Vitamin D status and risk for malignant cutaneous melanoma: recent advances

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Cutaneous malignant melanoma, whose incidence is increasing steadily worldwide, is the result of complex interactions between individual genetic factors and environmental risk factors. Ultraviolet radiation represents the most important environmental risk factor for the development of skin cancers, including melanoma. Sun exposure and early sunburn during childhood are the principal causes of cutaneous melanoma insurgence in adults, with double the risk relative to a nonexposed population. Consequently, ultraviolet protection has long been recognized as an important measure to prevent such a malignancy. Biological and epidemiological data suggest that vitamin D status could affect the risk of cancer and play a role in cancer prevention by exerting antiproliferative effects. Solar radiations are critical for vitamin D synthesis in humans; however, uncontrolled and intensive sun exposure is dangerous to skin health and may contribute toward the development of cutaneous malignant melanoma. An optimum balance between sun protection and exposure is thus advocated. Additional research is required to confirm the preventive role of vitamin D in melanoma incidence or a positive influence on patient outcome. *European Journal of Cancer Prevention* **00:000–000 Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

Cutaneous malignant melanoma (CMM) is one of the most frequent and aggressive forms of skin cancer that occurs in all age groups (Cossu *et al*., 2016). CMM arises from epidermal melanocytes, the cells responsible for the production of the melanin pigment. Risk factors associated with the development of malignant melanoma (MM) are multifactorial, with both genetic and environmental factors playing a role in its pathogenesis (Garbe and Leiter, 2009; Cossu *et al*., 2016). Epidemiological studies have identified risk factors including altered regulation of susceptibility genes (e.g. *CDKN2A*, *CDK4*, *MC1R*, *ATM*, and *MX2*), a family history of melanoma, hair and skin color, and ultraviolet (UV) light exposure (Holick, 2003; Nejentsev *et al*., 2004; Uitterlinden *et al*., 2004; Deeb *et al*., 2007; Casula *et al*., 2009; Welsh, 2011; Colombino *et al*., 2013, 2014; Pena-Chilet *et al*., 2013). Repeated sun exposure during childhood or adolescence resulting in sunburn is associated with an increased risk for melanoma; however, CMM can also arise in nonsun-exposed areas, also with different outcomes (Garbe and Leiter, 2009). Its incidence has increased markedly worldwide; in particular, the incidence of melanoma continues to increase in White populations irrespective of attempts to improve sun protection, underlining the need for further preventive measures and treatments (Linos *et al*., 2009; Rigel, 2010).

In recent years, attention has been focused on the possible role of vitamin D in cancer risk reduction and, in particular, in melanoma risk. Some studies suggest a protective role of vitamin D in melanoma, whereas results on the relationship between dietary intake of vitamin D and risk are controversial and there is inadequate evidence to suggest that vitamin D supplementation decreases the risk for melanoma. The relationship between vitamin D and melanoma seems to be more intricate compared with other cancers (Uitterlinden *et al*., 2004). In nonsun-exposed melanomas, systemic immunosuppression and other physiological aspects may influence the insurgence of the disease. A decrease in the

serum levels of vitamin D may play a critical role in these patients and it has been reported that higher circulating levels of vitamin D are associated with a better prognosis in melanoma patients (Ahn *et al*., 2010; Zhao *et al*., 2014). The aim of this review is to summarize the most recent advances on the relationship between vitamin D and melanoma.

Vitamin D biosynthesis

Vitamin D is a fat-soluble micronutrient, which plays a role in the maintenance of calcium and phosphate homeostasis, predominantly increasing the gut absorption of calcium and phosphate. Vitamin D is derived from two sources: endogenous (about 90% from synthesis in the skin – vitamin D_3) and exogenous [dietary (Table 1 and Fig. 1) or supplements – vitamins D_2 and D_3 for the remaining 10%]. The substrate, 7-dehydrocholesterol (7-DHC), the penultimate compound in the cholesterol synthesis pathway, accumulates in the epidermis (Deeb *et al*., 2007; Pena-Chilet *et al*., 2013). UVB radiation to the skin transforms 7-DHC into previtamin D_3, which undergoes nonenzymatic isomerization to form vitamin D_3 (Fig. 2). Vitamin D_3 is transferred into the blood stream by the vitamin D-binding protein, an α-globulin that has a high affinity for vitamin D and its metabolites.

To be physiologically active, vitamin D (either D_2 or D_3) must first be hydroxylated into 25-hydroxyvitamin D (25-OHD), predominantly in the liver (Figs 2 and 3) (Spina *et al*., 2006). Then, 25-OHD is subsequently hydroxylated into 1α,25-dihydroxyvitamin D [1,25$(OH)_2D_3$] or calcitriol (active form of vitamin D), predominantly in the kidneys, although many tissues (including the skin) possess the capacity to produce 1α,25-OHD (Figs 2 and 3) (Spina *et al*., 2006). In fact, the entire metabolic pathway can be performed in UVB-irradiated skin and 1,25$(OH)_2D_3$ can be produced within 16 h. Figure 2 shows the biosynthesis of vitamin D in detail. Briefly, vitamin D is mainly synthesized in sun-exposed skin, where UV radiation B (280–320 nm) induces the photolysis of the B ring in 7-DHC. Starting from the 7-DHC substrate, a cascade of compounds is sequentially generated: previtamin D_3 to vitamin D_3, which in turn is transformed into 25-OHD, and, finally, 1,25$(OH)_2D_3$. Skin phenotypes and the UVB dose influence vitamin D levels (Parkin, 2011).

Table 1 Food sources of vitamin D as international units per 100 g

Food types	IU/100 g
Raw, scrambled, and omelet eggs	30
Dairy alternatives (soy yogurt/milk)	53
Hard boiled eggs	87
Pork (extra lean ham, ribs)	93
Buttermilk and fortified yogurt	105
Dairy products	110
Butter	115
Caviar	117
Tofu	157
Herring and sardine	253
Fortified cereals (whole grain total)	333
Portobello mushroom	446
Salmon	632
Cooked trout	759
Swordfish	793
Smoked salmon	818
Maitake mushroom	913
Cod liver oil	100 00

IU, international unit.

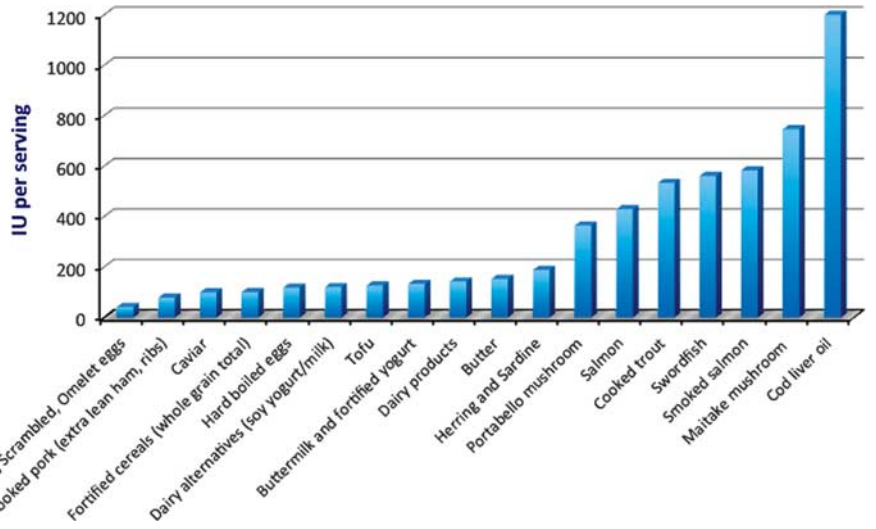
Biological activity

The primary role of vitamin D involves the regulation of bone metabolism and calcium–phosphorus homeostasis. However, over the past two decades, numerous in-vitro and in-vivo studies have evidenced several 'noncalcemic' or 'extraskeletal' effects of vitamin D (Holick, 2005). Reduced levels of vitamin D influence the onset and progression of several diseases such as autoimmune diseases, respiratory infections, diabetes mellitus type 1 and 2, hypertension and cardiovascular diseases, neuromuscular disorders, and cancer (Holick and Chen, 2008). The effects of vitamin D are largely correlated with the presence and activity of the nuclear vitamin D receptor (VDR). In fact, to exert its biological functions, 1,25$(OH)_2D_3$ binds to the VDR and heterodimerizes with the retinoid X receptor (Spina *et al*., 2006). The heterodimeric complex interacts in the nucleus with specific sequences in the promoter region of vitamin D-responsive genes, known as the vitamin D-responsive element (VDRE) (Spina *et al*., 2006). It regulates the expression of more than 900 genes, among which are genes of cell cycle progression, differentiation, and apoptosis (Valverde *et al*., 1995; Bonilla *et al*., 2005; Graf *et al*., 2005; Lamason *et al*., 2005). These biological activities suggest that high levels of vitamin D metabolites may be protective against cancer (Deeb *et al*., 2007).

Calcitriol exerts significant antitumoral activity *in vitro* and *in vivo* in murine squamous cell carcinoma (SCC), rat metastatic prostatic adenocarcinoma Dunning (MLL) model systems, human prostatic adenocarcinoma (PC-3 and LNCaP), human breast, colon, and pancreatic cancer, as well as in leukemia, myeloma, and lymphoma lines (Holick, 2004). In fact, calcitriol regulates multiple signaling pathways involved in proliferation, apoptosis, differentiation, and angiogenesis, and it therefore has the potential to influence cancer development and growth (Holick, 2007; Weinstein *et al*., 2015).

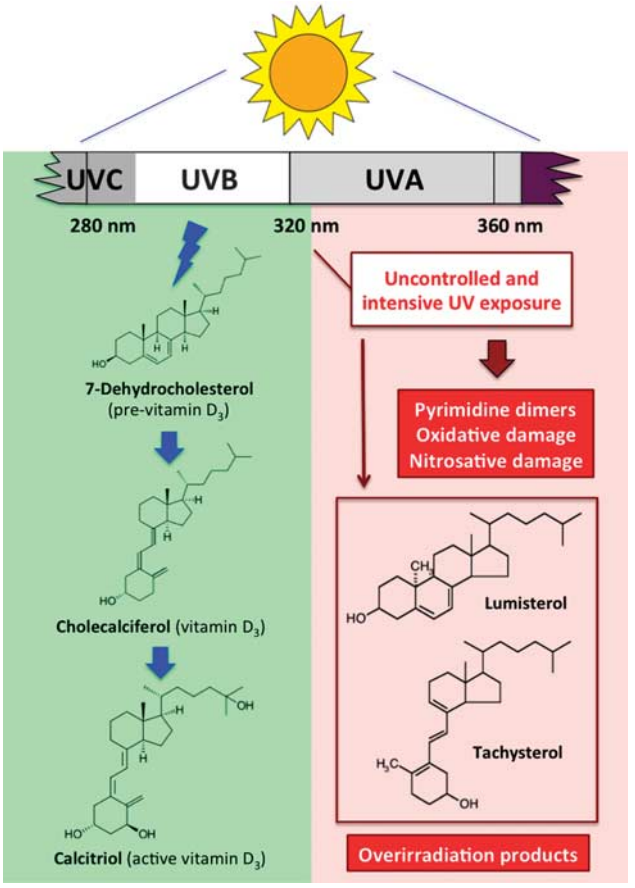
Numerous effects of 1,25$(OH)_2D_3$ also include improvement of DNA repair processes, defense against reactive oxygen species (ROS), and immunomodulation (Rass and Reichrath, 2008; Reichrath and Reichrath, 2012). Despite its antiproliferative properties, the use of 1,25$(OH)_2D_3$ as a therapeutic agent is limited because of its hypercalcemic effects. However, the reduction or elimination of the cholesterol-type side chain significantly reduces or abolishes the calcemic effects; furthermore, a certain number of analogs of vitamin D_3 have

Fig. 1



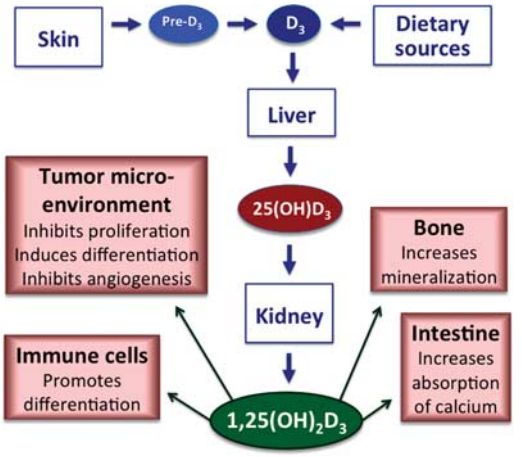
Food sources of vitamin D as international units (IU) per serving.

Fig. 2



Vitamin-D-related compounds at skin level. UV, ultraviolet.

Fig. 3



Systemic production of vitamin D compounds and biological effects. $1,25(OH)_2D_3$, 1,25-dihydroxycholecalciferol; $25(OH)D_3$, 25-hydroxycholecalciferol.

been synthesized, with the clinical activity of many of these still being investigated, both as single agents and in combination with other drugs (Rochel *et al.*, 2001).

As VDR is present in most cells in the body and calcitriol regulates 3–5% of the human genome, vitamin D activity is extensive, and can alter the defenses of the human body and most likely limit the progression of multiple diseases, including cancer (Fig. 3) (Stumpf *et al.*, 1979; Spina *et al.*, 2006). Among the many genes that are induced by calcitriol, *CYP24A1* (also known as

24-hydroxylase) is particularly important; it encodes the enzyme that catalyzes the degradation of both 1,25 $(OH)_2D_3$ (calcitriol) and 25(OH)D_3 (Prosser and Jones, 2004). Thus, the vitamin D activity is self-regulated as it may induce its own inactivation.

Vitamin D levels

UV radiation is the leading environmental factor involved in the development of skin cancer as well as melanoma. The synthesis of melanin in the skin represents a natural protective mechanism against UV-induced damage and carcinogenesis, but also limits vitamin D_3 synthesis. Vitamin D deficiency is a worldwide problem, with insufficient vitamin D levels increasing the risk of developing obesity, diabetes, asthma, autoimmune disorders, infectious diseases, and some cancers. Several meta-analyses support the evidence that low 25-hydroxyvitamin D_3 serum level is a risk factor for many chronic diseases including cancer (Guerrieri-Gonzaga and Gandini, 2013). The minimum desirable serum level of 25-hydroxyvitamin D_3 has been suggested to be between 20 and 30 ng/ml (Norman *et al.*, 2007). In 2011, the Institute of Medicine committee (Washington, DC, USA) reported guidelines stating that a level of 25-hydroxyvitamin D_3 higher than 20 ng/ml is needed for good bone and general health for almost all individuals (Ross *et al.*, 2011). However, the Institute of Medicine report did not consider clinical and demographic variables (race/ethnicity, body composition, sun exposure).

Recently, a meta-analysis from eleven prospective cohort studies suggests that the optimal 25-hydroxyvitamin D_3 concentrations of between 30 and 40 ng/ml (75 and 100 nmol/l) are needed to reduce mortality (Zittermann *et al.*, 2012). Nevertheless, the role of vitamin D in skin cancer risk is difficult to evaluate as serum levels of 25-OHD, as well as vitamin D intakes, are widely different from country to country from North to South Europe. Assays used to measure serum 25(OH)D_3 levels are not standardized and can yield variable results. This needs to be taken into consideration when interpreting the relationship between vitamin D and disease.

An important issue is how much exposure to sunlight is required to produce sufficient levels of circulating 25-(OH)D_3 for good health and whether this can be achieved without the deleterious effects of excessive sunlight exposure (such as skin cancer) or whether nutritional supplementation is needed. Several variables related to each individual (genetic constitution, skin type, area of sun-exposed skin, clothing, behavior) and environmental factors that influence the intensity and spectral range of UVB (such as latitude, season, and ozone layer) may be influential (Spina *et al.*, 2006). Guidelines have been developed for personal sunlight exposure to attain desirable levels of vitamin D (Holick *et al.*, 2011). 25-OHD is the major circulating form of vitamin D and, as a result, is used to determine the vitamin D status of both children and adults. The Endocrine Society's practice guidelines recommend that children 1 year and older receive 600–1000 IU daily and adults receive 1500–2000 IU daily, with the recommendation that obese individuals require 2–3 times more (Holick *et al.*, 2011).

Sun exposure and melanoma

Melanoma risk is associated with the skin color types (I, II, and III in north Europe and types III, IV in central Europe populations) and with the gradient of annual UVB–UVA radiation. Scandinavia is located at latitudes (>54°N) where the annual UVB (280–315 nm) exposures are moderate and only of the order of 25% of the equatorial UVB exposures (Moan and Dahlback, 1995). Despite this, CMM is a significant health problem in Scandinavia. In 2008, the estimated age-adjusted incidence rate of CMM for women in Norway was 16.5, compared with 8.9 in France, 5.6 in Spain, 8.7 in Italy, and 12.6 in Germany (Ferlay *et al.*, 2010).

The relationship between annual exposure to solar radiation (weighted using the CIE erythema action spectrum) and incidence rates of basal cell carcinoma (BCC), SCC, and CMM for Scandinavia, England, New Zealand, and Australia analyzed using logarithmic functions is similar with respect to skin types (Moan *et al.*, 2008). For SCC and BCC, the risk of disease is similar for men and women, whereas for CMM, the incidence rate is significantly higher for men than for women. The reason for this sex difference is likely to be that women in north Europe, New Zealand, and Australia tend to expose themselves intermittently to high doses during summer vacations compared with the men, who have lower exposure patterns (Moan *et al.*, 1990, 2013).

Individuals with dark, African skin (types V–VI) have a 20-fold lower risk of developing skin cancers than White individuals living at the same latitude (Wu *et al.*, 2011). Individuals of color living near the equator and exposed to sunlight on a daily basis have blood levels of 25(OH)D of 40–60 ng/ml. Their skin was designed to produce an adequate amount of vitamin and the melanin pigmentation prevents the damaging effects, minimizing the risk of nonmelanoma skin cancer (NMSC). Asians have an intermediate risk (Wu *et al.*, 2011). A recent investigation shows that the CMM mortality decreases with decreasing latitude in Europe as a whole (Spina *et al.*, 2006; Shipman *et al.*, 2011; Moan *et al.*, 2013).

In summary, the evidence that melanoma is caused by sun exposure comes from epidemiological data showing that melanoma incidence is highest where fair-skinned individuals (susceptible to sunburn) live at low latitudes. Furthermore, among white-skinned individuals, the risk is highest for those who report sunburn and sun exposure on holidays. The relationship between sun exposure and melanoma risk is, however, complex. Overall, the genetic–epidemiological data suggest that intermittent

sun exposure and sunburn are factors that are responsible for the marked increase in melanoma incidence.

Factors influencing vitamin D production

During exposure to sunlight, the UVB radiation enters the skin and activates the reactions for the production of previtamin D_3 as we reported before. The UVB photons also signal melanocytes to increase the synthesis of melanin. Melanin acts as a natural sunscreen and is efficiently packaged into melanosomes that migrate upward to the upper layers of the epidermis, where they efficiently absorb UVB and UVA radiation. An increase in skin pigmentation is inversely related to the number of UVB photons that can penetrate into the epidermis and dermis. Thus, the efficiency in utilizing UVB photons to produce vitamin D_3 in the skin is inversely related to the amount of skin pigmentation. An individual with deep skin pigmentation of African origin (skin type 5), who is exposed to the same amount of sunlight as an individual with minimum skin pigmentation of Celtic or Scandinavian origin (skin type 2), will produce less vitamin D_3 compared with that produced in the lighter-skinned individuals (Weinstein *et al.*, 2015). Aging causes a decrease in the amount of 7-DHC in the epidermis; elders exposed to the same amount of sunlight as a young adult will produce a lower amount of vitamin D compared with a young adult (Weinstein *et al.*, 2015).

The angle at which sunlight penetrates the Earth's atmosphere also influences the production of vitamin D in the skin. This angle, known as the zenith angle, is related to season, time of day, and latitude. At the lowest zenith angle, more UVB photons penetrate the Earth's surface. During the winter (i.e. November–February) above and below 35° latitude, the zenith angle is so oblique that UVB photons are absorbed by the stratospheric ozone layer. As a result, very little vitamin D can be produced in human skin. At very high latitudes, such as in Norway and Canada, little vitamin D is produced between the months of October and March. Latitude, season, and time of day influence the production of vitamin D in the skin (Weinstein *et al.*, 2015).

To solve the problem of these variables associated with sun-induced vitamin D synthesis including time of day, cloud cover, season, latitude, and skin type, a free app has been developed that provides the user not only with useful information for how much vitamin D is being produced but also alerts when they are at risk for overexposure to sunlight. Under some circumstances, dietary supplementation may be necessary and perhaps preferable to sunlight.

Vitamin D and cancer

Epidemiological studies have reported that individuals who live in higher latitudes are at a higher risk of developing breast, colon, ovarian, and prostate cancers; indeed, mortality rates in both men and women have been related to their exposure to sunlight (Grant, 2002). Several reports indicated that some of the most common cancers were significantly reduced in individuals with higher circulating levels of the antiproliferative hormone 25(OH)D, and that adequate vitamin D supplementation and sensible sunlight exposure are among the front-line factors of prophylaxis for a wide spectrum of tumors (Pludowski *et al.*, 2013). Indeed, the increased levels of 25(OH)D levels were found to prevent some cancers through downregulation of cell growth (Stumpf *et al.*, 1979; Spina *et al.*, 2006). However, it was difficult to understand how the increased exposure to sunlight could aid in decreasing the risk of common cancers as it is known that any significant increase in vitamin D intake or exposure to sunlight does not increase blood levels of 1,25(OH)D. Some clues to the pathological mechanisms were provided by observations that normal and tumor prostate cells may express a functional 1-OHase enzyme similar to that detected at the cutaneous level (Schwartz *et al.*, 1998). Since the initial observation, it is now recognized that normal and tumor cells in colon, breast, and other tissue types possess the enzymatic machinery to convert 25(OH)D directly into 1,25(OH)2D (Spina *et al.*, 2006). Thus, it seems that adequate levels of 25-(OH)D, probably above 30 ng/ml, considering that the demarcations between deficiency (< 20 ng/ml), insufficiency (20–30 ng/ml), and optimal (30–80 ng/ml) serum concentrations are controversial, may become the substrate for the extrarenal 1-OHase in several tissues (Haines and Park, 2012). The production of 1,25(OH)2D at multiple locations may be necessary to maintain and regulate genes involved in controlling cell growth and survival as well as to prevent cells from a carcinogenic transformation.

Observational studies on vitamin D levels and melanoma

Results on the relationship between vitamin D level and melanoma risk are controversial. Few studies have prospectively measured serum vitamin D before the development of melanoma, whereas most investigations have assessed serum vitamin D close to the time of diagnosis. In additional studies, vitamin D status at the time of diagnosis has been evaluated and patients were followed for survival. Moreover, the correlations between serum vitamin D levels and melanoma risk or serum vitamin D levels and survival of melanoma patients should be distinctly taken into consideration.

For a number of analyses, there was no evidence of increased melanoma risk associated with serum vitamin D. Epidemiological studies designed to look at the risk associated with vitamin D levels in the serum have many limitations including the fact that the levels likely change over time so that a single measure does not necessarily represent the complete picture. It is also not clear as to what is the most relevant measure (average vitamin D

over a period or the lowest level of vitamin D) to indicate the most critical period. The risk could also be modified by the individual's ability to utilize the circulating vitamin D. Major *et al.* (2012) carried out the first prospective study evaluating the relationship between prediagnostic serum vitamin D concentrations and subsequent melanoma risk. The results of this study indicate no statistically significant association between serum 25-(OH)D levels and melanoma, although there is a suggested protective association in the second quartiles compared with the lowest levels (Major *et al.*, 2012). In other studies, deeper Breslow thickness or higher stage was associated with lower serum vitamin D or both (Newton-Bishop *et al.*, 2009; Nürnberg *et al.*, 2009; Gambichler *et al.*, 2012). Newton-Bishop *et al.*, (2009) showed that serum 25-hydroxyvitamin D_3 levels were inversely correlated with tumor thickness among 941 patients with melanoma, although there was no difference in serum vitamin D levels between healthy controls ($n = 114$) and the patients. Another study showed no direct relationship between high or low levels of vitamin D and the occurrence and severity of melanoma (Garland *et al.*, 2003). In contrast, Nürnberg *et al.* (2009) evidenced that among patients with MM, significantly reduced serum 25(OH)D levels were found in stage IV patients compared with stage I patients, and those with low 25-(OH)D serum levels (< 10 ng/ml) probably develop earlier distant metastatic disease compared with those with higher 25 (OH)D serum levels (>20 ng/ml).

Data on the relationship between dietary intake of vitamin D and melanoma risk are also uncertain. Tang *et al.* (2011) found that daily supplementation with 1000 mg of calcium and 400 IU of vitamin D did not reduce the overall incidence of NMSC or melanoma in a large randomized double-blinded placebo-controlled trial. Vinceti *et al.* (2011) examined the association between vitamin D and melanoma risk through a population-based case–control study. They described an inverse association between dietary intake of vitamin D and melanoma risk, in particular among men and older patients. Finally, there is evidence that patients with CMM who strictly avoid sun exposure might benefit from 25-OHD supplements that are sufficient to maintain serum levels above 30 ng/ml (Gambichler *et al.*, 2012; Zittermann *et al.*, 2012). In a recent study, Fang *et al.* (2016) simultaneously examined vitamin D and C-reactive protein levels in a hospital-based cohort of patients with melanoma in relation to demographics, tumor histopathology, disease stage, and clinical outcome measures. They found that a lower level of vitamin D was associated with higher C-reactive protein, fall/winter months of blood draw, thicker and more ulcerated primary tumors, and advanced melanoma stage; they also found that a lower level of vitamin D was associated independently with poorer overall survival and disease-free survival (Fang *et al.*, 2016). More research is needed to determine whether 25(OH)D levels play a role in tumor control and progression of CMM.

As epidemiological studies have reported uncertain results and given the difficulties in assessing the risk of melanoma in response to serum levels of vitamin D, investigations have been directed toward the study of the vitamin D pathway, and in particular on the VDR receptor and its expression in melanocytes. Therefore, in recent studies, polymorphisms of *VDR* genes (which are often overexpressed in melanoma cells) are associated with the occurrence of several cancers including melanoma (Maruyama *et al.*, 2006; Hou *et al.*, 2015).

Vitamin D receptor

The most studied gene in the vitamin D pathway is the *VDR* gene located on chromosome 12q13.11. The gene has 11 exons, with more than 600 single-nucleotide polymorphisms (SNPs) having been identified within its coding region. This receptor is a nuclear transcription factor that belongs to the steroid hormone superfamily of receptors.

Nuclear receptors integrate hormonal, dietary, and other extracellular signals into cell fate decisions by regulation of gene expression and repression of a host of common gene targets. The VDR mediates the major cellular effects of vitamin D. Active hormone $1,25(OH)_2D_3$, by binding to VDR, regulates various signaling pathways involved in cell cycle progression, differentiation, and apoptosis, also implicated in cancer development and progression (Welsh, 2011).

Many epidemiological studies have evaluated associations between *VDR* variants and various types of cancer including those of the breast, colorectal region, ovary, and prostate (Maruyama *et al.*, 2006). Similarly, the associations of *VDR* polymorphisms with skin cancer risk are known. In a mouse model, dysfunctional *VDR* increased susceptibility to skin cancer following exposure to 7,12-dimethylbenz[a]anthracene or UV light (Pena-Chilet *et al.*, 2013). Given the genetic and environmental interactions between *VDR* and UV light exposure during the development of skin cancer, many epidemiological studies have also examined associations between *VDR* variants and melanoma risk. However, the results of these studies have not been consistent. Only a few SNPs, considered functional, occur at a high frequency in White populations and may influence receptor affinity, binding to nuclear DNA, RNA transcription, and protein synthesis (Uitterlinden *et al.*, 2004).

A recent study of *VDR* polymorphisms including *Fok1*, *Bsm1*, *Apa1*, and *Taq1* in a White population was carried out to define roles of the polymorphisms in skin cancer risk, incidence, and development. The results of this study show that *Fok1* polymorphism is associated with an overall significantly increased risk of skin cancer (Zhao *et al.*, 2014). The *Taq1* polymorphism could contribute

toward NMSC susceptibility, whereas the *Apa1* polymorphism is associated with the development of skin cancer. No significant association was observed between the *Bsm1* polymorphism and the risk of skin cancer. A meta-analysis study shows that *Fok1*, *Taq1*, and *Apa1* may be the susceptibility biomarkers for skin cancer in Whites (Zhao *et al.*, 2014).

A trend of cancer risk reduction with the *VDR BsmI B* allele was observed for many cancer sites. In particular, a meta-analysis was carried out to investigate the role of the *VDR BsmI* polymorphism in cancer risk, even according to different ethnicities (Raimondi *et al.*, 2014). The meta-analysis included 73 studies with 45 218 cases and 52 057 controls. A significant 6–7% reduction of cancer risk at any site was found, respectively, for carriers of *Bb* and for carriers of the *BsmI BB* genotype compared with *bb* carriers. For skin cancer, a significant risk reduction was observed for *Bb* carriers (summary odds ratio = 0.86; 95% confidence interval = 0.76–0.98). When the analysis was stratified by ethnicity, a significantly decreased risk for both *Bb* and *BB* compared with the *bb* genotype among Whites was still observed for any cancer site. Among other ethnic groups, the inverse association was still present, but did not reach statistical significance. In conclusion, a weak effect of the *BsmI B* allele in reducing cancer risk at any site, especially of the skin, was reported (Raimondi *et al.*, 2014).

VDR variants have been found to be associated with the risk of developing melanoma. In fact, in a recent epidemiological meta-analysis study, 10 eligible studies were identified and six *VDR* variants were evaluated (*Apa1*, *Bsm1*, *Cdx2*, *EcoRV*, *Fok1*, and *Taq1*) in a total of 4961 melanoma patients and 4605 controls (Hou *et al.*, 2015). It has been reported that the *VDR* variants *Fok1* and *Bsm1* may influence the susceptibility to developing melanoma, but not the other four variants *Apa1*, *Cdx2*, *EcorV*, and *Taq1*. The dominant genetic model suggested that the allele f carriers showed an 18% (pooled odds ratio = 1.18, 95% confidence interval = 1.07–1.29; $I^2 = 0\%$) increased risk for melanoma compared with homozygote FF, whereas for *BsmI*, under the dominant genetic model, a 15% decrease of melanoma risk was found for those with the *BB* or the *Bb* genotype compared with those with the *bb* genotype. The *VDR* variants *Fok1* and *Bsm1* may influence the susceptibility to the development of melanoma, although further studies are required to confirm these conclusions definitively (Hou *et al.*, 2015).

Genes involved in the vitamin D pathway

In addition to VDR, a key protein in vitamin D metabolism is the vitamin D-binding protein (DBP), and the gene encoding the DBP (*GC*, *group-specific component*) plays an important role in the vitamin D pathway. Recent studies using genome-wide association (GWAS) report that an SNP in the *GC* gene is associated with serum levels of vitamin D_3 (Wang *et al.*, 2010). Additional genes related to the production of the active form of vitamin D are probably involved in vitamin D status (Ahn *et al.*, 2010). In a previous GWAS of 4501 individuals of European ancestry, SNPs were identified in the gene encoding group-specific component (vitamin D binding) protein, *GC*, on chromosome 4q12-13, and suggestive signals for SNPs in or near three other genes involved in vitamin D synthesis or activation (Ahn *et al.*, 2010). Rs3829251 is on chromosome 11q13.4 in *NADSYN1*, encoding nicotinamide adenine dinucleotide synthetase, which was in high linkage disequilibrium with rs1790349, located in *DHCR7*, the gene encoding 7-DHC reductase that synthesizes cholesterol from 7-DHC. rs6599638, in the region with open-reading frame 88 (*C10orf88*) on chromosome 10q26.13 near *ACADSB* (acyl-coenzyme A dehydrogenase), is involved in cholesterol and vitamin D synthesis, and rs2060793, on chromosome 11p15.2 in *CYP2R1* (cytochrome P450, family 2, subfamily R, polypeptide 1), encodes a key C-25 hydroxylase that converts vitamin D_3 into an active vitamin D receptor ligand (Deeb *et al.*, 2007; Pena-Chilet *et al.*, 2013). SNPs in these four regions in 2221 additional samples were associated with 25(OH)D through meta-analysis with the GWAS data (Ahn *et al.*, 2010). In particular, SNPs for *GC*, *NADSYN1/DHCR7*, and *CYP2R*, but not for *C10orf88*, were associated with 25(OH)D concentrations (Ahn *et al.*, 2010). In the *GC* gene, at least six nonsynonymous SNPs are described, two of them with common frequency (rs7041 and rs4588) in White populations. The association between *GC* variants (rs12512631) and vitamin D levels in plasma has already been tested and proved in various lung diseases, but few studies support the association between polymorphisms on *GC* and melanoma (Ahn *et al.*, 2010; Pena-Chilet *et al.*, 2013; Hou *et al.*, 2015).

Ultimately, several studies investigated DBP serological levels and *GC* polymorphisms in association with cancer risk, with controversial results. Thus, a meta-analysis was carried out to investigate these associations and a total of 28 independent studies were included for the following tumors: BCC, bladder, breast, colon–rectum, endometrium, liver, esophagus, stomach, melanoma, pancreas, prostate, and kidney (Tagliabue *et al.*, 2015). The *GC* polymorphisms rs2282679, rs12512631, rs7041, rs4588, rs17467825, rs1155563, and rs1352844 were considered. Only a borderline decrease was found in cancer risk for patients with high levels compared with low levels of DBP, suggesting a role of DBP in cancer etiology, which should be confirmed in further studies (Tagliabue *et al.*, 2015).

VDR and the *p53* gene

Depending on the cell type and context, both VDR signaling and p53 signaling regulate many cellular functions that are of relevance for cancer development,

including proliferation, differentiation, apoptosis, and cell survival; consequently, vitamin D and p53-signaling pathways have a significant impact on spontaneous or carcinogen-induced malignant transformation of cells (Maruyama *et al*., 2006). Furthermore, the *VDR* and *p53* genes represent two important tumor suppressors in several tissues (Evans *et al*., 1998; Pálmer *et al*., 2004). Mutations in genes encoding proteins of the p53 pathway represent a hallmark of many if not all types of cancer.

Much of the tumor-suppressor function in the skin may be mediated through the interaction of the VDR and p53 pathways, either by mutual activation or by inhibition (Maruyama *et al*., 2006). UV radiation induces different forms of DNA lesions, which are generated either photochemically and directly or indirectly by UV activation of several photoreceptors, which have the capacity to modulate the cellular redox equilibrium, thereby generating ROS (Reichrath and Reichrath, 2012). Both UV-induced and ROS-induced damage activates p53. UV irradiation induces p53 to stimulate skin pigmentation by pro-opiomelanocortin derivatives including α-melanocyte-stimulating hormone and adrenocorticotropic hormone, reducing the cutaneous synthesis of vitamin D (Gupta *et al*., 2007). Cancer cells often contain abundant mutant p53 (mutp53) protein, which may contribute actively toward tumor progression by a gain-of-function mechanism. The VDR response element was identified as over-represented in promoter sequences bound by mutp53. Stambolsky *et al*. (2010) reported that mutp53 can interact functionally and physically with VDR. Mutp53 is recruited into VDR-regulated genes, augmenting the transactivation of some genes or decreasing the expression of others. Furthermore, mutp53 increases the nuclear accumulation of VDR and converts vitamin D into an antiapoptotic agent. Thus, p53 status can influence the biological impact of vitamin D on tumor cells and should be examined when considering vitamin D analogs for cancer therapy.

In summary, the *p53* tumor-suppressor gene, once mutated, not only loses its tumor-suppressor activity but gains oncogenic functions. Indeed, p53 has been considered an important target for cancer therapy. Similarly, vitamin D and its analogs are being evaluated as potential anticancer agents. An interaction between p53 and VDR has been reported, with clinical implications, suggesting that p53 status should be considered when contemplating vitamin D analogs for cancer therapy.

Genes influencing skin pigmentation and SLC45A2

In humans, only a few genes show effects on normal variations of skin pigmentation associated with UV radiation. The strongest evidence of pigmentation–mutation associations is found in the pigmentary genes *MC1R*, *ASIP*, *SLC24A5*, *SLC45A2*, *TYR*, *OCA2*, and *KITLG* (Bonilla *et al*., 2005; Graf *et al*., 2005; Lamason *et al*., 2005; Yuasa *et al*., 2007; Nan *et al*., 2009; de Gruijter et al., 2011). Among these, *SLC45A2* serves a major function in the process of melanin synthesis by controlling the activity and traffic of tyrosinase to the melanosomes and maintaining the melanosomal pH (Lucotte *et al*., 2010; Dooley *et al*., 2013). *SLC45A2*, also known as *MATP* or *AIM1*, is a membrane-associated transporter gene located on chromosome 5p and consists of seven exons spanning a region of ~40 kb. Human mutations in this gene can cause oculocutaneous albinism type 4 (Newton *et al*., 2001).

The involvement of genetic variants of *SLC45A2* in melanoma susceptibility has also been investigated. In fact, the variant 374L is protective against melanoma in different European populations (Dooley *et al*., 2013). An association of two common SNPs in *SLC45A2*, Leu374Phe (L374F, rs16891982), and Glu272Lys (E272K, rs26722) has been found with human pigmentation variations in European descendants from Australia (presumably of North European origin). The ancestral 374L allele, which is fixed in African populations, would contribute to an optimal eumelanin production, whereas the 374F allele, which is almost fixed in European populations, would give rise to an acidic melanosomal environment that negatively affects tyrosinase activity, hence leading to a lighter pigmentation (Lucotte *et al*., 2010).

The L374F SNP is significantly associated with melanoma, and the 374F (the 'light' pigmentation allele) represents a risk factor for melanoma (Cochran–Armitage trend test assuming an additive model, *P*: 4.36E – 06), although a nonuniform allele frequency is found across the geography according to the intensity of the incident UV radiation; this nonhomogeneous allele distribution suggests that positive selection has acted on the *SLC45A2* region (López *et al*., 2014).

Conclusion

In recent years, there has been growing interest in understanding the link between vitamin D status and melanoma as well as other cancers and chronic diseases. The relationship of serum levels and genetic factors of vitamin D with melanoma risk and melanoma mortality is actually not completely clear. There are few strong epidemiological studies to confirm the hypothesis that higher vitamin D levels might protect from melanoma, although a number of cohort studies have addressed a possible protective effect of vitamin D. In addition, there are insufficient indications to recommend vitamin D supplementation to decrease melanoma risk.

Certainly, vitamin D has clear antiproliferative activity on melanoma cell lines *in vitro*. There is evidence of reduced expression of the vitamin D receptor with progression from nevi through primary to metastatic melanoma. These observations suggest that if vitamin D is antiproliferative for melanoma cells *in vivo*, then those

cells might be less likely to respond to the antiproliferative effects of vitamin D as progression occurs. Other results indicate that high levels of vitamin D are correlated with the development of less aggressive tumors. Some studies report that normal levels of vitamin D_3 at the time of diagnosis are associated with a better prognosis in patients with melanoma. High circulating vitamin D concentration has been found to be associated with reduced melanoma progression and improved survival. Furthermore, reduced vitamin D serum levels have been reported in patients with stage IV melanoma compared with those with stage I.

An unresolved question remains as to which of the many described biological effects of vitamin D might have a protective effect for melanoma patients and what might be the optimal blood level to achieve those putative beneficial effects. The in-vitro antiproliferative effect of vitamin D added to melanoma cell cultures is convincing, and the Leeds Melanoma Cohort data suggest that primaries in individuals included in the study were thinner in patients with higher 25-hydroxyvitamin D_2/D_3 levels, which is consistent with an antiproliferative effect. Vitamin D, moreover, may exert pleomorphic effects including those on new blood vessel formation and immunity, and some of these effects might actually be deleterious for cancer patients. The reported effects of vitamin D on the immune system are extremely complex. If vitamin D supplementation suppresses adaptive immunity, then that would be a potentially harmful effect for melanoma patients. High doses of vitamin D should also be avoided. The evidence that vitamin D levels might influence melanoma risk remains uncertain; however, it should also be pointed out that studies of sufficient size to address this issue have not been carried out. In addition, there is evidence that patients with MM who strictly avoid sun exposure might benefit from 25-(OH)D supplements that are sufficient to maintain serum levels above 30 ng/ml. Given the interest in using vitamin D to reduce cancer risk, more research is warranted to establish its role in the control and progression of melanoma and whether vitamin D supplements can reduce risk and progression and improve outcomes.

Although the association between vitamin D and melanoma risk is still the object of considerable discussion, the potential effect of vitamin D on the risk of melanoma merits accurate consideration.

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Conflicts of interest

There are no conflicts of interest.

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