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24-Hydroxylase in Cancer: Impact on Vitamin D-based Anticancer Therapeutics

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Abstract

The active vitamin D hormone 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) plays a major role in regulating calcium homeostasis and bone mineralization. 1,25(OH)₂D₃ also modulates cellular proliferation and differentiation in a variety of cell types. 24-hydroxylase, encoded by the CYP24A1 gene, is the key enzyme which converts 1,25(OH)₂D₃ to less active calcitric acid. Nearly all cell types express 24-hydroxylase, the highest activity being observed in the kidney. There is increasing evidence linking the incidence and prognosis of certain cancers to low serum 25 (OH)D₃ levels and high expression of vitamin D 24-hydroxylase supporting the idea that elevated CYP24A1 expression may stimulate degradation of vitamin D metabolites including 25-(OH)D₃ and 1,25(OH)₂D₃. The over expression of CYP24A1 in cancer cells may be a factor affecting 1,25(OH)₂D₃ bioavailability and anti-proliferative activity pre-clinically and clinically. The combination of 1,25(OH)₂D₃ with CYP24A1 inhibitors enhances 1,25(OH)₂D₃ mediated signaling and anti-proliferative effects and may be useful in overcoming effects of aberrant CYP24 expression.

Keywords

CYP24; 24-hydroxylase; calcitriol and anti-tumor

1. Introduction

Vitamin D is a fat-soluble secosteroid which is synthesized in the body and has broad biological effects. 1,25(OH)₂D₃ is the most potent biologically active form of vitamin D₃ and plays a central role in regulating calcium and phosphorus homeostasis [1, 2]. Recent studies have shown that 1,25(OH)₂D₃ has a broad range of functions including modulating the immune system and regulating cellular differentiation and proliferation [1–5]. 1,25(OH)₂D₃ inhibits cancer cell proliferation, cell migration and invasion and induces apoptosis in a wide variety of cancer cell types [6–12], including leukemia, prostate, colon, breast, ovarian, pancreas and liver.

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Vitamin D₃ (cholecalciferol), a precursor of 1,25(OH)₂D₃, is available in the diet, but is primarily supplied through synthesis from 7-dehydrocholesterol in the skin after exposure to ultraviolet B-light. Once produced, vitamin D₃ is carried as a complex with the plasma vitamin D binding protein (DBP) to the liver[13], where it is hydroxylated by 25-hydroxylase to yield 25(OH)D₃, the major circulating form of vitamin D. 25(OH)D₃ is 1 α -hydroxylated to 1,25(OH)₂D₃, also known as calcitriol, by 1 α -hydroxylase (CYP27B1) mainly in kidney, and this is subject to tight control to avoid hypercalcemia. 1,25(OH)₂D₃ is also synthesized in many extrarenal sites that express CYP27B1[14–17].

24-hydroxylase encoded by CYP24A1 is the key enzyme to inactivate 1,25(OH)₂D₃ [18, 19]. The blood 1,25(OH)₂D₃ level is tightly controlled through feedback regulation of its biosynthesis and catabolism by CYP27B1 and CYP24A1, respectively. Both CYP27B1 and CYP24A1 are highly regulated enzymes and respond to modulating agents such as parathyroid hormone (PTH), calcitonin, calcium, phosphorus as well as 1,25(OH)₂D₃. CYP24A1 is constitutively expressed in kidney and gastrointestinal mucosa. In most other tissues, CYP24 is transcriptionally induced by 1,25(OH)₂D₃ [18–20]. This limits 1,25(OH)₂D₃ signaling and potential to cause hypercalcemia. Increased expression of CYP24A1 has been found in several human tumors[21, 22]. By stimulating 1,25(OH)₂D₃ degradation, over expression of CYP24A1 limits 1,25(OH)₂D₃ biologic activity and may diminish the effect of 1,25(OH)₂D₃ in modulating proliferation and motility of cancer cells. The understanding of CYP24A1 dysregulation in and cancers is likely to uncover potential ways to inhibit CYP24A1 in an effort to improve the anti-tumor efficacy of 1,25(OH)₂D₃.

2. Regulation of CYP24A1 expression

2.1 VDR/RXR binding to VDREs

The human CYP24A1 gene, located on chromosome 20q13.2 is highly conserved across all phyla[18, 23]. CYP24A1 is a mitochondrial protein and a member of the cytochrome class I P450 superfamily of enzymes. CYP24A1 expression is controlled by a 1,25(OH)₂D₃-vitamin D receptor (VDR) dependent process[24]. In VDR null mice, there are lower levels of CYP24A1 and 24-hydroxylated metabolites demonstrating that CYP24A1 expression is dependent on VDR[25]. In most normal tissues, CYP24A1 is expressed at very low basal levels and is induced by 1,25(OH)₂D₃ via positive transcriptional regulation.

CYP24A1 is regulated through binding of 1,25(OH)₂D₃ to the VDR and the subsequent interaction of the VDR-1,25(OH)₂D₃ complex with its heterodimeric partner, retinoid-X-receptor (RXR)[26]. Unliganded VDR/RXR binds to the vitamin D response elements (VDRE) to recruit corepressors, such as NCoR or Alien[27]. This results in recruitment of enzymes with HDAC activity and leads to a closed chromatin structure and gene repression. The binding of 1,25-(OH)₂D₃ to VDR leads to replacement of the repressor by a coactivator complex, such as nuclear coactivators (NCoAs)[28, 29]. These coactivators link the ligand-activated VDR to enzymes displaying histone acetyltransferase activity that cause chromatin relaxation and activate target gene transcription[30, 31]. A cluster of VDREs has been reported in the proximal promoter of both the human and rat CYP24 genes[32, 33]. Single nucleotide polymorphisms (SNPs) in the VDRE within the human CYP24A1 promoter have been identified and are associated with lower affinity for the VDR-RXR heterodimer, decreased transactivation, and reduced expression of CYP24A1[34].

2.2 Stimulation of intracellular pathways

1,25(OH)₂D₃ activation of protein kinase signaling pathways may also be involved in the regulation of CYP24A1 expression[18, 35, 36]. This rapid (minutes), non-genomic action involves intracellular signaling pathways such as protein kinase C (PKC) and mitogen activated protein kinase (MAPK), through a plasma-membrane-associated VDR. The

signaling mechanisms that regulate transcriptional activation of the CYP24A1 promoter are poorly defined and further investigation is needed. Some transcription factors that interact with VDR and enhance 1,25(OH)₂D₃-mediated induction of the CYP24A1 promoter through intracellular signaling pathways have been identified. For example, Ets-1 has been shown to induce CYP24A1 promoter activity[37, 38]. Ets-1 can be activated by the Ras/Raf signaling pathway through phosphorylation of threonine residue 38[39]. Furthermore, 1,25(OH)₂D₃ stimulates MAP kinase and ERK5 to activate Ets-1 through phosphorylation in COS-1 monkey kidney fibroblast cells[37]. Dwivedi et al.[40] reported that the interaction of VDR and Ets-1 caused a significant induction of CYP24A1 promoter activity in COS-1 cells. The direct interaction between VDR and Ets-1 protein was demonstrated by an *in vitro* GST-protein pull-down study[41]. Signal transduction-regulated CYP24A1 expression could be cell type specific. COS-1 cells treated with inhibitors for ERK1/2 and ERK5 abolished 1,25(OH)₂D₃ induction of the CYP24A1 promoter[40]. However, this was not found to be the case in human embryonic kidney 293T (HEK-293T) cells[37]. Cellular modulators, such as glucocorticoids also regulate CYP24A1 expression. The cooperation between CCAAT/enhancer-binding protein (C/EBP) and glucocorticoid receptor (GR) results in enhancement of 1,25(OH)₂D₃-induced CYP24A1 expression[42]. Glucocorticoids may also indirectly increase CYP24A1 transcription by increasing VDR gene expression[43].

2.3 Epigenetics

Epigenetic changes could operate on a more intermediate time scale to serve as an additional mechanism controlling CYP24 expression[44, 45]. Epigenetic regulation of steroid signaling is an emerging field of research[46]. However, at present, little is known about the consequences of epigenetic regulation of the vitamin D metabolism pathway. Novakovic et al. reported tissue-specific *CYP24A1* promoter methylation in human placenta, purified cytotrophoblasts, and primary cultures of chorionic villus tissue.[45]. They demonstrated that promoter methylation directly down-regulated basal promoter activity and abolished vitamin D-mediated gene activation. No methylation was detected in most normal human tissues including kidney, skeletal muscle, skin fibroblasts, brain (prefrontal cortex), sperm, whole blood, buccal mucosa, endometrial stroma, decidualized stroma, bone marrow, umbilical cord tissue[45] and peripheral blood lymphocytes[47].

3. CYP24A1 in Cancer

CYP24A1, which is a candidate oncogene[22, 48], is aberrantly expressed in numerous human tumor types[21, 22, 48]. High CYP24A1 levels seem to be a common feature of several solid tumors. Elevated tumor CYP24A1 expression is associated with a poorer prognosis[22, 49]. The increased intra-tumoral levels of CYP24A1 may lead to rapid degradation of 1,25(OH)₂D₃ and abrogation of its antiproliferative effects.

The mechanisms underlying the aberrant expression of CYP24A1 in tumors are not well defined. CYP24A1 overexpression does not correlate with VDR expression level in several types of tumors, since the latter are actually reduced or unchanged. This suggests that high CYP24A1 levels are not the result of the normal physiological transcriptional process observed when VDR-bound 1,25(OH)₂D₃ activates CYP24A1. There is evidence that multiple factors might be involved in dysregulation of CYP24 expression in cancer[14, 50–52].

One possibility is amplification at the CYP24A1 locus. Albertson et al.[53] found that CYP24A1 levels in breast tumors are higher in specimens with amplification at the CYP24A1 locus. Kallioniemi et al.[54] showed that a significant increase of regional copy number and amplification at the CYP24A1 locus was observed in approximately 18% of

primary breast tumors and 40% of breast cell lines. Alternatively, CYP24A1 regulation in tumors may be related to miRNAs that target the CYP24 gene. Komagata et al.[50] identified miR-125b, which binds at the 3' UTR of CYP24A1 mRNA to post-transcriptionally repress CYP24 translation. In these cells, CYP24A1 protein levels were increased by the inhibition of miR-125b and were decreased by miR-125b over-expression. Furthermore, CYP24A1 levels in breast cancer tissues were inversely associated with miR-125b levels. Additionally, the number of functional vitamin D receptor binding sites could be responsible for a difference in CYP24 gene expression level induced by vitamin D in malignant and normal mammary cells[52]. The number of functional VDREs is higher in malignant breast MCF-7 cells with high CYP24A1 expression than in normal breast MCF-10A cells. Lastly, CYP24 expression in cancer cells may be modified via epigenetic processes. CYP24 DNA promoter hypermethylation was observed in human choriocarcinoma cell lines[45], human prostate cancer cell lines[51, 55] and human prostate cancerous lesions. The promoter DNA methylation in cancer was inversely correlated with *CYP24A1* expression[51, 55]. In addition, studies indicate that repression of *CYP24A1* gene expression in human prostate cancer cells was mediated in part by promoter DNA methylation and repressive histone modifications. The tumor microenvironment also affects the methylation status of the CYP24 promoter. Differential methylation of the CYP24A1 gene promoter was also observed in endothelium from benign and malignant human prostate[56].

4. CYP24A1 inhibitors

As a key enzyme responsible for vitamin D catabolism, the aberrant expression of CYP24A1 in tumors may negatively impact vitamin D therapy. Furthermore, the induction of CYP24A1 expression by administration of 1,25(OH)₂D₃ through the negative feedback mechanism also limits the amount of 1,25(OH)₂D₃ available systemically and locally in tumor cells. In vitro studies demonstrated that differences in 1,25(OH)₂D₃-mediated growth inhibition among various cancer cell lines correlated inversely to levels of CYP24A1 expression in these cells[21, 57]. Data obtained using CYP24 inhibitors suggest that increased CYP24A1 expression in tumors restricts 1,25(OH)₂D₃ anti-tumor activity[58]. Inhibition of CYP24A1 slows the loss of 1,25(OH)₂D₃ and extends the half-life of 1,25(OH)₂D₃, thereby increasing 1,25(OH)₂D₃ exposure and anti-proliferative effects[58, 59].

Different types of CYP24A1 inhibitors have been developed. These include azole and non-azole inhibitors and vitamin D analogues. Azole inhibitors have a nitrogen heterocyclic ring, commonly an imidazole or triazole, and they bind with the Fe³⁺ heme of CYP24. Non-azole inhibitors lack this nitrogen heterocycle and instead bind within the CYP24 active site via hydrogen bonding and hydrophobic interactions.

Ketoconazole and liarozole[60, 61], are non-selective azole CYP24A1 inhibitors, which target the active sites of P450 enzymes[62]. Combination of 1,25(OH)₂D₃ with liarozole or ketoconazole increases the half-life of 1,25(OH)₂D₃ and potentiates its antiproliferative effects in human cancer cells exhibiting CYP24A1 activity.

The tetralone derivative (2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one), a non-azole CYP24 inhibitor, enhanced 1,25(OH)₂D₃ antiproliferative activity in DU145 cells and enhanced the upregulation of vitamin D target genes, p21waf1/cip1 and GADD45a[63]. Isoflavones are non-specific inhibitors of the CYP enzymes. Genistein is one of the isoflavones found in a number of plants[64]. Genistein inhibits the expression of CYP24, leading to an increase in the half-life of 1,25(OH)₂D₃ [65]. Combination of 1,25(OH)₂D₃ with genistein resulted in synergistic growth inhibition of prostate cancer cells

by activating vitamin D signaling[66]and enhanced 1,25(OH)₂D₃ mediated functional responses including upregulation of mRNA levels of p21/WAF1 and IGFBP-3[67].

The broad inhibitory effects of non-selective inhibitors such as ketoconazole on other P450 enzymes, particularly CYP27B1, limit their potential as CYP24A1 inhibitors. This has resulted in a decade long effort to develop CYP24 selective inhibitors. Schuster et al.[68] synthesized and screened a large number of azole compounds using primary human keratinocyte cultures as a source of CYP24 and CYP27B1, with 25(OH)D₃as substrate to analyze the complex metabolite profiles. Among them, VID400 ((R)-N-(2-(1H-Imidazol-1-yl)-2-Phenylethyl)-4-Chlorobiphenyl-4-Carboxamide), exhibited strong and selective CYP24A1 inhibitory activity (IC₅₀: 15 nM), with little effect on CYP27B1 activity (IC₅₀: 616nM). VID400 occupies the substrate site in the CYP24A1 enzyme. Indicative of its potential utility, the antiproliferative effect of 1,25(OH)₂D₃ was enhanced 100-fold in human keratinocytes by VID400[69].

Posner et al have developed CYP24 inhibitors that are 1,25(OH)₂D₃ analogs[70]. These include the sulfone and sulfoximine derivatives designated CTA018 and CTA091, respectively. CTA018, which also binds the VDR and functions as a VDR agonist, is currently in Phase II clinical trials for patients with end stage renal disease and secondary hyperparathyroidism. CTA019 significantly increases 1,25(OH)₂D₃-mediated gene expression and anti-proliferative activity in tumor cells and also significantly and specifically increases tumor exposure to co-administered 1,25(OH)₂D₃ in lung tumor xenograft models[71, 72].

5. Summary

The anti-proliferative and pro-apoptotic effects of 1,25(OH)₂D₃ have been demonstrated in various tumor model systems in vitro and in vivo. However, limited anti-tumor effects of 1,25(OH)₂D₃ have been observed in clinical trials. This may be attributed to a variety of factors including the over-expression of CYP24A1 in tumors, which likely leads to the rapid local inactivation of 1,25(OH)₂D₃. Efforts to dissect the mechanisms responsible for CYP24 over-expression in tumors are underway. Insights gained from these studies are expected to yield novel strategies to suppress CYP24 expression and improve the efficacy of 1,25(OH)₂D₃treatment. An alternative strategy for improving 1,25(OH)₂D₃ activity involves combining it with a selective CYP24 inhibitor. The validity of this approach is supported by numerous pre-clinical investigations, which demonstrate that CYP24 inhibitors suppress 1,25(OH)₂D₃ catabolism by tumor cells and increase the effects of 1,25(OH)₂D₃ on gene expression and cell growth. Studies are now required to determine whether selective CYP24A1 inhibitors can be used safely and efficaciously in the oncology clinic and how best to combine them with a vitamin D supplementation regimen.

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Highlights

An overview is presented on the role of 24-hydroxylase or CYP24 in cancer

CYP24 is overexpressed in cancer

CYP24 could influence the effectiveness of vitamin D therapeutics

The role of CYP24 inhibitors in cancer has not been explored