Vitamin D3 Hydroxyderivatives in Human

Subjects: Oncology

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itamin D3 (D3) is produced in the skin in two steps. Initially there is a photochemical reaction caused by the action of UVB radiation (290–315 nm) on 7-dehydrocholesterol (7DHC) in which the B ring is broken producing pre-vitamin D3. In the second reaction, vitamin D3 is formed from pre-vitamin D3 by its thermal isomerization at 37 °C over several hours. Both the UVB intensity and the level of skin pigmentation affect the rate of vitamin D3 production. Vitamin D3 is a fat-soluble prohormonal secosteroid that has endocrine, paracrine and autocrine functions. Melanin absorbs UVB limiting the production of D3, and the same effect is achieved with clothing and sunscreen. Skin, more specifically the epidermis, has the full capacity to produce and activate vitamin D3.

Keywords: melanoma ; vitamin D ; vitamin D receptor ; active forms of vitamin D ; malignancy

1. Overview

Active forms of vitamin D3, including 1,25$(OH)_2$D3, 20(OH)D3 and 1,20$(OH)_2$D3, inhibited cell proliferation, migration rate and the ability to form colonies and spheroids in the wild-type melanoma cell line, while cells with the vitamin D receptor (VDR) silenced showed an increased but not complete resistance to their action. Furthermore, silencing of the VDR in melanoma cells enhanced their proliferation as well as spheroid and colony formation and increased their migration rate. Previous clinicopathological studies have shown an inverse correlation between VDR expression, melanoma progression and poor outcome of the disease. Thus, the expression of VDR is not only necessary for the inhibition of melanoma growth by active forms of vitamin D, but the VDR can also function as a melanoma tumor suppressor gene.

Vitamin D3 is not only involved in calcium and phosphate metabolism in humans, but it can also affect proliferation and differentiation of normal and cancer cells, including melanoma. The mechanism of the anti-cancer action of vitamin D3 is not fully understood. The nuclear vitamin D receptor (VDR) is crucial for the phenotypic effects of vitamin D hydroxyderivatives. VDR expression shows an inverse correlation with melanoma progression and poor outcome of the disease. In this study we knocked out the VDR in a human melanoma cell line using CRISPR methodology. This enhanced the proliferation of melanoma cells grown in monolayer culture, spheroids or colonies and their migration. Activated forms of vitamin D, including classical 1,25$(OH)_2$D3, 20(OH)D3 and 1,20$(OH)_2$D3, inhibited cell proliferation, migration rate and the ability to form colonies and spheroids in the wild-type melanoma cell line, while VDR KO cells showed a degree of resistance to their action. These results indicate that expression of VDR is important for the inhibition of melanoma growth induced by activated forms of vitamin D. In conclusion, based on our previous clinicopathological analyses and the current study, we suggest that the VDR can function as a melanoma tumor suppressor gene.

2. Vitamin D3

Vitamin D3 (D3) is produced in the skin in two steps. Initially there is a photochemical reaction caused by the action of UVB radiation (290–315 nm) on 7-dehydrocholesterol (7DHC) in which the B ring is broken producing pre-vitamin D3 [1][2]. In the second reaction, vitamin D3 is formed from pre-vitamin D3 by its thermal isomerization at 37 °C over several hours [3]. Both the UVB intensity and the level of skin pigmentation affect the rate of vitamin D3 production [4]. Vitamin D3 is a fat-soluble prohormonal secosteroid that has endocrine, paracrine and autocrine functions [5]. Melanin absorbs UVB limiting the production of D3, and the same effect is achieved with clothing and sunscreen [6][7][8]. Skin, more specifically the epidermis, has the full capacity to produce and activate vitamin D3 [9][10][11].

The liver and kidneys are the main organs in which two-step activation of vitamin D3 occurs [1]. In the liver, vitamin D3 is metabolized by a vitamin D 25-hydroxylase (CYP2R1 or CYP27A1) to 25-hydroxyvitamin D3 (25(OH)D3), which is the main form of vitamin D in serum. 25(OH)D3 is further metabolized by 1α-hydroxylase (CYP27B1), mainly in the kidney proximal tubule, to 1α,25-dihydroxyvitamin D3 (1α,25$(OH)_2$D3), the major hormonally active form of vitamin D3 [8]. 1α,25$(OH)_2$D3 is transported via vitamin D binding protein (VDBP) in the bloodstream to target tissues such as the intestine, bones and kidneys where it regulates calcium and phosphate absorption and reabsorption, respectively. The

concentration of 1α,25(OH)$_2$D3 in the bloodstream regulates the expression of the inactivating enzyme, 25(OH)D3 24-hydroxylase (CYP24A1), which is induced when concentrations are high [12][13][14]. In addition to these classical pathways for vitamin D3 activation and inactivation, alternative metabolic pathways of vitamin D3 activation in the skin, including by keratinocytes [15] and dermal fibroblasts [16], are initiated by CYP11A1. Like 1,25(OH)$_2$D3, the products of these pathways display anti-proliferative and differentiating abilities [3][17][18]. CYP11A1 is well known for catalyzing the hydroxylation of cholesterol at C22 and C20, followed by cleavage of the bond between C20 and C22 to generate pregnenolone, a common precursor for steroid hormones [19]. As well as the gonads and adrenal cortex, CYP11A1 is expressed in peripheral tissues such as the gastrointestinal tract, nervous system, immune system and skin [20][21]. It has more recently emerged that it is a vitamin D metabolizing enzyme with vitamin D serving as an alternative substrate to cholesterol [22][23]. The main metabolites of vitamin D that are formed by a single hydroxylation by CYP11A1 are 20(OH)D3, 22(OH)D3 and 17(OH)D3. These metabolites can be further hydroxylated by CYP11A1 to form 20,23(OH)$_2$D3, 20,22(OH)$_2$D3, 17,20(OH)$_2$D3 and 17,20,23(OH)$_3$D3. Moreover, the major product of this pathway, 20(OH)D3, may also serve as a substrate for CYP27A1, CYP24A1, CYP2R1 and CYP3A4 with hydroxylation occurring at C24, C25 or C26, while CYP27B1 hydroxylates most of these products at C-1α to produce the corresponding trihydroxyvitamin D metabolites. Overall, it has been estimated that this alternative metabolic pathway can produce more than 21 vitamin D hydroxyl-metabolites [24][25].

In target tissues, 1,25(OH)$_2$D3 binds to the vitamin D receptor (VDR), a member of the nuclear receptor family, which includes ligand-activated transcription factors, and results in both genomic and non-genomic regulation of a variety of biological pathways [26][27][28]. The VDR is expressed in almost all tissues and cells, including the skin. Colston et al. [29] provided one of the first reports describing the presence of VDR outside of organs involved in calcium and phosphate homeostasis (intestines, kidneys and bone tissues), showing that the receptor is expressed in skin cell lines and in melanomas (malignant tumors originating from melanocytes) [30] and has anti-cancer properties. Subsequent experiments using human melanoma cell lines confirmed that the VDR is present in melanoma cells, although its expression level was heterogeneous between different cell lines [29]. The strongest expression of VDR was observed in normal skin, which decreased during progression of melanocytic lesions and during melanoma development. The VDR expression in perilesional skin was also significantly reduced in comparison to normal skin. Expression of VDR in various tumor tissues may suggest that it has an effect on tumorigenesis [31][32], for example in breast cancer [33] and lung cancer, and it might be related to the sex of patients [34]. The higher expression of VDR is also correlated with upregulated pathways that mediate the antitumor immunity and with downregulation of proliferative pathways [35].

Major types of skin cancer are basal and squamous cell carcinomas with melanoma being the most deadly skin neoplasm. The relationship between vitamin D and skin cancer is still under investigation. Vitamin D and novel vitamin D derivatives exhibit anti-proliferative activities on different skin cells, including melanoma cells [36][37][38][39][40][41]. Studies showed that vitamin D has a protective effect for patients with melanoma [42][43][44]. In the skin, 1,25(OH)$_2$D3 plays an important role in regulating the epidermal barrier function and in regulating the growth and cycle of the hair follicles and also has anti-cancer, anti-proliferative and anti-inflammatory effects. It has recently been confirmed that it can inhibit skin cell death and DNA damage induced by UVR exposure. Due to its calcemic toxicity, the pharmacological use of 1,25(OH)$_2$D3 is limited [45].

In 2010, Brożyna and co-authors showed that the level of tumor malignancy inversely correlates with VDR expression [31]. The strongest expression of VDR was observed in normal skin, and its expression decreased from normal skin through melanocytic nevi and melanoma to metastases. VDR expression in skin around moles and melanoma was also significantly reduced compared to normal skin [31], suggesting that it may serve as a marker of tumor progression [46][47]. Similar results were reported for breast cancer cells where VDR expression was inversely correlated to cancer malignancy [48], also seen in colon cancer [49]. In the current study we examined the effect of knocking out the VDR on melanoma malignant behavior.

3. Conclusions

Our studies demonstrating that knocking out VDR expression in human melanoma cells increases parameters of malignancy indicate that expression of VDR is connected with an increased malignant behavior in melanoma cells. This is consistent with clinicopathological studies showing an inverse correlation between melanoma progression and VDR expression, with very poor disease outcome in VDR negative melanomas. Therefore, we propose that VDR can act as a melanoma tumor suppressor gene.

Classical (1,25(OH)$_2$D3) and CYP11-derived (20(OH)D3, 1,20(OH)$_2$D3) hydroxyderivatives of vitamin D inhibited cell proliferation, migration rate and the ability to form colonies and spheroids in melanoma cells. Silencing the VDR

attenuated these actions, but not completely. Thus, vitamin D3 hydroxyderivatives are good candidates for melanoma therapy with their main mechanism of action involving VDR; however, action on other nuclear receptors cannot be excluded and remains to be investigated. These findings form a basis for future preclinical studies on the efficacy of CYP11A1-derivatives against human melanomas.

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