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The role of Vitamin D_3 metabolism in prostate cancer

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Abstract

Vitamin D deficiency increases risk of prostate cancer. According to our recent results, the key Vitamin D hormone involved in the regulation of cell proliferation in prostate is 25(OH) Vitamin D_3. It is mainly acting directly through the Vitamin D receptor (VDR), but partially also through its 1α-hydroxylation in the prostate. A deficiency of 25(OH) Vitamin D is common especially during the winter season in the Northern and Southern latitudes due to an insufficient sun exposure, but Vitamin D deficient diet may partially contribute to it. A lack of Vitamin D action may also be due to an altered metabolism or Vitamin D resistance. Vitamin D resistance might be brought up by several mechanisms: Firstly, an increased 24-hydroxylation may increase the inactivation of hormonal Vitamin D metabolites resulting in a Vitamin D resistance. This is obvious in the cancers in which an oncogenic amplification of 24-hydroxykase gene takes place, although an amplification of this gene in prostate cancer has not yet been described. During the aging, the activity of 24-hydroxylase increases, whereas 1α-hydroxylation decreases. Furthermore, it is possible that a high serum concentration of 25(OH)D_3 could induce 24-hydroxylase expression in prostate. Secondly, Vitamin D receptor gene polymorphism or defects may result in a partial or complete Vitamin D resistance. Thirdly, an overexpression or hyperphosphorylation of retinoblastoma protein may result in an inefficient mitotic control by Vitamin D. Fourthly, endogenous steroids (reviewed by [D.M. Peehl, D. Feldman, Interaction of nuclear receptor ligands with the Vitamin D signaling pathway in prostate cancer, J. Steroid Biochem. Mol. Biol. (2004)]) and phytoestrogens may modulate the expression of Vitamin D metabolizing enzymes. In summary, the local metabolism of hormonal Vitamin D seems to play an important role in the development and progression of prostate cancer.

Keywords: Calcidiol, Calcitriol, Cancer, Tissue concentration; Endocrine system

1. Introduction

Although Vitamin D is still best known from its role as a regulator of calcium and phosphorus balance, there is an increasing interest in other hormonal effects of Vitamin D in all organs and cells of the body. Regulation of many cell types with respect to their function, growth, differentiation and apoptosis are controlled by Vitamin D.

Vitamin D was originally found as a plant-derived antirachitic agent, which was named Vitamin D_2 (ergosterol). However, the subsequent characterization of structure and intrinsic synthesis of animal form, termed Vitamin D_3 (cholecalciferol), which formed a basis of the hormonal Vitamin D [2,3]. In human system, the production of Vitamin D_3 in skin is crucial, since nutrional supply of Vitamin D_3 as well as Vitamin D_2 is limited. The production of Vitamin D_3 begins from plasma membrane of skin cells where 7-dehydrocholesterol is photolyzed by UV light to produce previtamin D_3 which undergoes thermal isomerization to Vitamin D3. The synthesis of previtamin D_3 and Vitamin D_3 are self-controlled processes since further absorption of UV light causes isomerizations of these compounds to yield inactive products [4]. Conversion of 7-dehydrocholesterol to previtamin D_3 is decreased to less than half in elderly people [5]. From skin Vitamin D_3 enters to circulation where all Vitamin D compounds are mainly bound to Vitamin D binding protein (DBP) [6].

In the body, both Vitamin D_2 and D_3 undergo similar activation processes, which are prerequisite for their biological activity. Basic activation route of Vitamin D (hereafter Vitamin D_3) involves two successive hydroxylations catalysed by cytochrome p450 enzymes (Fig. 1). The first hydroxylation at the position C-25 occurs in liver by the mitochondrial sterol 27-hydroxylase (27-hydroxylase; CYP27A1) to yield 25-hydroxyvitamin D_3 (25OHD_3), which is the major circulating form of Vitamin D [7]. Second hydroxylation at po-

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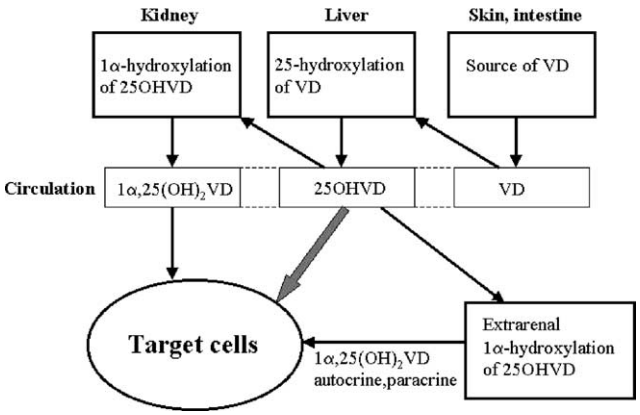

Fig. 1. A schematic presentation of Vitamin D activation metabolism. The figure points out the central role of 25-hydroxyvitamin D in extrarenal Vitamin D endocrine system.

sition C-1 occurring in kidney by 25-hydroxyvitamin D_3 1α-hydroxylase (1α-hydroxylase; CYP 27B1) produces the most active form of Vitamin D namely 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$) [8]. The serum values for $1\alpha,25(OH)_2D_3$ are approximately 1000 -fold less to that of $25OHD_3$. The above described route of Vitamin D activation has later been complicated by the studies revealing several tissue types to express 1α-hydroxylase and, thus, being capable for extrarenal production of $1\alpha,25(OH)_2D_3$ [9]. Among this skin has been shown to express all enzymes needed to exhibit autonomous production $1\alpha,25(OH)_2D_3$ [10]. In addition, there is evidence that it is actually microsomal 25-hydroxylase activity that plays a major role for production of $25OHD_3$ in liver [11]. Recently, a novel enzyme displaying microsomal 25–hydroxylase activity has been characterized in human by Cheng et al. [12].

Inactivation of $1\alpha,25(OH)_2D_3$ and $25OHD_3$ is catalyzed by mitochondrial 25-hydroxyvitamin D_3 24-hydroxylase (24-hydroxylase; CYP24) [13]. This enzyme sequentially hydroxylases $25OHD_3$ or $1\alpha,25(OH)_2D_3$ starting at C23 or C24 positions finally yielding more hydrophilic product for excretion [14]. 24-Hydroxylase is abundantly expressed in kidney but most probably all Vitamin D target cells express the enzyme [15]. In kidney, $1\alpha,25(OH)_2D_3$ itself among many other regulatory factors is involved in coordination its own synthesis and inactivation being strong inducer of 24-hydroxylase while inhibiting 1α-hydroxylase expression [15,16].

The hallmark in functional study of Vitamin D action was cloning of the respective receptor for $1\alpha,25(OH)_2D_3$, Vitamin D receptor (VDR) which was found to act as ligand-inducible transcription factor and mediate effects of Vitamin D on target gene transcription [17,18]. The studies revealing ubiquitous expression of VDR provided evidence for its central role in pleiotropic action of Vitamin D [19,20]. Later works including structural characterization of DNA binding regions for VDR and discovering of retinoid X receptor (RXR) as dimerization counterpart of VDR provided a base for current model of genomic action of VDR [21,22]. On the other hand, action of $1\alpha,25(OH)_2D$ has appeared to involve also rapid nongenomic effects including activation of protein kinase C and MAP-kinase [23]. These effects occurs within minutes after hormone administration and arise from cell plasma membrane by yet poorly understood mechanisms which may involve novel receptor systems for Vitamin D metabolites. Interestingly, both genomic and nongenomic signalling pathways of $1\alpha,25(OH)_2D_3$ has been, recently, shown to meet in controlling transcriptional induction of the rat 24-hydroxylase gene [24]. On the other hand, our laboratory has provided new evidence for biological activity of $25OHD_3$ with reference to transcriptional induction of 24-hydroxylase expression in human prostatic stromal cells [25].

2. Growth regulation by Vitamin D in normal and malignant prostate

The active form of Vitamin D, $1\alpha,25(OH)_2D_3$, in addition to its long recognized role in calcium homeostasis, has been identified as a secosteroid hormone with antiproliferative effect on normal and malignant cells and, thus, it and its less calcemic analogues are recommended as potential compounds for cancer treatment. The antiproliferative function of $1\alpha,25(OH)_2D_3$ is thought to be exerted mainly through nuclear Vitamin D receptor-mediated pathway to control the target gene expression, resulting in cell cycle arrest in G1/S phase, cell apoptosis and differentiation [26].

Prostate is a target organ of Vitamin D and VDR has been found to present in prostate epithelial [27,28] and cancer cells [27,29,30]. $1\alpha,25(OH)_2D_3$ is able to inhibit the growth of primary prostatic epithelial cells [27] and prostate cancer cells [30–32] as well as prostate cancer xenografts in animal models [33,34]. Some clinical studies have also been undertaken suggesting that Vitamin D might be effective in slowing the progression of prostate cancer [35,36]. Our epidemiological study also supports the idea that the Vitamin D is protective against prostate cancer development [37], however, a high serum concentration of 25OH D_3 (>75 nmol/L) may also increase the risk of prostate cancer [38].

Molecular mechanism of antiproliferative effect of Vitamin D has been under an intensive investigation. The development of microarray technology makes it possible to screen Vitamin D-responsive genes in cells and tissues. Due to its sensitivity to Vitamin D, the prostate cancer cell line LNCaP has been applied widely to study Vitamin D target genes. The growth of LNCaP cells can be almost completely inhibited by 10 nM $1\alpha,25(OH)_2D_3$ (Fig. 2). Vitamin D_3 causes an accumulation of LNCaP cells in G1 phase [39,40] and apoptosis [41–43]. Vitamin D_3 induced cell cycle arrest in prostate cancer cells is mediated mainly by cyclin dependent kinase inhibitor p21/WAF1 and p27 pathways. P21/WAF1 is upregulated by $1\alpha,25(OH)_2D_3$ in both mRNA and protein levels in LNCaP and ALVA-31 cells and resulting in cell cycle arrest in G0/G1 phase [44], whereas Vitamin D stimulates the expression of p27 in LNCaP cells by increasing the half life of p27

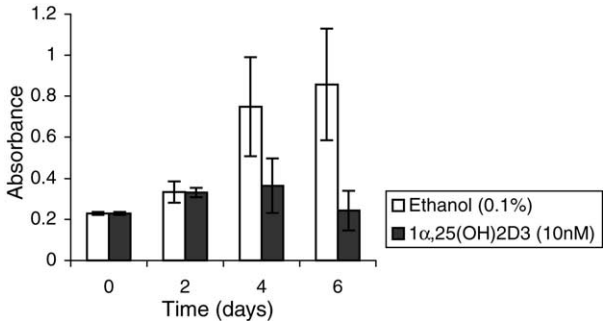

Fig. 2. Inhibition of prostate cancer LNCaP cell growth by 10 nM 1α,25(OH)2D3. LNCaP cells were seeded in 96-well plate and treated with or without Vitamin D. Cell growth was assayed by crystal violet staining. 10 nM 1α,25(OH)2D3 was shown to significantly inhibit the growth of LNCaP cells at Day 4 and 6.[48].

through lowering the cdk2 protein level in the nucleus where the p27 is degraded by cdk2, resulting in an inhibition of G1 to S phase progression [45]. Since the effect of Vitamin D on cell cycle is finally mediated by retinoblastoma protein, an over-expression or hyperphosphorylation of retinoblastoma protein might influence Vitamin D action [26,46]. Insulin-like growth factor binding protein 3 (IGFBP-3) has proposed to be a necessary component associated with $1\alpha,25(OH)_2D_3$-induced cell cycle arrest by increasing p21/WAF1 expression in LNCaP cells [47]. In our previous study, fatty acid synthetase (FAS) was found to be involved in the growth suppression of LNCaP cells by Vitamin D [48]. Knock-down of FAS gene results in LNCaP cell apoptosis [49]. This suggests that FAS might be associated with Vitamin D-induced growth inhibition/apoptosis in LNCaP cells. The effect of Vitamin D on cell growth and gene expression varies in different prostatic cells [30,50]. This might explain the different mechanisms whereby Vitamin D exerts its antiproliferative effect in different cells.

Androgen plays an important role in the antiproliferative effect of Vitamin D on some prostate cancer and normal cells. Our epidemiological study suggests that androgens might be involved in the anticancer activity of Vitamin D [37]. In vitro studies, the inhibitory effect of Vitamin D on LNCaP cell growth is shown to be androgen-dependent [51]. $1\alpha,25(OH)_2D_3$ has less effect on inhibition of the growth of AR-negative prostate cancer PC3 and DU145 cells (reviewed in more detail by Peehl and Feldman [1]). In vivo animal studies, Vitamin D showed no effect on prostatic growth when the endogenous androgens were removed by castration, whereas in intact animals, the prostatic growth was significantly inhibited by $1\alpha,25(OH)_2D_3$ [52]. These data strongly suggest that androgen is a necessary factor for Vitamin D-induced cell growth inhibition. We found that regulation of FAS gene expression by $1\alpha,25(OH)_2D_3$ was androgen-dependent and androgen was involved in the antiproliferative effect of Vitamin D on LNCaP cells [48]. The mechanism of the interaction between Vitamin D and androgen remains to be investigated. The inhibitory effect of Vitamin D is also shown to be androgen-independent in subset of prostate cancer cells [32]. These suggest that antiproliferative effect of Vitamin D is complex and the mechanism is different depending on cell type.

The synthetic less calcemic analogs of Vitamin D appear to be more potential in the treatment of prostate cancer when compared with $1\alpha,25(OH)_2D_3$ [53,54]. This suggests that the Vitamin D-derived compounds may be good candidates of human clinical trials in prostate cancer. The antiproliferative effect of Vitamin D can be enhanced by combination with some anticancer drugs, especially in hormone refractory prostate cancer [55].

3. Expression and regulation of Vitamin D_3 metabolizing enzymes in the prostate

3.1. 25-Hydroxyvitamin D_3 1α-hydroxylase

Human prostate cancer cell lines and primary cultures of noncancerous prostatic cells have 1α-hydroxylase activity [56]. A reduced 1α-hydroxylase activity in prostate cancer cells compared with cells derived from normal or benign prostatic hyperplasia tissues was observed [57], which seems to be due to a decreased promoter activity [54]. In colon cancer, the correlation between the differentiation grade and 1α-hydroxylase mRNA content remains controversial. One study reported that poorly differentiated cancers expressed low levels of 1α-hydroxylase mRNA [58], whereas other showed that the less-differentiated colon cancer tissues had more 1α-hydroxylase mRNA [59]. It is interesting that a hypermethylation of the CYP27B1 promoter CpG island was found in human breast cancer cell line MDA-MB-231 and in 41% of the breast tumors analyzed, which may result in the transcriptional inactivation of 1α-hydroxylase gene in vivo [60]. Studies on the regulation of the extrarenal 1α-hydroxylase suggest that the regulation of 1α-hydroxylase is different from that in the kidney. For instance, parathyroid hormone (PTH) and calcitonin, which are known regulators of renal 1α-hydroxylase, did not affect the expression of 1α-hydroxylase mRNA in macrophages [61] due to the lack of receptors. However, no feedback inhibition by $1\alpha,25(OH)_2D_3$ was seen in either macrophages or nonsmall cell lung carcinomas [61,62]. In a recent study [25], we demonstrated an expression of 1α-hydroxylase mRNA and protein in primary cultures of human prostatic stromal cells, but the enzyme activity was lower than that in primary cultures of human prostatic epithelial cells [57]. We also showed that a physiological concentration of $25OHD_3$ (100 nM) stimulated the expression of 1α-hydroxylase mRNA in one of the primary cultures tested, but $1\alpha,25(OH)_2D_3$ did not affect the expression of 1α-hydroxylase mRNA [25].

Since the discovery of the expression of 1α-hydroxylase in the prostate, the autocrine role of $1\alpha,25(OH)_2D_3$ has been attracting attention. $25OHD_3$ was shown to inhibit the proliferation of primary prostatic epithelial cells possessing

1α-hydroxylase [63]. Due to the intracellular conversion of $25OHD_3$ into $1\alpha,25(OH)_2D_3$, the systemic side effect of hypercalcemia is avoided, the use of $25OHD_3$ is thus under the consideration in the treatment of prostate cancer [64].

In conclusion, it is possible that the activation of $25OHD_3$ by 1α-hydroxylase in the prostate might be important in the antiproliferative action by Vitamin D. Therefore, the decrease of 1α-hydroxylase with aging might be one reason for the development/progression of prostate cancer [65,66].

3.2. 25-Hydroxyvitamin D_3 24-hydroxylase

Since the VDR was discovered in human prostate cancer cell lines, the induction of 24-hydroxylase by $1\alpha,25(OH)_2D_3$ has been demonstrated in DU145 and PC3 cells, but not in LNCaP cells [30]. Later, using real-time RT-PCR we showed that $1\alpha,25(OH)_2D_3$ significantly increases 24-hydroxylase mRNA in LNCaP cells and primary cultures of human prostatic stromal cells [25]. Human prostatic carcinoma cell lines were also shown to possess 24-hydroxylase activity, which was up-regulated by $1\alpha,25(OH)_2D_3$ [31]. In addition to $1\alpha,25(OH)_2D_3$, 24-hydroxylase was also induced by 9-*cis*-retinoic acid in human colon cancer cells HT-29 [67] and by dexamethasone in the presence of $1\alpha,25(OH)_2D_3$ in osteoclastic cells UMR-106 [68]. It has been suggested that the growth inhibition of $1\alpha,25(OH)_2D_3$ in human prostatic carcinoma cells was inversely proportional to the 24-hydroxylase activity [31].

Earlier studies have shown that the chromosome 20q13.2-q13.3 region, where CYP24 is located, was amplified in a variety of cancers, such as human ovarian cancer [69] and prostate cancer [70]. CYP24 was proposed to be a candidate oncogene or tumor-suppressor gene in human breast tumors [71] and mouse islet carcinomas [72]. Whether CYP24 is amplified and/or over-expressed in human prostate cancer needs further investigation. During aging the activity of 24-hydroxylase seems to increase at least in the experimental animals [73], which, in turn, may lead to a decreased sensitivity of a target organ to Vitamin D.

3.3. Levels of Vitamin D_3 metabolites in the prostate

The local concentrations of Vitamin D_3 metabolites naturally reflect the Vitamin D_3 metabolism in the prostate. However, only few animal studies are available. In male Sprague-Dawley rats, less than 1% of the serum level was detected in prostatic tissue within 24 h after the intravenous administration of $1\alpha,25(OH)_2D_3$ [74]. The study on the distribution of hydroxylated Vitamin D_3 metabolites in domestic pigs showed that the organ concentrations of $1\alpha,25(OH)_2D_3$ were much higher than the plasma levels whereas those of $25OHD_3$ lower [75]. We have measured the concentrations of Vitamin D_3 metabolites in human prostate after prostatectomy with a permission of the local ethical committee. Metabolites of Vitamin D were assayed using high performance liquid chromatography (HPLC) followed either by a competitive protein binding assay for $25OHD_3$ and $24,25(OH)_2D_3$ or a radioreceptor assay for $1\alpha,25(OH)_2D_3$. We found that the levels of $25OHD_3$ in the prostate are similar to or slightly higher than those in serum, however, those of $24,25(OH)_2D_3$ and $1\alpha,25(OH)_2D_3$ in the prostate are much higher than in serum suggesting a significant local metabolism of $25OHD_3$ (Table 1).

Table 1
Concentrations of three Vitamin D metabolites in serum and prostate of four prostatectomy patients

		$25OHD_3$ (nM)	$24,25(OH)_2D_3$ (nM)	$1\alpha,25(OH)_2D_3$ (pM)
1	Serum	100.5	4.2	99
	Prostate	85	26	195
2	Serum	48.5	12.1	65
	Prostate	74	20.5	<20
3	Prostate	16.5	9	100
4	Prostate	19.5	14.5	470

4. New 25OH D_3 hormonal system

We, recently, showed evidence for a new Vitamin D endocrine system [25]. $25OHD_3$ seems to be an active hormone in human prostatic stromal cells with respect to Vitamin D_3 response gene regulation and cell growth inhibition [25]. Our finding suggests that, in physiological concentration, $1\alpha,25(OH)_2D_3$ is inactive whereas $25OHD_3$ is active hormone in the prostate cells (Fig. 3). In our primary cultures of human prostatic stromal cells, $25OHD_3$ at a physiological concentration of 250 nM exhibited a clear transcriptional activity and an antiproliferative effect. The action of $25OHD_3$ was not due to 1α-hydroxylation, since its activity did not decrease significantly when a specific inhibitor of 1α-hydroxylase was used. $25OHD_3$ has been regarded as a prohormone having no significant biological activity because of its lower affinity for the VDR. However, the difference in the physiological concentration between $25OHD_3$ and $1\alpha,25(OH)_2D_3$ is 1000-fold. Although $1\alpha,25(OH)_2D_3$ has been demonstrated in vitro to regulate the growth, differentiation and function of a variety of cells including cancer cells, its actions in vivo and in vitro are achieved only at pharmacological concentrations. The physiological concentrations of $25OHD_3$ vary with the season and sun exposure whereas those of $1\alpha,25(OH)_2D_3$ are strictly controlled by calcium

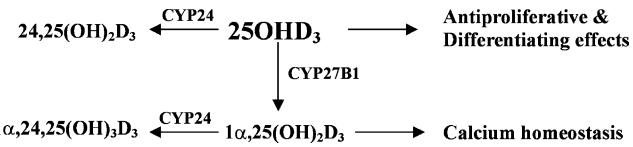

Fig. 3. Two endocrine systems of Vitamin D3. One is based on $1\alpha,25(OH)_2D_3$, mediating mainly calcium homeostasis; the other one is mediated by $25OHD_3$, regulating gene expression and cell proliferation in target tissues (e.g. prostate). Both $25OHD_3$ and $1\alpha,25(OH)_2D_3$ are inactivated by 24-hydroxylase in target tissues.

and PTH [76]. Therefore it is obvious that sunlight exposure and serum levels of $25OHD_3$ but not 1α25(OH)2D3 are associated with a decreased risk of prostate cancer [37,77,78]. Thus, a deficiency of $25OHD_3$ might be the important Vitamin D metabolite in the development and/or progression of prostate cancer. Paradoxically, a high serum concentration of $25OHD_3$ is also associated with an increased risk of prostate cancer [38], which may be due to its ability at high physiological concentrations to induce 24-hydroxylase [25] leading to a possible Vitamin D insensitivity.

5. Inhibitors for 24-hydroxylase in the treatment of prostate cancer

As mentioned above, 24-hydroxylase controls the first inactivation step of $1\alpha,25(OH)_2D_3$ and $25OHD_3$, therefore an inhibition of 24-hydroxylase activity could be beneficial and enhance Vitamin D action. The widely used inhibitors are antifungal imidazole derivatives, such as ketoconazole, liarozole and newly identified VID400. Imidazole derivatives, ketoconazole and liarozole, inhibit steroidogenesis by interfering with cytochrome P450 enzyme system [79]. They are promising in prostate cancer treatment not only because they inhibit the activities of Vitamin D_3 metabolizing enzymes, but also because they inhibit testosterone biosynthesis [80]. They exhibit a growth-inhibitory effect in both colon cancer cell line HT29-S-B6 and breast cancer cell lines MDA-MB-231 and Evsa-T [81] and a cytotoxic effect in prostate cancer cell lines PC3 and DU145 [82]. Thus, the combined treatment with $1\alpha,25(OH)_2D_3$ and imidazole derivatives could be in future a prostate cancer therapy. For example, ketoconazole and liarozole were shown to enhance the antiproliferative activity of $1\alpha,25(OH)_2D_3$ in breast cancer cells [83]. Liarozole caused a growth inhibitory response to $1\alpha,25\text{-}(OH)_2D_3$ and increased the half life of $1\alpha,25(OH)_2D_3$ in DU145 cells, which are $1\alpha,25(OH)_2D_3$-resistant cells due to high levels of 24-hydroxylase expression [84]. Likewise, ketoconazole decreased the activities of both 1α-hydroxylase and 24-hydroxylase and lowered the half maximal inhibitory dose of $1\alpha,25(OH)_2D_3$ in primary prostate cancer cells [85]. In primary cultures of human prostatic stromal cells, a specific inhibitor of 24-hydroxylase, VID400, markedly increased the transcriptional activities of $1\alpha,25(OH)_2D_3$ and $25OHD_3$ [25].

6. Phytoestrogens—novel modulators of Vitamin D metabolism in the prostate

Phytoestrogens have been shown to have a beneficial effect on cancers in both in vitro [86–88] and in epidemiological studies [89,90]. Recently, their inhibiting effect on Vitamin D metabolizing enzymes has also been established [91–93].

Phytoestrogens are a subclass of flavonoids, phenolic compounds present in all plants. The two main groups of phytoestrogens are the isoflavonoids and the lignans. Isoflavonoids, such as genistein, daidzein and glycitein, can be found in some vegetables, fruits and in especially high concentrations in most soy-protein products. Lignans (e.g. enterodiol and enterolactone) are derived from plants such as rye, berries, whole grains and flaxseed. Both isoflavonoids and lignans exist in the plants as precursors, which undergo metabolism by the gut microflora. Both metabolites and precursors are absorbed in varying amounts.

6.1. In vitro studies

In vitro studies have shown phytoestrogens to have several effects on the metabolism of sex steroids, the proliferation of several cell lines and the function of enzymes.

6.2. Effects on sex steroid metabolism

Phytoestrogens have been found to have both estrogenic [94] and possibly antiestrogenic [95] effects as well on estrogen binding sites. The function as antiestrogen can be explained on the basis of the finding that phytoestrogens may compete with estradiol for nuclear estrogen-binding sites [96]. It has been reported that the action of phytoestrogens in the inhibition of growth could be independent of the presence of estrogen receptors on the cell [87]. Phytoestrogens are also able to lower the amount of estrogens in blood. This is accomplished by their stimulating effect on the synthesis of sex hormone binding globulin in liver [97], thus decreasing the amount of free circulating estrogen, as well as the inhibition of the aromatase enzyme [98]. Genistein can also enhance the conversion of estradiol to weaker estrogens or inactive metabolites in breast cancer cells [99]. Phytoestrogens have not been found to have any effect on the testosterone levels in blood [100,101].

6.3. Effects on cell proliferation

In vitro studies have shown that phytoestrogens affect the proliferation of various cancer cell lines. In prostate, the isoflavonoid, genistein, has been found to inhibit growth by having an apoptotic and an antimitotic effect in both androgen-dependent [86,88] and androgen-independent [87,88] prostate cancer cell lines. Genistein has been reported to arrest the cell cycle in the phase G2/M [102]. Phytoestrogens also have several other possible anticarcinogetic activities. Genistein is able to inhibit topoisomerase [103] and tyrosine protein kinases [104], which mediate the effects of growth factors in cells. Genistein also inhibits angiogenesis, which is a crucial element in tumour formation [105].

6.4. Epidemiological studies

Population migration studies show that the diet plays an important part in the development of cancers. The risk of prostate cancer among Japanese men immigrated into the

United States increases up to the level of non-Japanese men born in the United States, this might be at least partly due to the change in the diet [106]. The epidemiological results on the protective effect of phytoestrogens against cancer are conflicting. In breast cancer, both protective and non-protective effects have been reported [89,107,108]. Rodent suggests that a diet rich in isoflavones may be protective against breast cancer only when consumed before puberty or during adolescence [109]. Some studies on prostate cancer have shown the beneficial effect of a large soy intake, rich in isoflavonoids, against the cancer [90,110]. There is less evidence about the beneficial qualities of the lignans, mainly because of lack of data. Stattin et al. reported recently that the lignan, enterolactone, had no protective effect against prostate cancer in a cohort among Nordic men [111], but more research is needed. Furthermore, it can be speculated, whether it truly is the phytoestrogens of the diet, which protect from cancer or whether they only are indicators of a generally healthy diet.

6.5. Phytoestrogens and Vitamin D

It has been known for some time that Vitamin D has a beneficial effect against cancer. Thus, when Kállay et al found that the phytoestrogen genistein increased the amount of $1\alpha,25(OH)_2D_3$ in the mouse colon, they suggested that the antitumorigenic effects of phytoestrogens could be at least partially mediated by Vitamin D [112]. The effects of genistein on the Vitamin D metabolism has probably been well studied. Genistein inhibits the function [92] and the transcriptional activity [93] of 24-hydroxylase in DU-145 prostate cancer cells. Recently, Farhan et al also reported that genistein inhibits the expression of 24-hydroxylase in the prostate cancer cell line PC-3, as well as the expression of 1α-hydroxylase in the DU-145 cell line (Fig. 4) [91]. Genistein also inhibits the induction of 24-hydroxylase caused by a treatment with $1\alpha,25(OH)_2D_3$ in PC-3 cells [91]. Genistein and $1\alpha,25(OH)_2D_3$ have been found to synergistically inhibit the growth of primary human prostate epithelial cells as well as some prostate cancer cell lines [113]. Inhibition of growth was also found in primary prostate epithelial cells with the combination of genistein and $25OHD_3$ [113]. Both treatments caused a cell cycle arrest in both phases G2/M and G1/G0 and the inhibition of growth was greater with the combination of the compounds than with any of them alone. Genistein has also been found to increase VDR protein expression in breast cancer cells [114] and therefore, it might increase their sensitivity to Vitamin D.

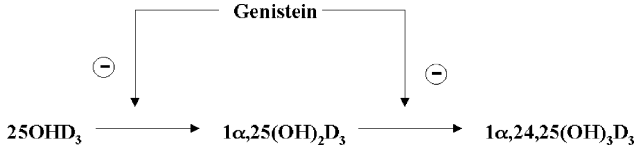

Fig. 4. Effect of genistein on Vitamin D metabolism. Genistein inhibits both 1α-hydroxylase and 24-hydroxylase [91].

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