin D. Normal and malignant prostatic epithelial cells express VDR, and activation of VDR by calcitriol generally results in inhibition of proliferation and cell cycle arrest [10]. There is some evidence that calcitriol increases differentiation of prostatic epithelial cells, stimulates apoptosis and inhibits invasive and migratory properties of prostate cancer cell lines [10]. The ability of calcitriol to inhibit prostate growth has been demonstrated in primary cultured cells from normal tissues, benign prostatic hyperplasia (BPH) and prostate cancer, multiple prostate cancer cell lines and several xenograft models of prostate cancer [10]. The mechanism of growth inhibition by calcitriol appears to be mutifactorial but

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induction of $p21^{WAF1/CIP1}$ and/or $p27^{Kip1}$ seems to be a major pathway [11–13].

Initial clinical trials of calcitriol in prostate cancer patients indicated some anti-cancer activity in that the increase in rise of serum prostate-specific antigen (PSA), a biochemical marker of progression of prostate cancer, was slowed by treatment in some individuals or declined in others [10]. However, the ability to raise serum levels of calcitriol was limited by hypercalciuria. Trump and co-workers [14] used intermittent dosing of calcitriol (three times per week) combined with glucocorticoids to diminish hypercalcemia with some success (see below). This group has also combined calcitriol with various chemotherapy agents and shown some promising results [15,16]. Recently, Beer et al. [17] have devised an intermittent method of dosing once weekly that permits treatment with extremely high doses of calcitriol, while causing only transient hypercalcemia apparently without toxicity. The responses of patients receiving this weekly bolus delivery are encouraging, and other recent trials suggest that combination of high doses of calcitriol with drugs such as docetaxol may be even more efficacious [18].

2.2. Vitamin D analogs

Analogs of Vitamin D that are less calcemic but equally or more effective than calcitriol at inhibiting growth or mediating other anti-tumor effects have been synthesized and studied [10]. The mechanism for the differential activity is not completely clear but relates to several different activities. These include pre-receptor differences in pharmacokinetics, as well as differences in the functional conformation of the ligand-bound VDR complex which can alter properties of RXR hybridization, DNA binding and co-activator recruitment [19] (see below). Very recently Bettoun et al. [20] described a “phantom ligand effect” whereby VDR ligands enabled the recruitment of the p160 family of co-activators to RXR itself. This phenomenon may account for the ability of some Vitamin D analogs to elicit enhanced VDR heterodimer formation with RXR and contribute to increased potency of the analogs.

Vitamin D analogs have been extensively tested in vitro and in xenograft models [10]. Many can be used at higher concentrations than calcitriol and yet still exhibit less hypercalciuria and hypercalcemia, while inducing antiproliferative effects that exceed those of calcitriol. The ability to use higher concentrations than calcitriol with less toxicity bodes well for this approach. Clinical trials are currently underway with several analogs [15].

3. Retinoids

3.1. All-trans retinoic acid (ATRA)

ATRA, the physiologically active retinoid, is a ligand for retinoic acid receptor (RAR)-α, -β and -γ [21]. As a single agent, ATRA generally inhibits the proliferation of cultured normal and malignant prostatic epithelial cells at concentrations of 1 nM or higher, but instances of growth-stimulatory activity of lower concentrations of ATRA have been reported [22–24]. Like calcitriol, ATRA may increase differentiation of prostatic epithelial cells. When primary cultures of normal or malignant prostatic epithelial cells were treated with ATRA, cytokeratins 8 and 18, markers of differentiated secretory epithelial cells, were increased [25]. ATRA, like calcitriol, also increased the expression of PSA in the prostate cancer cell line LNCaP [22,24,26]. Another example of similar effects of retinoids and Vitamin D compounds on prostate cells is the increase in synthesis of insulin-like growth factor (IGFBP)-3 that occurs in response to ATRA [27] or calcitriol [28–31]. IGFBP-3 has been implicated as a primary mediator of the growth inhibition of the prostate cancer cell line LNCaP by calcitriol [30]. IGFBP-3 has recently been shown to enter the nucleus and bind to RXR, enhancing RXR-mediated activity [32]. Therefore, IGFBP-3 may be a key element in a loop in which nuclear receptor ligands induce IGFBP-3 expression, then IGFBP-3 enhances the activity of nuclear receptor heterodimers with RXR. Since IGFBP-3 is induced by calcitriol, these downstream events may relate to calcitriol’s action in prostate cells. Similarly, IGFBP-3 may be the molecular focal point of cross-talk between nuclear receptors. Synergistic growth-inhibitory interactions of ATRA and Vitamin D compounds on normal and malignant prostatic epithelial cells have been reported, for example [33], and possible mechanisms of interaction, including induction of IGFBP-3, are discussed below.

3.2. 9-cis retinoic acid (9cRA)

9cRA binds to RARs as well as to retinoid X receptors (RXR)-α, -β and -γ [21]. Many studies show additive or synergistic effects of Vitamin D compounds and 9cRA on prostate cancer as well as other types of cells [34,35]. In a detailed analysis, Elstner et al. [36] showed that 9cRA in combination with a Vitamin D analog caused synergistic and irreversible growth inhibition of LNCaP cells, increased cell cycle arrest in G0/G1, elevated expression of the cell cycle regulators $p21^{WAF1/cip1}$ and $p27^{kip1}$, and prevented the formation of LNCaP colonies on bone marrow stroma. Like ATRA and calcitriol, 9cRA increases PSA in LNCaP cells [24]. 9cRA and a specific RAR agonist, but not an RXR agonist, also induced IGFBP-3 in LNCaP cells [27]. 9cRA, like calcitriol, also increased AR protein in LNCaP cells, and growth inhibition and induction of PSA in LNCaP cells by 9cRA was blocked by Casodex, an antagonist of AR [35] (see below).

Ikeda et al. [37] recently reported that treatment of prostate cancer cell lines with calcitriol and 9cRA in combination inhibited human telomerase reverse transcriptase (hTERT) transcription as well as telomerase activity. This phenomenon did not occur with either agent alone, and was shown to depend on direct interaction of the VDR/RXR heterodimer with

the DR3′ sequence in the hTERT gene promoter. Combination of calcitriol with an RXR selective ligand, PA024, achieved the same result. These investigators also showed that calcitriol and 9cRA together inhibited growth of PC-3 cell cultures and xenografts better than either agent alone. The effects of these agents may be an illustration of the induction of senescence through inhibition of telomerase as a tumor suppressor mechanism.

3.3. Other retinoids

Fenretinide [*N*-(4-hydroxyphenyl)retinamide] has been tested in clinical trials to treat prostate cancer with rather limited effect [38], despite antiproliferative activity against prostate cancer cell lines [39–41]. Chemopreventive activity of fenretinide was demonstrated in a rat model of androgen-promoted carcinomas of the seminal vesicle and prostate by some investigators [42]. The Vitamin D analog Ro24-5531 was also moderately effective at inhibiting the development of carcinomas in this model, but the retinoid and Vitamin D analog were not evaluated in combination for possible additive or synergistic activity. Others did not find that fenretinide protected rats from prostate cancer [43], and the results of a chemopreventive trial to reduce the incidence of prostate cancer with fenretinide were not encouraging [44].

Nuclear receptors indirectly regulate other transcriptional pathways via AP-1 repression. Dimerization of the AP-1 proteins, c-jun and c-fos, into an active form is generally associated with transactivation of genes associated with proliferation. Some nuclear receptors, including RAR-α, negatively regulate AP-1 activity by down-regulating c-fos. A synthetic retinoid, SR11238, with predominately anti-AP-1 activity and minimal ability to transactivate through a retinoic acid response element (RARE), synergistically inhibited the growth of both the androgen-responsive prostate cancer cell line LNCaP as well as the androgen-independent cell line PC-3 in combination with a Vitamin D analog [45]. Transactivation of the osteocalcin Vitamin D response element (VDRE) by the Vitamin D analog was not enhanced by SR11238, but the expression of E-cadherin was additively upregulated by both compounds. Since loss of E-cadherin is associated with poor prognosis for prostate cancer [46,47], restoration of E-cadherin might contribute to tumor suppression by this combination therapy [48].

3.4. Mechanisms of interaction of retinoids and Vitamin D compounds

Both VDR and RAR form heterodimers with RXRs [49]. Of the three subtypes of RXR (-α, -β and -γ), RXRα is the most important for VDR activity [20,50]. Responses of individual cell types to combinations of Vitamin D compounds and retinoids depend on the constituency of nuclear receptors in the cells. The absence of RAR-β, the loss of which is common in prostate and other cancers [51], alters the outcome of treatment with Vitamin D as well as retinoids [52].

The mechanisms responsible for additive or synergistic activities of Vitamin D compounds and retinoids are likely multifactorial. Some effects might occur through activation of VDRE and RARE in combination in target genes. For example, $p21^{WAF1/CIP1}$ has both VDRE and RARE and is upregulated by both Vitamin D and retinoids in some cells.

A molecule implicated in growth-inhibitory activity of both retinoids and Vitamin D compounds in prostate cells is transforming growth factor (TGF)-β. Either ATRA or calcitriol inhibited the growth of an immortal but nonmalignant rat prostatic epithelial cell line, NRP-152, and increased synthesis of TGF-β [53]. Combined treatment resulted in additive growth inhibition and increased production of TGF-β. Regulation of expression of TGF-β by calcitriol is probably mediated by VDRE in the promoter of the TGF-β gene [54].

Some interactions, although not all, necessitate activation through RXR. Levels of RXR have been shown to influence cellular sensitivity to both Vitamin D compounds and retinoids [55]. In some cases, potentiation of activity occurs through reciprocal upregulation of the receptor. Zhao et al. [56] investigated the relationship between the ability of Vitamin D analogs to induce heterodimerization of VDR with RXR and the level of transcriptional activation of the analogs. They concluded that the level of transcriptional activation correlated well with the strength of VDR–RXR heterodimerization for the most part, but that the potency of the analogs was not fully revealed by heterodimerization activity.

Modifications of RXRα may affect signal transduction by calcitriol. For example, mitogen-activated protein kinase (MAPK) was shown to inhibit growth-inhibitory activity of calcitriol by phosphorylating RXRα [57]. Phosphorylation of RXRα disrupts VDR–RXRα heterodimer formation [58]. This finding likely explains resistance of *ras*-transformed prostate cell lines to growth inhibition by calcitriol, and may be a factor in the resistance of other prostate cancer cell lines as well. This observation is also relevant to therapeutic activity of calcitriol, since several publications suggest that MAPK activity is elevated in advanced prostate cancer [59,60].

Synergistic activity of retinoids and Vitamin D compounds may also occur through modulation of receptor levels. ATRA, for example, upregulates VDR protein [61].

4. Androgens

4.1. Androgen activity in the prostate

Androgens are essential for the development of the prostate and for maintenance of normal growth and differentiation of the adult prostate [62]. Androgens are also required for the initiation and progression of prostate cancer, and androgen deprivation therapy has long been the mainstay of treatment for advanced prostate cancer [1]. Androgen action is mediated by the AR, which is expressed predomi-

nantly in differentiated secretory cells of the benign prostatic epithelium and in cancer cells. Prostatic stromal cells also express AR [63] and may release cytokines when stimulated by androgens that promote growth and differentiation of epithelial cells [64]. Eventual failure of prostate cancer patients to respond to androgen deprivation and their progression to androgen-independent disease is usually not accompanied by loss of AR, but rather by mutations in the AR that alter ligand specificity or other alternate means of activating the AR [65].

4.2. Interactions of androgen and Vitamin D

Many of the effects of Vitamin D compounds on androgen-responsive prostate cancer cells appear to be mediated through effects of Vitamin D on the AR. Treatment of the androgen-responsive prostate cancer cell line LNCaP with calcitriol resulted in upregulation of AR mRNA and protein, increased AR nuclear localization, and increased ligand binding [24,66–68]. The AR is not a direct target of Vitamin D, however, and induction of AR by calcitriol is mediated by an unidentified intermediate protein [35]. In the LNCaP cell line, recent cDNA microarray experiments revealed that some target genes regulated by calcitriol are also androgen target genes [69]. In particular, both calcitriol and androgens decreased growth, increased expression of PSA [10] and decreased expression of fatty acid synthase (FAS) [70]. These responses to Vitamin D compounds all appear to be mediated, at least in part, by androgen action via the AR. Down-regulation of FAS could be an important element in anti-cancer activity of Vitamin D, since FAS is overexpressed in prostate cancer [71] and has been associated with promotion of cancer growth.

The induction of IGFBP-3 was discussed above as a mediator of the antiproliferative effects of Vitamin D in prostate cells. If calcitriol's effects on prostate cells occur through upregulation of the AR, then it might be expected that androgen would also upregulate IGFBP-3. Androgen, however, reportedly decreased expression of IGFBP-3 in LNCaP cells regardless of whether cells were treated with growth-stimulatory or -inhibitory concentrations of androgen [27], although others found that IGFBP-3 was enhanced in similar studies [72]. The effects of androgen on IGFBP-3 expression in prostate cells require further investigation.

Whether Vitamin D affects AR in nonmalignant prostatic epithelial cells is unclear. Treatment of the immortal but nonmalignant prostatic epithelial cell line 267B-1 (SV_{40}-transformed neonatal cells) with supraphysiologic levels of calcitriol increased levels of AR protein at 24 h [73]. Calcitriol was noted to upregulate its own receptor as has been the usual pattern [61]. The active metabolite of androgen in the prostate, dihydrotestosterone (DHT), increased VDR binding activity. These studies, therefore, suggest cross-talk between nuclear receptor signaling pathways at the level of receptor regulation. Another group reported that a calcitriol analog blocked androgen-induced growth proliferation in stromal cells cultured from BPH, but effects of the Vitamin D analog on levels of AR were not investigated [74].

The interaction of androgen and Vitamin D in regulating the growth and development of the adult rat prostate was also investigated [75]. Administration of calcitriol for 3 weeks to intact animals decreased prostate size by 40%. Histological changes were interpreted as loss of epithelium as well as stroma. In rats that had been castrated 1 week prior to the administration of calcitriol, prostate size was not changed. However, histological examination indicated that there was a loss of cytoplasmic area of the epithelium, but an increase in the stroma. A growth-stimulatory effect of calcitriol on cultured human prostatic stromal cells was previously reported by these investigators [76], although others found that calcitriol inhibited similar cells [77]. AR and VDR protein levels were both increased in the prostates of Vitamin D-treated, intact rats, whereas only AR was increased by Vitamin D in the prostates of castrated rats.

Other studies suggest that calcitriol has an "imprinting" effect on the prostate [78], as has been described for estrogen [79]. Prenatal exposure to a high dose of calcitriol influenced growth and development of the rat prostate [78]. At prepuberty, the prostatic weight of calcitriol-exposed animals was 35% greater than controls. In adults, prostatic weight of exposed animals was 68% greater than controls. Administration of calcitriol just before puberty was not found to significantly influence prostatic growth in the presence of endogenous or exogenously administered DHT [80].

In adult rats, calcitriol cooperated with testosterone to induce differentiation of normal prostatic epithelial cells [81]. In intact or castrated rats, calcitriol had a regressive effect on the epithelium as well. Interestingly, stromal volume was increased in treated animals, especially in castrated rats, as mentioned above.

4.3. Androgen-independent activity of calcitriol

Certainly all activity of Vitamin D on prostatic epithelial cells is not mediated through effects on the AR, since AR-negative cells, such as primary cultures or PC-3 cells, are responsive to Vitamin D [10]. Even in cells that express AR, some or all of the actions of Vitamin D occur by AR-independent mechanisms. For example, MDA PCa cells express AR but respond to calcitriol in an androgen-independent manner [82]. LNCaP-104R1, an AR-expressing, androgen-independent variant of LNCaP cells, remained responsive to growth inhibition by calcitriol and this effect was not blocked by the AR antagonist Casodex [83]. Similarly, introduction of AR into a Vitamin D-resistant prostate cancer cell line, ALVA 31, did not restore sensitivity to Vitamin D [83].

It is interesting that introduction and stable expression of AR in PC-3 cells enhanced sensitivity to ATRA, but not to calcitriol [84]. However, in this study, androgen and calcitriol additively inhibited growth of AR-expressing PC-3, as noted previously for LNCaP cells.

5. Glucocorticoids

5.1. Glucocorticoids and prostate cancer

Glucocorticoids are often used to treat advanced prostate cancer, either as a single agent or in combination with other drugs [6]. Despite the widespread application of this therapeutic approach, the mechanism of anti-tumor activity is not clear [6]. Glucocorticoids suppress adrenal androgen secretion, and therefore, may contribute to suppression of prostate cancer growth by enhancing androgen deprivation. Alternatively, glucocorticoids might directly affect prostate cancer growth through the glucocorticoid receptor (GR). While some prostate cancer cell lines express GR [85,86], GR expression in prostate cancer tissues may be low [87]. In addition, the observed effects of glucocorticoids on prostate cancer cells are not always consistent with anti-tumor activity. For example, glucocorticoids may stimulate, rather than inhibit, growth of prostate cancer cells in culture [88]. A striking example is the effect of glucocorticoids on the prostate cancer cell lines MDA PCa 2a and 2b. Derived from a bony metastasis of an individual with androgen-independent cancer, the cell lines have a doubly mutated AR (L701H and T877A) that confers high affinity for cortisol and cortisone [89]. These glucocorticoids, acting via the mutant AR, stimulate proliferation and secretion of PSA in MDA PCa cell lines, and one speculates that glucocorticoids were driving the progression of cancer in the patient of origin.

The T877A mutant AR found in LNCaP cells [90] is thought to be quite common in androgen-independent prostate cancer, since it can be selected for by treatment with the anti-androgen flutamide [91]. The mutation widens the range of hormones that bind to the AR and allows flutamide to act as an agonist [90]. Recently, it was shown that cells with the T877A mutation also can be stimulated to grow by adrenal corticosteroids, such as deoxycorticosterone (DOC) and corticosterone [92]. Suppression of these adrenal secretion products by exogenous glucocorticoid administration, which suppress adrenocorticotrophic hormone (ACTH) secretion from the pituitary, is another pathway by which the administration of exogenous glucocorticoids may inhibit the growth of androgen-independent prostate cancers harboring mutant ARs [65].

Recently, it was shown that whereas cortisol, cortisone and dexamethasone stimulated the growth of MDA PCa 2a and 2b cells carrying the double AR mutation (T877A and L701H), triamcinolone did not. This finding raised the possibility that triamcinolone might be an especially effective synthetic glucocorticoid for patients with mutant ARs [89].

5.2. Effects of glucocorticoids on Vitamin D activity

Glucocorticoids inhibit calcium absorption [93], modulate VDR concentration [61] and have been used to treat hypercalcemia [93]. Because of their therapeutic and/or palliative activity against prostate cancer and ability to reduce the hypercalcemic toxicity of Vitamin D, glucocorticoids combined with calcitriol are being tested in clinical trials to treat prostate cancer [15]. However, it is worth considering that effects of glucocorticoids on Vitamin D activity are varied and species-specific as well as cell-specific. Although a glucocorticoid response element (GRE) has been identified in the promoter region of the VDR gene [94], VDR levels are not consistently changed by glucocorticoids among different types of cells. The VDR in mouse and rat tissues exhibit divergent responses to glucocorticoids [61] with VDR in rat bone and intestine up-regulated, while mouse bone and intestine VDR were down-regulated. On the other hand, dexamethasone was reported to enhance anti-tumor activity of calcitriol and increase VDR protein in murine squamous cell carcinoma cells [95]. In human cells, dexamethasone potentiated the antiproliferative activity of calcitriol in PC-3 prostate cancer cells [96]. In contrast, hydrocortisone reduced the growth-inhibitory action of calcitriol on primary cultures of human prostate cancer cells but did not change VDR levels [33]. Interactions of glucocorticoids and Vitamin D may occur at levels other than nuclear receptor cross-talk, and Bernardi et al. [97] have suggested involvement of Erk/Akt signaling pathways in antiproliferative effects of combined glucocorticoids and calcitriol.

Nevertheless, initial findings in a Phase II clinical trial of combined dexamethasone and calcitriol in patients with advanced (androgen-independent) prostate cancer are promising. A >50% reduction in serum PSA was seen in 5 of 24 patients and a decrease in PSA velocity was noted in the remaining 19 patients [15].

6. PPARγ agonists

6.1. Activity of PPARγ in the prostate

Ligands of PPARγ, such as thiazolidinediones, have recently attracted interest as potential cancer therapeutic agents. These drugs are currently in clinical use as anti-diabetic agents due to their enhancement of insulin sensitivity and regulation of lipid and lipoprotein metabolism. However, the many anti-cancer functions of PPARγ agonists have prompted clinical trials to assess chemotherapeutic activity of these agents.

In contrast to retinoids or Vitamin D compounds, PPARγ agonists decrease, rather than increase, PSA production in LNCaP cells [98]. This effect on PSA may be due to the ability of PPARγ agonists to inhibit androgen activation of an androgen response element (ARE) in the regulatory region of the PSA gene. PPARγ agonists do not, however, bind AR. The phenotype induced in prostate epithelial cells upon activation of PPARγ is distinctive and unlike that induced by retinoids or Vitamin D compounds. PPARγ agonists inhibit growth similarly to retinoids or Vitamin D, but after an extended period of exposure to PPARγ ligands, numerous vacuoles develop in the cytoplasm of treated prostatic cells [9,99]. These vacuoles are negative for lipids, unlike those

that develop in adipocytes after activation of PPARγ, and their significance is not yet clear.

PPARγ, like VDR and RAR, heterodimerize with RXR. PPARγ ligands in combination with 9cRA have been shown to synergistically inhibit proliferation of myeloid leukemic cell lines [9], but retinoids or Vitamin D compounds in combination with PPARγ agonists have not yet been tested on prostate cancer cells to our knowledge.

7. Nuclear receptor cofactors

Co-activator and co-repressor proteins modulate the activity of nuclear receptors, including the VDR, and determine their ability to increase the expression of target genes by regulating RNA polymerase [100]. One important function of co-activators is to remodel chromatin to loosen the repressive effect of chromatin on gene expression. The SRC/p160 family of co-activators contains histone acetyl-transferase (HAT) activity that allows VDR and other nuclear receptors to activate target genes. This activity is reversed by molecules with histone deacetylase (HDAC) activity. The relative insensitivity of some prostate cancer cell lines to growth-inhibitory activity of calcitriol or Vitamin D analogs was increased by co-treatment with inhibitors of HDAC [101]. This finding suggests that the dysregulation of nuclear receptor co-activators and co-repressors that occurs in cancer may attenuate response to Vitamin D and other hormones, but that this may be overcome through the use of HDAC inhibitors, such as sodium butyrate or trichostatin A. Inhibitors of HDAC, such as phenylacetate, have shown activity in clinical trials against prostate cancer [102].

8. Proteasomes

Both RXR and VDR proteins are degraded by proteasomes [103,104]. The use of proteasome inhibitors for cancer therapy has been of growing interest [105]. The potent and specific inhibitor of the 26S proteasome, Bortezomib, has shown anti-tumor activity in prostate cancer cell lines and is currently in clinical trials [106]. Such inhibitors may have a role in combination therapy with Vitamin D and/or retinoids. Prufer et al. [55] demonstrated the efficacy of such an approach by showing that proteasome inhibitors increased expression of RXR and VDR and restored the response of osteosarcoma cells to growth-inhibitory effects of calcitriol and 9cRA.

9. Conclusions

As knowledge of molecular targets and signaling pathways of nuclear receptors becomes more detailed, investigators will be able to implement rationale strategies to use agonists or antagonists of these receptors to prevent or treat prostate cancer more effectively. An important consideration will be the cell-specific effects of these receptors and their co-factors, and additional preclinical studies are needed with realistic cell culture and animal models of prostate cancer. Recent clinical trials of calcitriol in prostate cancer are promising, and combination therapies that target nuclear receptors that impact Vitamin D signaling pathways may enhance activity.

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