Vitamin D as an adjuvant in melanoma therapy

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Metastatic melanoma: a formidable challenge for clinicians & researchers

Recent advances in our understanding of the principles underlying melanoma behavior [1,2] have led to an explosion in the strategies proposed to treat cutaneous metastatic melanomas [1–3]. In effect, new molecules and treatment modalities are being developed [1–4] to treat this devastating disease. Unfortunately, these strategies only retain efficacy for a few months before the disease relapses, plus the drugs involved have toxic side effects and are very expensive [5], so the only reliable therapy is proper surgical excision when the disease is localized to the skin.

Therefore, the management of metastatic melanoma represents a significant challenge for both clinicians and patients, and there is an urgent need to clarify the mechanisms responsible for resistance to

therapy against metastatic melanoma [5]. These challenges undoubtedly will be addressed by basic and clinical melanoma researchers for years to come. However, to help patients with these devastating diseases we need immediate, nontoxic and economical adjuvant strategies for improvement of patient survival, with relatively limited side effects. These requirements can be met by the application of natural products and their derivatives in an educated fashion, to not only restrict melanoma growth and progression but also to restore the body's homeostatic capabilities to control tumor behavior [5]. Examples of such compounds are vitamin D that can be activated in the body by the classical pathway to $1\alpha,25$ -dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) [6,7], and newly discovered forms of vitamin D [8,9] that show antiproliferative, anti-inflammatory and anticancer properties [10].

KEYWORDS

• adjuvant therapy • CYP11A1
• CYP27B1 • melanoma
• metabolism • vitamin D • vitamin D receptor

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Source & activation of vitamin D

Exposure of human skin to ultraviolet (UV) wavelengths of solar radiation, including UVA ($\lambda = 320\text{--}400\text{ nm}$) and UVB ($\lambda = 280\text{--}320\text{ nm}$), has cytotoxic and mutagenic effects, and induces damage at the subcellular, cellular and tissue levels leading to a variety of skin pathologies including, but not limited to, epidermal skin cancers and melanoma [11,12]. In addition, UVB also has a positive effect on systemic homeostasis through induction of vitamin D₃ (D₃) formation in the skin [6], which after transport to the liver undergoes hydroxylation to 25-hydroxy-vitamin D₃ (25(OH)D₃). This serves as a pro-hormone that is activated to 1,25(OH)₂D₃ in the kidney or peripheral tissues that express the required enzyme, CYP27B1 [6]. The epidermis can also activate D₃ locally through the same sequence of reactions: $D \rightarrow 25(OH)D \rightarrow 1,25(OH)_2D$ [7]. Although the vast majority of systemic D₃ derives from the skin, an additional source can be from dietary supplementation [6].

Over the last decade, we have identified novel pathways of vitamin D metabolism, initiated by the action of CYP11A1, that produce a number of hydroxy derivatives as follows: $D_3 \rightarrow 20(OH)D_3 \rightarrow 20,23(OH)_2D_3 \rightarrow 17,20,23(OH)_3D_3$ and $D_2 \rightarrow 20S(OH)D_2 \rightarrow 17,20(OH)_2D_2 \rightarrow 17,20,24(OH)_3D_2$ [8,10]. Our studies indicate that these pathways operate *in vivo* with CYP27B1 being capable of hydroxylating some of the products in the 1 α -position [8,9]. In tissues with high expression of CYP11A1, 20(OH)D₃ is the major metabolite and is more abundant than 25(OH)D₃ [8]. Similarly, tissues expressing low levels of CYP11A1 such as skin can also produce 20(OH)D₃ and its hydroxy derivatives [8,9]. The initial and major metabolite of the pathway, 20(OH)D₃, is detectable in human serum and epidermal keratinocytes [8].

Defects in vitamin D signaling are associated with melanomagenesis & melanoma progression

There is accumulating evidence that melanoma development may be facilitated by vitamin D deficiency and/or defects in the functioning of the vitamin D receptor (VDR) [13–17]. Specifically, VDR polymorphisms are associated with an increased risk of developing melanoma (reviewed in [13]). Also, serum levels of 25(OH)D₃ decrease with progression of metastatic melanoma [14]. Importantly, a negative correlation between melanoma progression and

VDR expression was shown with there being a remarkable decrease in VDR immunoreactivity in nuclei of melanoma cells at vertical versus radial growth phases, with metastatic melanomas showing the lowest receptor expression [15,17]. Also, the presence of ulceration and the lack of nonbrisk tumor infiltrating lymphocytes were accompanied by significantly lower VDR expression [17]. Furthermore, lack of or low expression of the VDR in primary melanomas and metastases was related to shorter overall patients' survival [15,17].

Similar observations were made concerning CYP27B1 [16], with expression being significantly lower in melanomas at the vertical growth phase and in metastatic melanomas compared with melanocytic nevi and melanomas at the radial growth phase. Expression was also significantly lower in more advanced stages (Clark's levels III–V, and Breslow's thickness $\geq 2.1\text{ mm}$) [16]. In addition, there was an inverse correlation between CYP27B1 and Ki-67 expression [16]. Furthermore, CYP27B1 expression was reduced in primary melanomas that developed metastases compared with nonmetastasizing melanomas. Importantly, the lack of or low expression of CYP27B1 was associated with shorter overall survival and disease-free survival time.

The above information indicates that defects in VDR signaling due to reduced receptor expression, low levels of 25(OH)D₃ or its reduced activation (1 α -hydroxylation) by CYP27B1 in the tumor environment, can be linked to melanomagenesis and tumor progression leading to shorter overall survival and disease-free survival time [16].

Vitamin D & growth of melanoma in experimental models

Active forms of vitamin D, including calcitriol (1,25(OH)₂D₃) and its derivatives, have been proven to display potent anticancer activity [6,7,11] with the inhibition of melanoma growth (reviewed in [13,18]). Unfortunately, the hypercalcemic effect of high levels of 1,25(OH)₂D₃ attenuates the use of pharmacological doses for cancer therapy. However, its precursor, vitamin D₃, has been successfully used at high doses (50,000 units per week or 10,000 units per day) in adjuvant treatment of autoimmune diseases (reviewed in [13]). Thus, vitamin D₃/2 can potentially be used in the therapy of melanoma at relatively high doses providing patients are monitored for changes in serum calcium levels to prevent hypercalcemia.

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Importantly, we have discovered a new pathway of vitamin D metabolism initiated by CYP11A1, which produces novel analogs including 20-hydroxyvitamin D₃/2 (20(OH)D₃/2). These show strong antileukemia and anticancer activities *in vitro*, while being non-calcemic and nontoxic *in vivo* [10,19]. We have already tested the *in vitro* activity of the major product of this pathway, 20(OH)D₃/2 and its metabolites, and found that they show antiproliferative activity against cultured melanoma cells, acting through the VDR [10,18,19]. 20(OH)D₃/2 and 20,23(OH)₂D₃ are also nontoxic *in vivo* at pharmacologically relevant doses [10], and are produced *in vivo* [8], defining them as an endogenous/natural products. These novel secosteroids demonstrate biological potency, equal or higher than that of 1,25(OH)₂D₃, with antiproliferative, prodifferentiation and anti-inflammatory activities on epidermal keratinocytes, melanocytes, melanoma cells and dermal fibroblasts. Importantly, 20(OH)D₃ is noncalcemic and nontoxic at the highest pharmacological doses tested in rats (3 µg/kg) and in mice (30–60 µg/kg), which are up to 100-fold higher than doses of 1,25(OH)₂D₃ or its precursor, 25(OH)D₃, that are toxic [10]. Thus, we have identified a family of novel secosteroids that are excellent candidates for treatment of melanoma, since they can be used at very high doses that are nontoxic in animal models. The mechanism of the action of these secosteroids involves activation of the VDR [10,19] and/or action on alternative receptors including retinoic acid orphan receptor α and γ [20].

Vitamin D as a perfect adjuvant in therapy of melanoma

Since vitamin D has anticancer and protective properties (reviewed in [6,7,11,13]), it can be used as an adjuvant during any type of therapy for stage III or IV melanoma. This proposal is substantiated by evidence showing that defects

in vitamin D signaling are associated with melanomagenesis and its progression and that the majority of patients with advanced melanoma are vitamin D deficient or insufficient [13–17]. Furthermore, we propose the use of high doses of vitamin D₃ (50,000 units per week or 10,000 units per day) for patients at disease stages I and II that have low levels of 25(OH)D₃ (<30 ng/ml) as a prophylactic agent against recurrent or progressing disease. In this scenario, once serum levels of 25(OH)D₃ reached 50–100 ng/ml, the daily dose of vitamin D would be decreased to 4000–600 units per day, with the precise dose defined by serum calcium levels. This strategy could benefit melanoma patients at risk of developing metastatic disease (mitotically active ulcerated melanomas at the vertical growth phase that are relatively thick or with a positive sentinel lymph node biopsy).

Conclusion & future perspective

Based on the antitumor properties of vitamin D and the link between defects in vitamin D signaling and melanoma development, we propose the use of high doses of vitamin D, accompanied by monitoring of serum 25(OH)D₃/2 and calcium levels, as an adjuvant or prophylactic strategy for disease stages III and IV or stages I and II, respectively. Preclinical testing of novel vitamin D analogs that are noncalcemic and can serve as natural products is mandatory to develop alternative or complementary strategies to treat this devastating disease.

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