Send Orders of Reprints at bspsaif@emirates.ae

Anti-Cancer Agents in Medicinal Chemistry, **2013***, 13,* **83-97**

Sunlight Vitamin D and Skin Cancer

Rebecca S. Mason[1,*] and Jörg Reichrath[2]

[1]*Physiology & Bosch Institute, University of Sydney, Sydney, Australia, and* [2]*Department of Dermatology, The Saarland University Hospital, Homburg, Germany*

Abstract: Today, there is a controversial debate in many scientific and public communities on how much sunlight is appropriate to balance between the positive and negative effects of solar UV-exposure. UV exposure undoubtedly causes DNA damage of skin cells and is a major environmental risk factor for all types of skin cancers. In geographic terms, living in parts of the world with increased erythemal UV or high average annual bright sun results in increased risks of skin cancers, with the greatest increased risk for squamous cell carcinoma, followed by basal cell carcinoma and then melanoma. On the other hand, sunlight exerts positive effects on human health, that are mediated in part *via* UV-B-mediated cutaneous photosynthesis of vitamin D. It has been estimated that at present, approximately 1 billion people worldwide are vitamin D-deficient or –insufficient. This epidemic causes serious health problems that are still widely under-recognized. Vitamin D deficiency leads to well documented problems for bone and muscle function. There are also associations between vitamin D-deficiency and increased incidence of and/or unfavourable outcome for a broad variety of independent diseases, including various types of malignancies (e.g. colon-, skin-, and breast cancer), autoimmune diseases, infectious diseases, and cardiovascular diseases. In this review, the present literature is analyzed to summarize our present knowledge about the important relationship of sunlight, vitamin D and skin cancer.

Keywords: UV irradiation, Vitamin D, Skin cancer, Vitamin D receptor.

1. INTRODUCTION

1.1. The Physiologic Role of the Vitamin D Endocrine System (VDES) in the Human Body

Today, there is a controversial debate in many scientific and public communities on how much sunlight is appropriate to balance between positive and negative effects of solar UV-exposure [1]. During the last decades, it has been convincingly shown that at least some of the positive effects of sunlight are mediated *via* UV-B-mediated cutaneous photosynthesis of vitamin D. In this review, the present literature is analyzed to summarize our present knowledge about the important relationship of sunlight, vitamin D and skin cancer.

It has been estimated that for more than 500 million years during evolution, phytoplankton and zooplankton have been producing vitamin D [2]. While the physiologic function of vitamin D in lower non-vertebrate organisms is at present still not well understood, it is well known that most vertebrates need an adequate source of vitamin D, in order to develop and maintain a healthy mineralized skeleton [2]. While up to 10 % of the human organism`s requirements for vitamin D can be obtained by the diet (under most living conditions in the US and Europe), approximately 90 % of all needed vitamin D has to be photosynthesized from 7-dehydrocholesterol (7-DHC) in the skin through the action of the sun (UV-B) [2].

1.2. Consequences of Vitamin D Deficiency

It has been estimated that at present, approximately 1 billion people worldwide are vitamin D-deficient or –insufficient [2]. This epidemic causes serious health problems that are still widely under-recognized. Associations between vitamin D-deficiency and unfavourable outcomes of a broad variety of independent diseases including various types of malignancies (e.g. colon-, skin- and breast cancer), autoimmune diseases, infectious diseases, and cardiovascular diseases have been mostly confirmed in a large number of studies [2]. Animal experiments, and epidemiological data from many countries associate risk for and survival of various malignancies including colon- and lung cancer with solar UV-exposure, latitude and cutaneous vitamin D_3-synthesis, probably indicating a causal relationship [2,3]. Moreover, laboratory investigations analyzing the role of the vitamin D endocrine system (VDES) for cancer pathogenesis and progression, support the so-called vitamin D/cancer hypothesis. Remarkably, an increasing body of evidence now demonstrates an association between numerous vitamin D receptor (VDR) polymorphisms and cancer risk and outcome [4,5].

Interestingly, many reports have been published that associate the diagnosis of skin cancer (as an indicator of life time UV exposure) with overall positive health benefits, including decreased risk of prostate cancer and reduced cancer mortality rates [6-9].

During the last decades, great progress has been made in laboratory investigations that searched for the "missing link" between the vitamin D and cancer connection. Of particular importance was the discovery that in contrast to earlier assumptions, skin, prostate, colon, breast, and many other human tissues not only express the VDR but also express the key enzyme (vitamin D-1αOHase, CYP27B1) to convert 25(OH)D to its biologically active form, $1,25(OH)_2D$ [1,2,10]. The biologically active vitamin D metabolite $1,25(OH)_2D$, a seco-steroid hormone, is now considered as an not exclusively calciotropic endocrine hormone, but additionally as a locally produced potent autocrine hormone regulating various cellular functions including cell growth and differentiation [2,11]. As an example of its multiple important biologic effects, it has been shown that $1,25(OH)_2D$ is a direct regulator of antimicrobial innate immune responses [2,12,13]. $1,25(OH)_2D$ induces antimicrobial peptide gene expression in human keratinocytes, monocytes and neutrophils, and $1,25(OH)_2D$ along with lipopolysaccharides (LPS), synergistically induce cathelicidin antimicrobial peptide (camp) expression in neutrophils [2,12,13]. Moreover, it has been reported that Toll-like receptor (TLR) activation of human macrophages up-regulates expression of VDR and CYP27B1, leading to induction of cathelicidin and killing of intracellular Mycobacterium tuberculosis [2,12-13]. Taken these data together, the effects of solar UV radiation on the innate and adapted immune system are not exclusively immunosuppressive, but also stimulate distinct immune responses.

*Address correspondence to this author at the Physiology & Bosch Institute, University of Sydney, Sydney, NSW 2006, Australia; Tel: 61 2 93512561; Fax; 61 2 93512510; E-mail: rebeccam@physiol.usyd.edu.au

1875-5992/13 $58.00+.00

1.3. "Sunlight Dilemma"

Vitamin D is mainly produced in the skin through the actions of energetic UVB wavelengths, which convert 7-dehydrocholesterol (7DHC) into pre-vitamin D, which eventually isomerizes to vitamin D. There is a great deal that is not understood about vitamin D synthesis in skin including why efficiency is so low (<15% of 7DHC is converted), whether all areas of the skin respond to the same extent to UVB and whether, as assumed, a larger exposed skin area means that a lower UVB dose is required to produce a similar 25-hydroxyvitamin D (25OHD) outcome. Although there is some evidence that vitamin D compounds in skin contribute to UV adaptation (see below), UV exposure undoubtedly causes DNA damage of skin cells and is a major risk factor for all types of skin cancers. While there is some evidence that currently recommended sun exposures do produce adequate vitamin D in most people [14-16], the risk of encouraging sun exposure is a greater incidence of skin cancer. More research is needed before practical guidelines that lead to adequate vitamin D with minimal increased risk of skin cancer, can be properly developed. Given all the variables which affect UVB and vitamin D synthesis, it is difficult to distil a simple message for health professionals and the public. The task may be impossible.

2. PHOTOCARCINOGENESIS OF SKIN CANCER

2.1. Epidemiology and Risk Factors

There are three major types of skin cancer – squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and cutaneous malignant melanoma (CMM). Squamous cell carcinoma and melanoma can metastasize, while basal cell carcinomas can be locally invasive. The precursor of squamous cell carcinoma is the actinic keratosis or sun-spot.

In geographic terms, living in parts of the world with increased erythemal UV or high average annual bright sun results in increased risks of skin cancers, with the greatest increased risk for squamous cell carcinoma, followed by basal cell carcinoma and then melanoma [17].

In relation to personal exposure, higher levels of occupational and total UV exposure increase the risk of non-melanoma skin cancer, with greater correlation with squamous cell carcinoma than basal cell carcinoma. "Intermittent" sun exposure, such as high exposure only at weekends or holidays tends to be associated with increased risk of melanoma and to some extent basal cell carcinoma [17]. Sunburn at any age increases the risk of all skin cancers, with greatest effect on melanoma, followed by basal cell carcinoma and then squamous cell carcinoma [17,18]. The age at which high exposures occur may also matter, since there is epidemiological data showing that the risks of all major skin cancers are reduced by half in people who migrate to a high solar UV environment, like Australia after the age of 10 years [19,20]. Pale skin increases the risk of all types of skin cancer, while ability to tan lessens the risk, with decreases greatest in risk of squamous cell carcinoma followed by basal cell carcinoma and then melanoma. Squamous cell carcinomas and basal cell carcinomas tend to occur on constantly sun-exposed areas like the face, ears, neck and back of the hands, while melanomas occur in these areas but also on shoulders, backs and limbs.

2.2. UV-induced DNA Damage

UV-induced DNA damage is an important molecular trigger for skin cancer development as gene mutations can occur in damaged DNA leading to cellular transformation and malignancy [21-25]. DNA damage also initiates the events that suppress immune surveillance responsible for detecting and eliminating transformed cells [26,27]. Various forms of DNA lesions are produced either photochemically or indirectly by UV activation of other photoreceptors that can alter the cellular redox equilibrium, generating reactive oxygen species (ROS). ROS induced by UV radiation can interact with DNA causing oxidative damage to DNA, as well as lipid peroxidation. Excess levels of nitric oxide (NO) are also induced by UV-upregulation of nitric oxide synthase [28-30], and also by UVA decomposition of NO stores in nitrosothiols and nitrite [31,32]. Pathophysiological increases in NO and ROS combine to form genotoxic NO derivatives such as peroxynitrite that cause oxidative and nitrosative modifications to the sugar-phosphate scaffold and bases of DNA.

Promutagenic pyrimidine dimers are the major forms of DNA damage produced directly by UV [33]. The predominant form of pyrimidine dimer found after UV exposure in human skin is the thymine-thymine dimer, a cys-syn cyclobutane pyrimidine dimer (CPD), while thymine-cytosine, cytosine-cytosine bipyrimidines, and 6-4 photoproducts are less common [34-37]. CPDs are produced by the perturbation of the 5-6 double bonds in two adjacent pyrimidines, followed by abnormal covalent bonding that joins the 2 pyrimidines by a stable ring configuration forming a bipyrimidine product [38,39]. It is generally acknowledged that CPD production requires the higher energy wavelengths of UVB (290-320 nm). However there is some evidence for thymine dimer by UVA wavelengths below 330 nm [37, 40-43,]. Wavelengths of UVA are less energetic but considerably more abundant (20-fold higher) than UVB in sunlight, and can penetrate to deeper levels in skin [44-46] where actively dividing stem and transit amplifying cells of the epidermis are situated. The shorter, more highly energetic UVC wavelengths below 290 nm can also form CPDs, but as they are absorbed by the stratosphere they are only potentially hazardous if stratospheric ozone levels are depleted. CPDs have also been produced chemically in isolated DNA. This has been suggested to be *via* a triplet energy transfer mechanism [47,48], which may explain the production of CPDs in skin cells by a nitric oxide donor in the absence of UV [49], and a reduction in UV-induced CPDs in skin cells treated with inhibitors of nitric oxide synthase [50].

Another major promutagenic photolesion found in human skin is 8-hydroxy-2'deoxyguanosine, which is induced indirectly by oxidation of the base guanine by peroxynitrite [51,52]. Peroxynitrite can also cause nitrosative damage to form 8-nitroguanine [53-56], which is converted to a promutagenic abasic site within a few hours. DNA strand breaks are also caused by nitrosation of primary amines by another NO derivative, nitrous anhydride [57].

Photolesions resistant to DNA repair will contribute to deleterious gene mutations that form either by deletion, base mispairing, or substitutions during DNA replication when adenine is inserted as the default base. Mutations that modify cellular function can lead to aberrant behaviour leading to skin cancer development. Sequence alterations such as C to T transitions are associated with bipyrimidine sites, and correlate with mutations found in the *p53* tumor suppressor gene in various types of tumors including skin cancers and their precursors [25,52,58-61]. G to T transversions are associated with 8-hydroxy-2'deoxyguanosine [62] and occur in isolated DNA exposed to peroxynitrite [63].

2.3. DNA Damage Response

In order to protect genome integrity, cells respond to DNA damage by initiating signal transduction pathways that lead to cell cycle arrest before the affected cells can replicate. This facilitates either DNA-repair or the elimination of severely damaged cells by apoptosis [64,65].

CPDs are repaired by the nucleotide excision repair (NER) pathway that has a relatively long half-life of 7-12 hours [66-69]. Individuals with the inherited disorder xeroderma pigmentosum have a defect in one of the key enzymes of NER pathway and are highly prone to sunlight-induced skin carcinogenesis [70,71].

Oxidative DNA damage is eliminated by a more rapid alternate base excision repair (BER) pathway [72-76]. However, the repair enzyme human 8-oxoguanine-DNA glycolase 1 is less abundant in the basal layers than the superficial layers of the skin, which indicates that repair of oxidative damage in the dividing cells of the epidermis is less efficient [76].

The tumor suppressor protein p53, a key regulator of the DNA damage response, is activated by DNA damage [43,52,77,78]. Physiological doses of UVA and UVB can induce inactivating mutations in p53. Mutations in the tumor suppressor p53 in engineered human skin were found to be predominantly UVA finger print mutations induced by oxidative damage located in the basal layer of the epidermis [52]. A positive association between mutations in the tumor suppressor p53 gene in UV damaged skin in mouse and human skin before skin tumors appear, provides evidence for their involvement in skin carcinogenesis [52]. Activation of p53 is by post-translational phosphorylations and acetylations on multiple sites. These modifications enhance p53 accumulation by inhibiting degradation by a negative regulator MDM2, and/or by increasing its transcription. These processes, lead to nuclear accumulation of p53 that reaches maximum levels 12 hours after UV radiation [79]. p53 regulates the transcription of genes that control cell growth [65], nucleotide excision repair [80,81] and base excision repair [74] pathways, as well as pro- or anti-apoptotic pathways [82]. p53 mediates the gene transcription of GADD45, that assists DNA repair by binding to DNA, increasing accessibility to repair enzymes [83]. DNA repair was blocked in cells transfected with a dominant negative p53 [84], and early onset tumor formation increased in homozygote p53 knock-out mice [85]. The gene for the DNA strand sensor protein kinase (ATM) acts by phosphorylating p53 at serine 15 and is inactivated in patients with the genetic disorder ataxia telangiectasia. These patients suffer from genome instability, immunodeficiency and cancer [86-88]. Inactivation of the p53 phosphorylation site at serine 392, is a mutational hotspot in p53, resulting from UV-induced DNA damage. A knock-in mutation in mice that blocks the phosphorylation of serine 398 (the murine equivalent of human serine 392) promoted photocarcinogenesis in mice [89].

2.4. UV-induced Immune Suppression

Cutaneous immune responses that would normally detect and prevent the development of tumors in skin are suppressed by low doses of UV. This was demonstrated by the progressive growth of tumors transplanted into irradiated mice, while the tumors were rejected in unirradiated mice [90]. Pyrimidine dimers are important mediators of photoimmune suppression [26,27]. This was first demonstrated in the opossum where pyrimidine dimers are normally repaired in the presence of visible light by photolyase, an endogenous photoreactivating enzyme, which is present in most living organisms, but lost in mammals. A reduction in pyrimidine dimers correlated with a reduction in photoimmune suppression in the opossum after treatment with visible light immediately after UV irradiation [26]. Photommune suppression was also reduced in irradiated mice after reduction of CPDs by application of encapsulated T4 endonuclease, the specific repair enzyme for pyrimidine dimers [27].

There are other mediators of photoimmune suppression which include the release of pro-inflammatory cytokines that inhibit the antigen presenting function of Langerhan's cells, resulting in decreased T cell differentiation and activation and the suppression of T-cell-mediated responses [91,92]. Cis-urocanic acid formed by UV isomerisation of the photoreceptor trans-urocanic acid located in the outermost layers of skin also inhibits antigen presentation by Langerhan's cells [93,94]. Depletion of Langerhan's cells in the skin by DNA damage and oxidative stress reduces their antigen-presenting propensity [95]. Free radicals generated by UV contribute to immune suppression by releasing platelet-activating factor (PAF) from epidermal cells. The peroxidation of lipids by peroxynitrite and PAF is implicated in prostaglandin and cytokine production and release, which in turn modulate regulatory T cells (Tregs) suppressing immune responses at distant sites [91,96]. Tregs are involved in immune homeostasis by maintaining the balance between immunosuppression and autoimmunity, and reside in skin as well as skin draining lymph nodes [92] and therefore could also be subjected to DNA damage and oxidative stress.

Antioxidant treatment has been shown to abolish immune suppression mediated by the lipid peroxidation pathway in irradiated mice [97]. UV activation of Src, located on the inner surface of the keratinocyte plasma membrane, triggers signalling cascades that activate the transcription factors AP-1 and NF-κB that regulate immune regulatory cytokines, which is also blocked by antioxidant treatment [91,98,99]. Activation of an alternate complement pathway has also been implicated in inflammatory and immune modulating activities [100,101].

Both UVB and UVA components of sunlight are immuno-suppressive in mice and humans [102-105] while certain wavelengths of UVA have been shown to have a protective effect against UVB-induced immunosuppression in mice [106]. However there is some conflicting data from studies in mice and humans regarding the particular wavelengths of UVA and their immuno-modulatory effects [105-107].

2.5. Melanoma

There is evidence that inadequately repaired DNA damage produced by both UVB and UVA, together with UV-induced immune suppression also contribute to the pathogenesis of melanoma, particularly on sun-exposed skin [108-110]. Inadequately repaired DNA damage in melanocytes may lead to mutations or amplifications of genes involved in a variety of growth and survival pathways, such as *BRAF*, *Kit* and *cyclin D1* [111]. Melanocytes are different from keratinocytes in that they do not proliferate much and have reduced DNA repair capacity, but tend to be more resistant to apoptosis, despite significant DNA damage. These differences may help to explain the somewhat different patterns of UV exposure associated with melanoma compared with squamous cell carcinoma [108,112].

3. THE VITAMIN D ENDOCRINE SYSTEM (VDES) IN SKIN CANCER

3.1. VDES in Non-melanoma Skin Cancer

Increasing evidence indicates an important role of the VDES for skin carcinogenesis. It has been stated that the VDR, mostly due to its ligand-induced growth-regulatory effects, may act as a tumor suppressor in skin [113]. Using immunohistochemical techniques and real-time PCR, strong expression of key components of the VDES (VDR, CYP24A1, CYP27A1, CYP27B1) has been demonstrated in most cell types present in the skin (including keratinocytes, melanocytes and immune cells), and in BCCs and SCCs previously [10,114,115]. Interestingly, expression of VDR, CYP24A1, and CYP27B1 is stronger in BCCs and SCC as compared to unaffected, normal skin [10,114,116]. These findings provide supportive evidence for the concept that endogeneous synthesis and metabolism of vitamin D compounds as well as VDR expression may regulate growth characteristics of BCCs and SCCs. In BCCs, many splicing variants of CYP27B1 have been detected, however the functional significance of this finding is still unknown [10]. It has been shown that mouse and human BCC and SCC cell lines respond well to the antiproliferative effects of biologically active vitamin D compounds [117,118]. The Hedgehog (Hh) signaling pathway, that involves Patched (Ptch), Gli1 and Smo, has been shown to regulate cellular functions that are of importance for embryonic development and carcinogenesis [117]. It is now well accepted that mutations in the Ptch gene are a key event of BCC

carcinogenesis [117]. Interestingly, it has been demonstrated that calcitriol inhibits proliferation and growth of BCCs of Ptch mutant mice *in vitro* and *in vivo* [117]. As assessed by reduced Gli1 transcription, it has recently shown that calcitriol inhibits canonical Hh signaling independently of VDR signaling and downstream of Ptch. An obvious molecular target of this VDR-independent effect of calcitriol is Smo, because Smo-deficient cells show no decreased Gli1 transcription in response to this substance. A similar observation has been made for the inactive form of calcitriol, vitamin D3 [119]. According to this work, Ptch might function as an efflux pump for vitamin D-related compounds with Hh-inhibitory potential.

Considering the importance of the VDES for carcinogenesis of BCCs and SCCs that is outlined in this review, it is no surprise that low serum 25(OH)D concentrations and genetic variants of the VDES have recently drawn attention as potential risk factors for occurrence and prognosis of non-melanoma skin cancer. Expression and function of the VDR protein can be affected by genetic variants (polymorphisms) in the VDR gene [4,120]. These single nucleotide polymorphisms (SNPs), defined as mutations with an allelic frequency of at least 1% in a given population, are subtle DNA sequence changes which occur often and can have biologic effects [4]. Because of their abundance in the human genome as well as their high frequencies in the human population, SNPs have often been studied with the aim of explaining variations in the risk and prognosis for common diseases [4]. Associations indicate that the Apa1 and Taq1 genotypes of VDR may be of importance for carcinogenesis of BCCs, but not for SCCs [121] though associations with risk of solar keratoses have been reported [122]. Associations of the BSM1 polymorphism with BCC [123] and SCC [124] have also been reported. In conclusion, an increasing body of evidence now indicates that the VDES is of relevance for carcinogenesis and progression of non-melanoma skin cancer and that vitamin D compounds may hold promise as effective agents for the prevention and treatment of these malignancies.

3.2. VDES in Melanoma

A contribution of the VDES to the pathogenesis and prognosis of malignant melanoma has been recognized for several decades [125]. The expression of key components of the vitamin D endocrine system (VDR, CYP27A1, CYP27B1, CYP24A1) in normal melanocytes and in malignant melanoma has been characterized *in vitro* and in situ [116,126]. These findings, that include strong expression of VDR, CYP27B1 and CYP24A1, provide convincing evidence for the concept that endogeneous synthesis and metabolism of vitamin D metabolites as well as VDR expression may regulate growth characteristics and function of normal melanocytes and melanoma cells *in vitro* and *in vivo* [118, 126-129]. Using array CGH, amplification of the $1,25(OH)_2D$-metabolizing enzyme CYP24A1 was detected as a likely target oncogene of the amplification unit 20q13.2 in breast cancer cell lines and tumors [130]. It has been speculated that over-expression of CYP24A1 due to gene amplification may abrogate $1,25(OH)_2D$-mediated growth control. Additionally, amplification of the CYP27B1 gene has been reported in human malignant glioma [131]. The significance of these findings remains to be investigated. One study analyzing metastases of malignant melanomas found no evidence of amplification of CYP27B1 or CYP24A1 genes using Southern analysis [116].

One study investigated the effects of 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$], its analog seocalcitol (EB 1089), and 25-hydroxyvitamin D3 [25(OH)D3], on the growth of seven melanoma cell lines *in vitro* [126]. While three cell lines (MeWo, SK-Mel-28, SM) responded to antiproliferative effects of active vitamin D analogs, the others (SK-Mel-5, SK-Mel-25, IGR, MelJuso) were resistant. A strong induction (7000-fold) of CYP24A1 mRNA was detected in responsive cell lines along with $1,25(OH)_2D_3$-treatment, indicating functional integrity of VDR-mediated transcription. In contrast, induction of CYP24A1 was much lower in resistant melanoma cells (70-fold). VDR mRNA was induced up to 3-fold both in responsive and resistant cell lines along with $1,25(OH)_2D_3$-treatment. In that study, RNA for vitamin D-activating enzymes CYP27A1 and CYP27B1 was detected in all melanoma cell lines analyzed, additionally splicing variants of CYP27B1 were shown in SK-Mel-28 cells. Expression of CYP27A1 and CYP27B1 was marginally modulated along with treatment. Proliferation of melanoma cells was not inhibited by treatment with $25(OH)D_3$, indicating no significant stimulation of endogeneous production of antiproliferative acting $1,25(OH)_2D_3$. In conclusion, the vitamin D endocrine system has been characterized in melanoma cells and it was demonstrated that the majority of melanoma cell lines analyzed is resistant to antiproliferative effects of $1,25(OH)_2D_3$. The authors concluded, that only a minority of cases with metastasizing melanoma may represent a promising target for palliative treatment with new vitamin D analogs that exert little calcemic side effects or for pharmacological modulation of $1,25(OH)_2D_3$-synthesis/metabolism. Interestingly, it has been shown previously that $1,25(OH)_2D_3$-sensitivity of melanoma cells can, at least in part, be restored by co-treatment with the histone deacetylase inhibitor (HDACI) trichostatin A (TSA) or with the DNA methyltransferase inhibitor (DNMTI), 5-azacytidine (5-Aza) [132]. It was shown that treatment with $1,25(OH)_2D_3$ and/or epigenetic drugs (5-Aza, TSA) modulated the VDR mRNA expression in $1,25(OH)_2D_3$-responsive and -resistant melanoma cell lines and in cultured normal human melanocytes (NHM). Treatment with 5-Aza, but not with TSA, reduced the expression of a VDR-regulating microRNA (miR-125b) in $1,25(OH)_2D_3$-responsive and -resistant melanoma cell lines and in NHM. Treatment with $1,25(OH)_2D_3$ and/or epigenetic drugs (5-Aza, TSA) reduced the expression of another VDR-regulating microRNA (miR 27b) in three out of four melanoma cell lines. It was concluded that responsiveness to $1,25(OH)_2D_3$ corresponds to the expression level of VDR mRNA which in turn might be regulated by VDR microRNAs (miR-27b, miR-125b) or by epigenetic modulation [132].

Considering the importance of the VDES for cancer, it is no surprise that low 25(OH)D serum concentrations and genetic variants of the VDES have drawn attention as potential risk factors for occurrence and prognosis of melanoma. In 2000, an association of Fok 1 restriction fragment length polymorphisms (RFLP) of the VDR gene with occurrence and outcome of malignant melanoma, as predicted by Breslow thickness, was reported [133]. The same laboratory demonstrated thereafter that a SNP in the promotor region of VDR (A-1012G, adenine-guanine substitution -1012 bp relative to the exon 1a transcription start site) is associated in melanoma patients with greater Breslow thickness and with the development of metastatic disease [134]. The authors concluded that polymorphisms of the VDR gene, which can be expected to result in impaired function of biologically active vitamin D metabolites, are associated with susceptibility and prognosis in malignant melanoma. In recent years, many studies have convincingly reported an association of VDR SNPs with occurrence and outcome of malignant melanoma, although it has to be noted that a few investigations showed negative results. Recently, the interaction between VDR polymorphisms and sun exposure was investigated in a population-based multinational study comparing 1138 patients with a multiple (second or subsequent) primary melanoma (cases) to 2151 patients with a first primary melanoma (controls) [135]. This was essentially a case-control study of melanoma in a population of melanoma survivors. Sun exposure was assessed using a questionnaire and interview, and was shown to be associated with multiple primary melanoma. VDR was genotyped at the FokI and BsmI loci and the main effects of variants at these loci and their interactions with sun exposure were

analyzed. The authors found that only the BsmI variant was associated with multiple primary melanoma (OR=1.27, 95% CI 0.99-1.62 for the homozygous variant genotype) and concluded that these results suggest that risk of multiple primary melanoma is increased in people who have the BsmI variant of VDR.

The association of VDR polymorphisms and the risk of cutaneous melanoma has been analyzed in a meta-analysis [136]. Six studies (cases, 2152; controls, 2410) that investigated the association between 5 VDR polymorphisms (TaqI, FokI, BsmI, EcoRV, and Cdx2) and the risk of melanoma were retrieved and analyzed. The model-free approach was applied to meta-analyze these molecular association studies. Available data suggested a significant association between the BsmI VDR polymorphism and melanoma risk (pooled odds ratio [OR], 1.30; 95% confidence interval [CI], 1.11-1.53; P= .002; heterogeneity Cochran Q test, P> .1), and the population-attributable risk was 9.2%. In contrast, the FokI polymorphism did not appear to be associated with such risk (OR, 1.09; 95% CI, 0.99-1.21; P= .07; heterogeneity Cochran Q test, P> .1). For the TaqI and the EcoRV polymorphisms, significant between-study heterogeneity did not support genotype data pooling. Only 1 study investigated the Cdx2 variant, and the findings were negative. Current evidence is in favor of an association between 1 VDR gene polymorphism (BsmI) and the risk of developing melanoma [136]. These findings prompt further investigation on this subject and indirectly support the hypothesis that sun exposure may have an antimelanoma effect through activation of the vitamin D system [136].

Several studies reported a strong inverse correlation between serum 25-hydroxyvitamin D concentrations and Breslow thickness [137-139]. Among the patients with malignant melanoma, significantly reduced serum 25(OH)D levels were found in the stage IV patients as compared to stage I patients, and those with low 25(OH)D serum levels (<10 ng/ml) may develop earlier distant metastatic disease compared to those with higher 25(OH)D serum levels (>20 ng/ml) [137].

Berwick *et al*. reported 2005 upon the association between sun exposure and mortality from melanoma [140]. They showed that melanoma incidence and survival are positively associated, both geographically and temporally. Solar elastosis, a histologic indicator of cutaneous sun damage, has also been positively associated with melanoma survival. Although these observations raise the possibility that sun exposure increases melanoma survival, they could be explained by an association between incidence and early detection of melanoma. Berwick *et al*. therefore evaluated the association between measures of skin screening and death from cutaneous melanoma. Case subjects (n = 528) from a population-based study of cutaneous melanoma were followed for an average of more than 5 years. Data, including measures of intermittent sun exposure, perceived awareness of the skin, skin self-screening, and physician screening, were collected during in-person interviews and review of histopathology and histologic parameters (i.e., solar elastosis, Breslow thickness, and mitoses) for all of the lesions. Competing risk models were used to compute risk of death (hazard ratios [HRs], with 95% confidence intervals [CIs]) from melanoma. All statistical tests were two-sided. Sunburn, high intermittent sun exposure, skin awareness histories, and solar elastosis were statistically significantly inversely associated with death from melanoma. Melanoma thickness, mitoses, ulceration, and anatomic location on the head and neck were statistically significantly positively associated with melanoma death. In a multivariable competing risk analysis, skin awareness (with versus without, HR = 0.5, 95% CI = 0.3 to 0.9, P = .022) and solar elastosis (present versus absent, HR = 0.4, 95% CI = 0.2 to 0.8, P = .009) were strongly and independently associated with melanoma death after adjusting for Breslow thickness, mitotic index, and head and neck location, which were also independently associated with death [140]. They concluded that sun exposure is associated with increased survival from melanoma. Conversely, diagnosis in winter, which is likely to be associated with low 25(OH)D concentrations, has been reported to be associated with poorer survival [141].

However, it has to be noted that most of these investigations are association studies that do not allow a conclusion of a causal relationship and that randomized controlled trials are still lacking. One such pilot trial (MelD) is being conducted at the Sydney Melanoma unit [142]. For this trial, patients who recently had a melanoma removed and who are at high risk of recurrence, are randomized to receive 500,000IU of oral vitamin D3, followed by monthly doses of 50,000IU for 2 years, or placebo tablets for loading and maintenance. The requirement to raise 25(OH)D levels quickly was considered to outweigh possible negative effects of a large loading dose [143]. While the primary outcomes of this pilot study are safety and ability to raise blood 25(OH)D concentrations, survival over 5 years is being monitored.

In light of inverse relationships reported in observational studies of vitamin D intake and serum 25(OH)D levels with risk of non melanoma skin cancer (NMSC) and melanoma, the effects of vitamin D (400 IU daily) combined with calcium supplementation (1000 mg daily) on skin cancer were recently evaluated in a randomized placebo-controlled trial analyzing postmenopausal women age 50 to 79 years (N = 36,282) enrolled onto the Women's Health Initiative (WHI) calcium/vitamin D clinical trial (mean follow-up period of 7.0 years) [144]. Neither incident NMSC nor melanoma rates differed between treatment hazard ratio [HR], 1.02; 95% CI, 0.95 to 1.07) and placebo groups (HR, 0.86; 95% CI, 0.64 to 1.16). In subgroup analyses, women with a history of NMSC assigned to calcium + D had a reduced risk of melanoma versus those receiving placebo (HR, 0.43; 95% CI, 0.21 to 0.90; P(interaction) = .038), which was not observed in women without a history of NMSC. The authors concluded that vitamin D supplementation at a relatively low dose plus calcium did not reduce the overall incidence of NMSC or melanoma. However, in women with history of NMSC, calcium + D supplementation reduced melanoma risk, suggesting a potential role for calcium and vitamin D supplements in this high-risk group [144]. The authors concluded that results from this post hoc subgroup analysis should be interpreted with caution but warrant additional investigation [144].

4.1. Vitamin D Compounds in the Prevention of Skin Cancer: Results from *in vitro* Investigations and Animal and Human Studies

The photoprotective effects of vitamin D compounds against thymine dimers and apoptosis demonstrated in mouse and human skin, and protection against photoimmune suppression and photocarcinogenesis in mice has led to the proposal that photosynthesis of vitamin D from UVB in skin and its local conversion to the active hormone $1,25(OH)_2D_3$ is an adaptive mechanism for cellular defence against further UV exposures.

4.1.1. Vitamin D Compounds Reduce UV-induced DNA Damage

There is considerable evidence for the photoprotective effect of vitamin D compounds against thymine dimers, which are reduced in irradiated skin cells treated immediately after irradiation with $1,25(OH)_2D_3$. This has been demonstrated by immuno-histochemistry using a monoclonal antibody specific for thymine dimers. Quantification by image analysis showed reduced nuclear staining in $1,25(OH)_2D_3$-treated irradiated skin cells in culture [50, 145-148] and in mouse and human skin *in vivo* [147,149-151]. The reduction in DNA damage after UV in the presence of $1,25(OH)_2D_3$ has been reported in keratinocytes [50,145,146,148,151] skin fibroblasts [145] and melanocytes [147]. Thymine dimers increased after UV, reaching a maximum level after 6 hours [50]. In the presence of $1,25(OH)_2D_3$, thymine dimers were reduced 30 minutes after UV exposure. A reduction in thymine dimers with silibinin

was also reported one hour after irradiation in Skh:hr1 hairless mice [152]. A reduction of thymine dimers by $1,25(OH)_2D_3$ within this short time frame is inconsistent with improved DNA repair, as the rate of repair by the NER pathway is relatively slow (6-24 h) [66-69]. The increase in thymine dimers after irradiation and their suppression by the vitamin D hormone within 30 minutes leads to a proposal that thymine dimers may be produced by a metabolic processes, which is suppressed by vitamin D compounds, in addition to being produced by direct DNA absorption of UV.

4.1.2. Vitamin D Compounds and Sunburn Cell Formation

DNA damage that is irreparable leads to the elimination of the damaged cells by apoptosis [65,153]. $1,25(OH)_2D_3$ protects against UV-induced cell loss in cultured human skin cells [50,149,154-157] and in mouse skin [50,151]. and human skin [150] which is attributed to a reduction in DNA damage [50]. Apoptosis was also inhibited in irradiated cultured skin cells in culture by the vitamin D analogs, calcipotriol [158], $1\alpha,25(OH)_2$lumisterol$_3$ (JN) and $1\alpha,25(OH)_2$-7-dehydrocholesterol (JM) [145]. Apoptotic cells in irradiated skin are identified as sunburn cells by their pyknotic nuclei and eosinophilic cytoplasm in stained skin sections [159]. Sunburn cells are reduced by $1,25(OH)_2D_3$ after systemic or topical treatment in mice immediately after UV-irradiation [148,151,160] and in human subjects treated topically after irradiation [145].

4.1.3. Vitamin D Compounds and UV-induced Immune Suppression

Vitamin D compounds have an immunomodulatory role in skin. The proinflammatory cytokine, IL-6 is reduced in cultured human skin cells by 125D [157] and ergocalciferol [161]. 125D and the JN analog also reduced IL-6 and edema in irradiated skin of Skh:hr1 hairless mice [49,151] and reduced cell-mediated immune suppression measured by contact hypersensitivity (CHS) to oxazalone in UV-irradiated Skh:hr1 hairless mice [148,151,162].

As UV-induced immune suppression is associated with an increase in pyrimidine dimers and reactive nitrogen species [27,102] it is reasonable to propose that the reduction of CPD and nitric oxide derivatives by $1,25(OH)_2D_3$ mediates, at least in part, its photoprotective effect against immune suppression [49].

Immune protection by $1,25(OH)_2D_3$ demonstrated in some mouse models is, however, not consistently found [162]. In human subjects, a recall delayed type hypersensitivity (DTH) Mantoux test showed basal immune suppression when treated with high doses of $1,25(OH)_2D_3$ [150]. Immune suppression was at a similar level to that obtained with solar-simulated UV irradiation [163]. Additionally, an increased suppressive activity of $CD4^+CD25^+$ regulatory T cells in the skin-draining lymph nodes of BALB/c mice following a single topical application of $1,25(OH)_2D_3$ has been reported [164]. Conversely, CHS studies in other mouse species apart from Skh:hr1 and including BALB/c mice displayed no basal immunosuppressive response with $1,25(OH)_2D_3$ (unpublished observations). The vitamin D immunomodulatory effect in skin appears to be elicited by a number of factors, including the inhibition of antigen-presenting Langerhans cell maturation and function by suppressing relB a component of NF-κB, and modification of cytokine expression patterns [165-167]. Also, $1,25(OH)_2D_3$ and the analog 20-hydroxycholecalciferol were found to increase IkappaB alpha, an inhibitor to NF-κB activity in keratinocytes [168]. Recent reports have shown that $1,25(OH)_2D_3$ induced an increased production of the immune suppressor cytokine IL-10 in mast cells [169]. and activated regulatory T cells that suppress T-helper type 1 (Th1) responses [164]. In contrast to this, innate immunity is compromised in humans with low vitamin D status [170,171]. Identification of the VDR in Langherhans cells, monocytes, macrophages and activated T and B cells [172] supports the idea that $1,25(OH)_2D_3$ produced locally in skin cells may have a physiological role in modulating these cells and therefore skin immunity. The discrepancies in immune responses to $1,25(OH)_2D_3$ between humans and mouse strains and models may depend on a number of factors, such as genetic differences between species, dose, and the protocol used. It has also been suggested that the effects are due to the pathway used to elicit the response. Photoprotection is likely mediated, at least in part, *via* the non-genomic pathway, while the immunosuppressive effect of $1,25(OH)_2D_3$ could be mediated by the genomic pathway [162].

4.1.4. Vitamin D and Skin Carcinogenesis Including Chemical and Photocarcinogenesis

Photocarcinogenesis studies in mice have demonstrated protection with vitamin D compounds against tumor formation induced by chronic and low doses of solar simulated UV. Topical treatment with $1,25(OH)_2D_3$ immediately after each UV exposure resulted in a significant reduction in size and number of tumours [151]. Tumours were also reduced, but to a lesser extent, by the rapid acting cis-locked analog JN [151] suggesting that $1,25(OH)_2D_3$ activation of the genomic pathway may be also important for photoprotection [151]. Reduction of UV-induced tumours by vitamin D compounds is in accord with other studies that demonstrated protection from chemically-induced skin carcinogenesis by $1,25(OH)_2D_3$ [173] and related analogs [174]. These studies also complement reports showing increased susceptibility to photocarcinogenesis in VDR knock-out mice [175,176]. Interestingly, human susceptibility to solar keratoses [122] and increased risk of SCC [124] are related to VDR polymorphisms.

4.2. *In Vitro* Investigations (Including Work on Mechanisms)

4.2.1. Evidence for Partial Non-genomic Mechanism

In vitro investigations provide evidence that the mechanism for photoprotection by 125D occurs, at least in part, through the nongenomic steroid pathway.

$1,25(OH)_2D_3$, like other steroid hormones, can function not only by a relative slow genomic pathway *via* the vitamin D receptor (VDR) in the nucleus and subsequent transactivation of target genes, but also by a non-genomic pathway [177,178]. Responses elicited by the genomic pathway normally emerge over hours or days, whereas the non-genomic pathway tends to be much faster acting, eliciting responses within seconds or minutes [179,180]. It has been proposed that the photoprotective effects of $1,25(OH)_2D_3$ are mediated, at least in part, *via* the non-genomic pathway [162,180]. This is supported by studies with synthetic vitamin D analogs with different but fixed conformations. A 6-s-cis locked rapid-acting agonist 1,25-dihydroxylumisterol$_3$ (JN), which has been reported to have no gene transactivating capacity [181] inhibited the formation of UV-induced thymine dimers in skin cells to the same extent as $1,25(OH)_2D_3$ [145,147,180]. By contrast, the photoprotective effects of $1,25(OH)_2D_3$ were completely abolished by a non-genomic antagonist 1β, 25dihydroxyvitamin D3 (HL) [145,147] while an antagonist of the genomic pathway, (23S)-25-dehydro-1α-hydroxyvitamin D3-26,23-lactone (TEI-9647) did not alter photoprotection [147]. Nevertheless, the non-genomic analog 1,25-dihydroxylumisterol$_3$ (JN) was not as effective as $1,25(OH)_2D_3$ in reducing photocarcinogenesis in mice [151].

4.2.2. Receptors

It has been reported that non-genomic activation results in a rapid opening of voltage-gated chloride and calcium channels [178,182] and triggers extranuclear signal cascades probably *via* a cell surface receptor complex of the VDR translocated to the cell surface [178,182], possibly in association with the membrane-associated rapid response steroid binding protein (MARRS), also known as ERp57/GRp58/ERp60 [183]. Further details on the actions of these two receptors in the non-genomic pathway are discussed in [178,184]. Photoprotection by $1,25(OH)_2D_3$ requires the presence of the classical vitamin D receptor (VDR) for its action, as photoprotection against thymine dimers was ineffective in

fibroblasts from patients with hereditary vitamin D-resistant rickets (VDRR) carrying a null mutation in the VDR gene. Photoprotection against DNA damage was not abolished in VDRR fibroblasts expressing VDR protein with a defective DNA-binding domain or a defect in the classical ligand-binding site, providing further evidence that the photoprotective DNA damage response to $1,25(OH)_2D_3$ is nongenomic, and furthermore suggesting that $1,25(OH)_2D_3$ binds to the alternate pocket of the VDR for this activity. Protection against UV-induced thymine dimers also appears to require the presence of the ERp57 protein, with evidence that these proteins co-immunoprecipitate [184].

Nevertheless, the definitive molecular mechanisms involved in photoprotection by vitamin D compounds remain to be elucidated. A number of physiological responses to vitamin D treatment in UV-irradiated cells have been reported, which may contribute to its photoprotective effect. These cellular responses include the upregulation of p53 and metallothionein, and a reduction in nitric oxide metabolites as measured by nitrite and 3-nitrotyrosine [50,151].

4.2.3. Nuclear p53

After UV, levels of expression of p53, which accumulates in nuclei of skin cells, are increased, indicating transactivating activity that may facilitate DNA repair [78]. This UV-induced accumulation of nuclear p53 is amplified in irradiated $1,25(OH)_2D_3$-treated cells [142,151]. However, as noted earlier, the reduction of thymine dimers by $1,25(OH)_2D_3$ occurs within 30 minutes [50]. This 30 minute time period is inconsistent with the 7-12 hour half-life of CPD repair by NER [66] and also the 12 hour peak of transactivating activity of p53 [79]. It is probable that any increased repair of CPDs by $1,25(OH)_2D_3$ through upregulated NER pathway genes mediated by p53, is exerted *via* the genomic pathway. No differences were reported in levels of transcription of one of the key NER genes, xeroderma pigmentosum complementation group G (XPG), in irradiated skin cells in the presence or absence of $1,25(OH)_2D_3$ [49]. However, $1,25(OH)_2D_3$ interactions with the many other NER pathway genes still remain to be explored. $1,25\text{-}(OH)_2D_3$ up-regulated expression of the tumor suppressor p53 in normal fibroblasts. This up-regulation of p53, however, was observed in all mutant fibroblasts, including those with no VDR, and with Ab099, which is a neutralizing antibody to ERp57, so VDR and ERp57 are not essential for p53 regulation in skin fibroblasts. GADD45, which is regulated by p53, and which binds to DNA and promotes its accessibility to repair enzymes [83] has been shown to be induced by $1,25(OH)_2D_3$ resulting in growth arrest in prostate cancer cells [185].

4.2.4. Nitric Oxide

As noted earlier, upregulated nitric oxide synthase activity by UV, along with release from pre-formed stores, increases NO in skin [28-30]. NO combines with ROS to form potent genotoxic and cytotoxic derivatives such as peroxynitrite causing DNA damage, nitrosylation of tyrosine residues in proteins, and initiating lipid peroxidation [186]. Peroxynitrite also activates poly(ADP-ribose) polymerase that converts NAD+ to nicotinamide and ADP-ribose, thus reducing NAD+ and ATP formation and resulting in energy depletion. This disrupts cellular functions and leads to cell death [186]. NO overproduction induced by UV also inhibits CPD repair by preferentially inhibiting excision and ligation steps during NER [187,188]. and this can be reversed by inhibitors of nitric oxide synthase [189].

There is evidence to suggest that $1,25(OH)_2D_3$ diminishes the incidence of oxidative and nitrative DNA damage by reducing the production of NO and other toxic reactive nitrogen species, which may well improve DNA repair mechanisms. Two relatively stable end products of the nitric oxide pathway, nitrite and 3-nitrotyrosine, used as measures for NO production, were significantly reduced in irradiated skin cells in the presence of $1,25(OH)_2D_3$ when measured by the Griess assay (for nitrite) or a whole cell ELISA using a nitrotyrosine antibody [50,151]. Similarly, nitric oxide synthase inhibitors, such as aminoguanidine and L-N-monomethylarginine (L-NMMA) reduced nitrite and thymine dimer production in irradiated cells to an extent that was comparable to that produced by $1,25(OH)_2D_3$ treatment [50].

4.2.5. Metallothionein and Other Factors

Another likely pathway for photoprotection against UV-induced DNA damage is an increase in antioxidant systems. UV radiation increases the rate of ROS and reactive nitrogen species production by the activation of photoreceptors in skin. Photoreceptor activation generates genotoxic and cytotoxic free radicals by electron transfer or hydrogen abstraction processes in other molecules, or by energy transfer to molecular oxygen, forming ROS [39]. Low levels of ROS are produced during normal cellular metabolism, but are converted to less damaging molecules by intrinsic antioxidant enzyme systems and scavengers that maintain the redox balance in cells. The antioxidant enzyme systems regulating ROS production include superoxide dismutase and catalase. Superoxide dismutase converts superoxide anions to hydrogen peroxide, which is then converted to water and oxygen by catalase. Insufficient enzyme activity increases the levels of superoxide or hydrogen peroxide, and the latter can form highly toxic hydroxyl ions. Free radical scavengers are also present in skin, and include glutathione, metallothionein, thioredoxin, vitamin C and E, and carotenoids. An imbalance between ROS and antioxidant production can cause genetic modifications and inflammation that lead to skin cancer. Inflammatory cells that migrate into irradiated skin may also contribute to an increase in ROS [186].

UV induced a decrease in expression levels of glutathione peroxidase, superoxide dismutase and catalase lasting for several days, although a late transient increase in mitochondrial superoxide dismutase was reported. In addition, ROS and reactive nitrogen species induced by UV can inactivate endogenous free radical scavengers, thus enabling ROS and reactive nitrogen species to increase exponentially. A reduction in antioxidant enzymes and free radical scavengers by UV limits the capacity for cellular defence against oxidation of DNA and protein residues, and the peroxidation of membrane lipids [190].

$1,25(OH)_2D_3$ protects keratinocytes from TNFα and hydrogen peroxide cytotoxicity induced by oxidative stress [156,191] possibly by increasing inherent antioxidant systems. Metallothionein, a cysteine-rich protein responsible for metal detoxification and an oxygen radical scavenger has been observed in UVB irradiated skin treated with cadmium chloride, and reported to have a photoprotective effect by reducing sunburn cells, cell death and photo-damage [192]. Metallothionein also reduced superoxide and hydroxyl radicals in irradiated mice [193]. Furthermore, a deficiency in the antioxidant action of metallothionein increased UV-induced immune suppression in a metallothionein knockout mouse [194,195]. Transcription of metallothionein is upregulated by $1,25(OH)_2D_3$ [196] along with a reduction in UV-induced sunburn cells [160,149], which may be some evidence for a genomic pathway involvement in photoprotection.

5. CONCLUSIONS

At present, there is an ongoing debate on how much vitamin D we need to achieve a protective effect against cancer and other diseases [1,2,197]. It was recommended by some experts in the field that adults may need 1,000 IU daily, to be adequately protected against bone fractures, some cancers and derive other broad-ranging health benefits [1]. However some experts even suggested that daily oral doses of 2,000 IU may not deliver the amount of vitamin D that may be optimal [1,2]. To evaluate putative risks that may be associated with vitamin D-supplementation, one should first look at the physiological capacity of the human skin to synthesize vitamin D. On a sunny summer

day, total body sun exposure produces in the skin approximately more than 10,000 IU vitamin D per day [2]. Some authors propose that people need 4,000-10,000 IU vitamin D daily and that toxic side effects are not a concern until a 40,000 IU/day dose [198]. According to estimations an intake of 1,000 IU daily would bring 25(OH)D serum level concentrations of at least 50 % of the population up to advantageous ranges of 75 nmol/L [199].

However, one has to keep in mind that the literature is somehow controversial on how much vitamin D is needed to achieve certain 25(OH)D target ranges [200]. Moreover, it is still a matter of debate what the optimal 25(OH)D level for the prevention of individual diseases is. Zittermann *et al*. showed in a meta-analyses that the optimal 25(OH)D levels with regard to mortality are 75 to 87.5 nmol/L [201].

In 2011, the Institute of Medicine (IOM) published *Dietary Reference Intakes for Calcium and Vitamin D* [202]. They defined a recommended dietary allowance (RDA) of 600 IU vitamin D daily for life stage groups of 1-70 years [202]. Based on their definition of vitamin D deficiency, i.e. 25(OH)D less than 50nmol/L (20 ng/mL), the IOM concluded that concerns about widespread vitamin D deficiency in North American population are not well founded [202]. However, the IOM report has been criticized by other experts in the field including a workgroup of the American Endocrine Society, that recommended a daily intake of 400-2000 IU vitamin D for individual age groups [203].

The vitamin D–cancer dose–response relations have been investigated in several studies. A meta-analysis of five observational studies of serum 25(OH)D found that it takes about 1500 IU of vitamin D_3 per day to reduce the risk by 50 % for colorectal cancer, based on the assumption that 25(OH)D-level concentrations of the population are low [204]. In a cohort study of male health professionals, it was found that taking 1500 IU of vitamin D3 daily should reduce all-cancer mortality rates by approximately 30 % for males in the United States [205,206]. For breast cancer, based on two studies of 25(OH)D-serum level concentrations and breast cancer risk, it was concluded that it takes about 4000 IU/day for a 50 % reduction in risk for breast cancer [207]. At present, many experts in the field agree that the evidence to date suggests that daily intake of 1000-2000 IU/day of vitamin D could reduce the incidence of vitamin D-deficiency-related diseases with minimal risk in Europe, the US, and other countries. Against this, there are potential hazards of relatively high doses of oral vitamin D, which are rarely discussed but need to be considered. Animal studies have shown that moderate intakes of vitamin D, well below the level causing classical vitamin D toxicity, are associated with the development of atherosclerotic vascular damage in rats [208], rabbits, pigs [209] and squirrel monkeys [210]. The applicability of these studies for humans is unclear [211], but there have been no relevant studies with this outcome.

The benefit of an increased vitamin D status in reducing the economic burden of disease in western Europe has been estimated [212]. In that study, vitamin D dose-disease response relations were estimated from observational studies and randomized controlled trials. The reduction in direct plus indirect economic burden of disease was based on increasing the mean serum 25(OH)D level concentration to 100 nmol/L, which could be achieved by a daily intake of 2000-3000 IU of vitamin D. For 2007, the reduction was estimated at 187,000 million Euro/year. The estimated cost of 2000-3000 IU of vitamin D3/day along with ancillary costs such as education and testing might be about 10,000 million Euro/year. The authors suggested that sources of vitamin D could include a combination of food fortification, supplements, and natural and artificial UVB- irradiation, if properly acquired [212].

5.2. How Much Sunlight do We Need?

There is no doubt that UV-radiation is mutagenic and is the main reason for the development of skin cancer. Therefore, excessive solar UV-exposure has to be avoided, particularly burning in childhood. To reach this goal, the use of sunscreens as well as the wearing of protective clothes and glasses is absolutely important. Additionally, sun exposure around midday should be avoided during the summer in most latitudes, though solar noon, with the highest proportionate UVB dose, may be the optimal time for exposure outside summer, particularly in higher latitudes [14]. However, it has been assumed that the net effects of solar UV-B-radiation on human health are beneficial at or near current levels in the US and in most countries in Europe [206,213]. It has been speculated that the beneficial (protective) effect of less intense solar radiation outweighs its negative (mutagenic) effect. In agreement with this assumption, some authors concluded that many lives could be prolonged through careful exposure to sunlight or possibly more safely, vitamin D-supplementation, especially in non-summer months [213,214]. Previously, the economic burdens of insufficient UV-B-irradiation and vitamin D insufficiency as well as excess UV-irradiation for related diseases and conditions have been estimated in the United States. It was estimated that approximately 50,000-63,000 individuals in the United States and 19,000-25,000 in the UK die prematurely from cancer annually due to insufficient vitamin D. The U.S. economic burden due to vitamin D insufficiency from inadequate exposure to solar UV-B-irradiance, diet, and supplements was estimated at $40-56 billion in 2004, whereas the economic burden for excess UV-irradiance was estimated at $6-7 billion. The authors concluded that increased vitamin D through UV-B-irradiance, fortification of food, and supplementation could reduce the health care burden in the United States, UK, and elsewhere [214].

To summarize, it is important that recommendations of health campaigns on sun protection represent a balanced view of positive and negative effects of solar UV-exposure. As Michael Holick reported previously [2,215], we have learned that at most latitudes, even Boston, USA, very short and limited solar UV-exposure is sufficient to obtain "adequate" vitamin D-level concentrations, at least in summer. It is likely that relatively short, frequent exposures may be more useful for improving vitamin D status, since prolonged irradiation degrades the pre-vitamin D and vitamin D produced by UVB [2] and there is some evidence that UV-damage repair is better after short exposures [216]. Exposure of the body in a bathing suit to one minimal erythemal dose (MED) of sunlight is equivalent to ingesting at least about 10,000 IU of vitamin D and Dr. Holick estimated that exposure of less than 18 % of the body surface (hands, arms, and face) two to three times a week to a third to a half of an MED; (about 5 min for skin-type-2 adult in Boston at noon in July) in the spring, summer, and autumn is likely to be adequate. There are some further studies which support this general proposal [16]. Anyone intending to stay exposed to sunlight longer than recommended above should apply a broad-spectrum sunscreen with a sufficient sun protection factor to prevent sunburn and the damaging effects of excessive exposure to sunlight.

5.3. How to Treat and Prevent Vitamin D Deficiency?

What conclusions do we draw from the findings reported above, most importantly the need for adequate vitamin D but the problem that sun exposure increases the risk of various types of skin cancers? The important take home message for dermatologists and other clinicians is that health campaigns promoting strict sun protection procedures to prevent skin cancer may increase the substantial health risk of vitamin D-deficiency. Especially dermatologists have to know about the importance of an adequate vitamin D-status if solar UV-exposure is seriously curtailed. It has to be emphasized that in groups that are at high risk of developing vitamin D-deficiency (e.g. nursing home residents; patients with skin type V, VI, transplant recipients or other patients under immunosuppressive therapy), vitamin D-status need to be monitored . As a consequence of the severe health risks that are associated with vitamin D-deficiency, vitamin D-deficiency has to

be treated, e.g. by giving vitamin D orally as recommended previously. It has been shown that a single dose of 50,000 IU vitamin D once a week for 8 weeks is efficient and safe in the short term to treat vitamin D-deficiency. Another means of guaranteeing vitamin D-sufficiency, especially in nursing home residents, is to give 50,000 IU of vitamin D once a month. To prevent vitamin D deficiency in the general population, at present, most experts in the field agree that the evidence to date suggests that daily intake of 1000-2000 IU/day of vitamin D could reduce the incidence of vitamin D-deficiency-related diseases with minimal apparent risk in Europe, the US, and other countries [2]. As an alternative, advice to go outside for a walk in summer with sleeves rolled up, mid-morning or mid-afternoon for around 6-8 minutes, most days or in winter, to try to get out at lunchtime most days for about 7- 50 minutes or so depending on the latitude, might be helpful to maintain vitamin D status [197]. If people walk briskly, they can perhaps roll their sleeves up a bit. While this is not practical for everyone, the idea of getting outside to get a bit of exercise, perhaps clear the head and maybe improve people's mood, as well as to improve vitamin D status, is a goal worth considering.

If we follow the recommendations discussed above carefully, they will help to ensure an adequate vitamin D-status, thereby protecting us against adverse effects of strict solar UV-protection. Most importantly, these measures will protect us sufficiently against the multiple negative effects of vitamin D-deficiency on health without greatly increasing our risk of developing UV-radiation-induced skin cancer. To reach this goal it is important that health campaigns transfer this information to the general population and to every clinician, especially to dermatologists.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The assistance of Dr Clare Gordon-Thomson and Ms Myriam Abboud with manuscript preparation is gratefully acknowledged. The work was supported by the National Health and Medical Research Council of Australia (RM), by the Australian Research Council (RM), and by the Deutsche Krebshilfe (JR).

REFERENCES

[1] Reichrath, J. The challenge resulting from positive and negative effects of sunlight: how much solar UV exposure is appropriate to balance between risks of vitamin D deficiency and skin cancer? *Prog. Biophys. Mol. Bio*., **2006**, *92* (1), 9-16.

[2] Holick, M. F. Vitamin D deficiency. *N. Engl. J. Med*., **2007**, *357* (3), 266-81.

[3] Grant, W. B. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. *Cancer*, **2002**, *94* (6), 1867-1875.

[4] Kostner, K.; Denzer, N.; Muller, C. S.; Klein, R.; Tilgen, W.; Reichrath, J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. *Anticancer Res.*, **2009**, *29* (9), 3511-36.

[5] Raimondi, S.; Johansson, H.; Maisonneuve, P.; Gandini, S. Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. *Carcinogenesis*, **2009**, *30* (7), 1170-1180.

[6] de Vries, E.; Soerjomataram, I.; Houterman, S.; Louwman, M. W.; Coebergh, J. W. Decreased risk of prostate cancer after skin cancer diagnosis: a protective role of ultraviolet radiation? *Am. J. Epidemiol*., **2007**, *165* (8), 966-72.

[7] Grant, W. B. An ecologic study of cancer mortality rates in Spain with respect to indices of solar UVB irradiance and smoking. *Int. J. Cancer*, **2007**, *120* (5), 1123-8.

[8] Jensen, A. O.; Lamberg, A. L.; Jacobsen, J. B.; Braae Olesen, A.; Sorensen, H. T. Non-melanoma skin cancer and ten-year all-cause mortality: a population-based cohort study. *Acta. Derm. Venereol*., **2010**, *90* (4), 362-7.

[9] Rukin, N. J.; Zeegers, M. P.; Ramachandran, S.; Luscombe, C. J.; Liu, S.; Saxby, M.; Lear, J.; Strange, R. C. A comparison of sunlight exposure in men with prostate cancer and basal cell carcinoma. *Br. J. Cancer*, **2007**, *96* (3), 523-8.

[10] Mitschele, T.; Diesel, B.; Friedrich, M.; Meineke, V.; Maas, R. M.; Gartner, B. C.; Kamradt, J.; Meese, E.; Tilgen, W.; Reichrath, J. Analysis of the vitamin D system in basal cell carcinomas (BCCs). *Lab. Invest*., **2004**, *84* (6), 693-702.

[11] Lehmann, B.; Abraham, S.; Meurer, M. Role for tumor necrosis factor-alpha in UVB-induced conversion of 7-dehydrocholesterol to 1alpha,25-dihydroxyvitamin D3 in cultured keratinocytes. *J. Steroid Biochem. Mol. Biol*., **2004**, *89-90* (1-5), 561-5.

[12] Gombart, A. F.; Borregaard, N.; Koeffler, H. P. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D-3. *Faseb J*., **2005**, *19* (9), 1067-1077.

[13] Wang, T. T.; Nestel, F. P.; Bourdeau, V.; Nagai, Y.; Wang, Q.; Liao, J.; Tavera-Mendoza, L.; Lin, R.; Hanrahan, J. W.; Mader, S.; White, J. H. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J. Immunol*., **2004**, *173* (5), 2909-12.

[14] Webb, A. R.; Engelsen, O. Calculated ultraviolet exposure levels for a healthy vitamin D status. *Photochem. Photobiol*., **2006**, *82*, 1697-1703.

[15] Armas, L. A.; Dowell, S.; Akhter, M.; Duthuluru, S.; Huerter, C.; Hollis, B. W.; Lund, R.; Heaney, R. P. Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: the effect of UVB dose and skin color. *J. Am. Acad. Dermatol.*, **2007**, *57* (4), 588-93.

[16] Rhodes, L. E.; Webb, A. R.; Fraser, H. I.; Kift, R.; Durkin, M. T.; Allan, D.; O'Brien, S. J.; Vail, A.; Berry, J. L. Recommended summer sunlight exposure levels can produce sufficient (> or =20 ng ml(-1)) but not the proposed optimal (> or =32 ng ml(-1)) 25(OH)D levels at UK latitudes. *J. Invest. Dermatol*., **2010**, *130* (5), 1411-8.

[17] Armstrong, B. K.; Kricker, A. The epidemiology of UV induced skin cancer. *J. Photochem. Photobiol. B*., **2001**, *63* (1-3), 8-18.

[18] Cust, A. E.; Jenkins, M. A.; Goumas, C.; Armstrong, B. K.; Schmid, H.; Aitken, J. F.; Giles, G. G.; Kefford, R. F.; Hopper, J. L.; Mann, G. J. Early-life sun exposure and risk of melanoma before age 40 years. *Cancer Cause. Control*, **2011**, *22* (6), 885-97.

[19] English, D. R.; Armstrong, B. K.; Kricker, A.; Winter, M. G.; Heenan, P. J.; Randell, P. L. Demographic characteristics, pigmentary and cutaneous risk factors for squamous cell carcinoma of the skin: a case-control study. *Int. J. Cancer*, **1998**, *76* (5), 628-34.

[20] Holman, C. D.; Armstrong, B. K. Cutaneous malignant melanoma and indicators of total accumulated exposure to the sun: an analysis separating histogenetic types. *J. Natl. Cancer I.*, **1984**, *73* (1), 75-82.

[21] Hart, R. W.; Setlow, R. B.; Woodhead, A. D. Evidence that pyrimidine dimers in DNA can give rise to tumors. *Proc. Nat. Acad. Sci. U. S. A.*, **1977**, *74* (12), 5574-5578.

[22] Sutherland, B. M.; Blackett, A. D.; Feng, N. I.; Freeman, S. E.; Ogut, E. S.; Gange, R. W.; Sutherland, J. C. Photoreactivation and other ultraviolet/visible light effects on DNA in human skin. *Ann. NY. Acad. Sci.*, **1985**, *453*, 73-79.

[23] Brash, D. E.; Rudolph, J. A.; Simon, J. A.; Lin, A.; McKenna, G. J.; Baden, H. P.; Halperin, A. J.; Ponten, J. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc. Nat. Acad. Sci. U. S. A.*, **1991**, *88* (22), 10124-10128.

[24] Agar, N. S. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc. Natl Acad. Sci. U.S.A.*, **2004**, *101*, 4954-4959.

[25] Besaratinia, A.; Kim, S.; Pfeifer, G. P. Rapid repair of UVA-induced oxidized purines and persistence of UVB-induced dipyrimidine lesions determine the mutagenicity of sunlight in mouse cells. *FASEB J*., **2008**, *22* (7), 2379-2392.

[26] Applegate, L. A.; Ley, R. D.; Alcalay, J.; Kripke, M. L. Identification of the molecular target for the suppression of contact hypersensitivity by ultraviolet radiation. *J. Exp. Med.* **1989**, *170* (4), 1117-1131.

[27] Kripke, M. L.; Cox, P. A.; Alas, L. G.; Yarosh, D. B. Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. *Proc. Nat. Acad. Sci. U. S. A.*, **1992**, *15, 89(16)*, 7516-7520.

[28] Deliconstantinos, G.; Villiotou, V.; Stravrides, J. C. Release by ultraviolet B (u.v.B) radiation of nitric oxide (NO) from human keratinocytes: a potential role for nitric oxide in erythema production. *Br. J. Pharmacol.*, **1995**, *114* (6), 1257-1265.

[29] Bruch-Gerharz, D.; Ruzicka, T.; Kolb-Bachofen, V. Nitric oxide in human skin: current status and future prospects. *J. Invest. Dermatol.*, **1998**, *110* (1), 1-7.

[30] Cals-Grierson, M. M.; Ormerod, A. D. Nitric oxide function in the skin. *Nitric Oxide Biol. Ch.*, **2004**, *10* (4), 179-193.

[31] Paunel, A.; Dejam, A.; Thelen, S.; Kirsch, M.; Horstjann, M.; Gharini, P.; Muertz, M.; Kelm, M.; de Groot, H.; Kolb-Bachofen, V.; Suschek, C. UVA induces immediate and enzyme-independent nitric oxide formation in healthy human skin leading to NO-specific signalling. *J. Invest. Dermatol.*, **2005**, *125* (1), A3-A3.

[32] Mowbray, M.; McLintock, S.; Weerakoon, R.; Lomatschinsky, N.; Jones, S.; Rossi, A. G.; Weller, R. B. Enzyme-independent NO stores in human skin: quantification and influence of UV radiation. *J. Invest. Dermatol.*, **2009**, *129* (4), 834-842.

[33] Wikonkal, N. M.; Brash, D. E. Ultraviolet radiation induced signature mutations in photocarcinogenesis. *J. Invest. Dermatol.,. Symp. P.*, **1999**, *4* (1), 6-10.

[34] Douki, T.; Court, M.; Sauvaigo, S.; Odin, F.; Cadet, J. Formation of the main UV-induced thymine dimeric lesions within isolated and cellular DNA as measured by high performance liquid chromatography-tandem mass spectrometry. *J. Biol. Chem.*, **2000**, *275* (16), 11678-11685.

[35] Cooke, M. S.; Podmore, I. D.; Mistry, N.; Evans, M. D.; Herbert, K. E.; Griffiths, H. R.; Lunec, J. Immunochemical detection of UV-induced DNA damage and repair. *J. Immunol. Methods*, **2003**, *280* (1-2), 125-133.

[36] Courdavault, S.; Baudouin, C.; Charveron, M.; Favier, A.; Cadet, J.; Douki, T. Larger yield of cyclobutane dimers than 8-oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells. *Mutat. Res.*, **2004**, *556* (1-2), 135-142.

[37] Mouret, S.; Baudouin, C.; Charveron, M.; Favier, A.; Cadet, J.; Douki, T. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc. Nat. Acad. Sci. U. S. A.*, **2006**, *103* (37), 13765-13770.

[38] Ravanat, J. L.; Douki, T.; Cadet, J. Direct and indirect effects of UV radiation on DNA and its components. *J.Photochem. Photobiol. B.*, **2001**, *63* (1-3), 88-102.

[39] Pattison, D. I.; Davies, M. J. Actions of ultraviolet light on cellular structures. *EXS*, **2006**, (96), 131-57.

[40] Applegate, L. A.; Scaletta, C.; Panizzon, R.; Niggli, H.; Frenk, E. *In vivo* induction of pyrimidine dimers in human skin by UVA radiation: initiation of cell damage and/or intercellular communication? *Int. J. Mol. Med.*, **1999**, *3* (5), 467-72.

[41] Rochette, P.; Therrien, J.-P.; Drouin, R.; Perdiz, D.; Bastien, N.; Drobetsky, E. A.; Sage, E. UVA-induced cyclobutane pyrimidine dimers form predominantly at thymine–thymine dipyrimidines and correlate with the mutation spectrum in rodent cells. *Nucleic Acids Res.*, **2003**, *31*, 2786-2794.

[42] Courdavault, S.; Baudouin, C.; Sauvaigo, S.; Mouret, S.; Candeias, S.; Charveron, M.; Favier, A.; Cadet, J.; Douki, T. Unrepaired cyclobutane pyrimidine dimers do not prevent proliferation of UV-B-irradiated cultured human fibroblasts. *Photochem. Photobiol.*, **2004**, *79* (2), 145-51.

[43] Jiang, W.; Ananthaswamy, H. N.; Muller, H. K.; Kripke, M. L. p53 protects against skin cancer induction by UV-B radiation. *Oncogene*, **1999**, *18* (29), 4247-53.

[44] Kochevar, I. E., Pathak, M.A. and Parrish, J.A. Photophysics, Photochemistry and Photobiology. In *Fitzpatrick's Dermatology in General Medicine*, 5th ed.; Freedberg, I. M., Eisen,A, Wolff K, Austen, K.F., Goldsmith, L, Katz S and Fitzpatrick, T.B., Eds. McGraw-Hill: New York, 1999; Vol. 1, pp 220-230.

[45] Nishigori, C.; Yarosh, D. B.; Ullrich, S. E.; Vink, A. A.; Bucana, C. D.; Roza, L.; Kripke, M. L. Evidence that DNA damage triggers interleukin 10 cytokine production in UV-irradiated murine keratinocytes. *Proc. Natl. Acad. Sci. U. S. A.*, **1996**, *93* (19), 10354-9.

[46] Sequiera, V. B.; Dixon, K. M.; Mason R. S. Resisting the sun with vitamin D. In special edition Newly recognized actions of vitamin D3. *Immunology, Endocrine and Metabolic Agents in Medicinal Chemistry*, **2009**, 129-136.

[47] Lamola, A. A. Production of pyrimidine dimers in DNA in the dark. *Biochem. Bioph. Res. Co.*, **1971**, *43* (4), 893-8.

[48] Lhiaubet-Vallet, V; Cuquerella, C. M.; Castell J. V.; Bosca, F.; Miranda, M. A. Triplet excited fluoroquinolones as mediators for thymine cyclobutane dimer formation in DNA. *J. Phys. Chem. B.*, **2007**, 7409-7414.

[49] Mason, R. S.; Sequeira, V. B.; Dixon, K. M.; Gordon-Thomson, C.; Pobre, K.; Dilley, A.; Mizwicki, M. T.; Norman, A. W.; Feldman, D.; Halliday, G. M.; Reeve, V. E. Photoprotection by 1alpha,25-dihydroxyvitamin D and analogs: further studies on mechanisms and implications for UV-damage. *J. Steroid Biochem. Mol. Biol.*, **2010**, *121* (1-2), 164-8.

[50] Gupta, R.; Dixon, K. M.; Deo, S. S.; Holliday, C. J.; Slater, M.; Halliday, G. M.; Reeve, V. E.; Mason, R. S. Photoprotection by 1,25 dihydroxyvitamin D3 is associated with an increase in p53 and a decrease in nitric oxide products. *J. Invest. Dermatol.*, **2007**, *127* (3), 707-15.

[51] Kvam, E.; Tyrrell, R. M. Induction of oxidative DNA base damage in human skin cells by UV and near visible radiation. *Carcinogenesis*, **1997**, *18* (12), 2379-2384.

[52] Agar, N. S.; Halliday, G. M.; Barnetson, R. S.; Ananthaswamy, H. N.; Wheeler, M.; Jones, A. M. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: a role for UVA in human skin carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101* (14), 4954-9.

[53] McAndrew, J.; Patel, R. P.; Jo, H. J.; Cornwell, T.; Lincoln, T.; Moellering, D.; White, C. R.; Matalon, S.; DarleyUsmar, V. The interplay of nitric oxide and peroxynitrite with signal transduction pathways: Implications for disease. *Semin. Perinatol.*, **1997**, *21* (5), 351-366.

[54] Burney, S.; Caulfield, J. L.; Niles, J. C.; Wishnok, J. S.; Tannenbaum, S. R. The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat. Res.*, **1999**, *424* (1-2), 37-49.

[55] Niles, J. C.; Wishnok, J. S.; Tannenbaum, S. R. Peroxynitrite-induced oxidation and nitration products of guanine and 8-oxoguanine: structures and mechanisms of product formation. *Nitric oxide Biol. Ch.*, **2006**, *14* (2), 109-21.

[56] Pacher, P.; Beckman, J. S.; Liaudet, L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev.*, **2007**, *87* (1), 315-424.

[57] Wink, D. A.; Kasprzak, K. S.; Maragos, C. M.; Elespuru, R. K.; Misra, M.; Dunams, T. M.; Cebula, T. A.; Koch, W. H.; Andrews, A. W.; Allen, J. S. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* **1991**, *254* (5034), 1001-1003.

[58] Nataraj, A. J.; Trent, J. C., 2nd; Ananthaswamy, H. N., p53 gene mutations and photocarcinogenesis. *Photochem. Photobiol.*, **1995**, *62* (2), 218-230.

[59] Berg, R. J.; van Kranen, H. J.; Rebel, H. G.; de Vries, A.; van Vloten, W. A.; Van Kreijl, C. F.; van der Leun, J. C.; de Gruijl, F. R. Early p53 alterations in mouse skin carcinogenesis by UVB radiation: immunohistochemical detection of mutant p53 protein in clusters of preneoplastic epidermal cells. *Proc. Nat. Acad. Sci. U. S. A.*, **1996**, *93* (1), 274-278.

[60] Brash, D. E.; Ziegler, A.; Jonason, A. S.; Simon, J. A.; Kunala, S.; Leffell, D. J. Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion. *J. Invest. Dermatol. Symp. P.*, **1996**, *1* (2), 136-42.

[61] Soehnge, H.; Ouhtit, A.; Ananthaswamy, O. N. Mechanisms of induction of skin cancer by UV radiation. *Front. Biosci.*, **1997**, *2*, d538-51.

[62] Besaratinia, A.; Synold, T. W.; Xi, B.; Pfeifer, G. P. G-to-T Transversions and Small Tandem Base Deletions Are the Hallmark of Mutations Induced by Ultraviolet A Radiation in Mammalian Cells†. *Biochemistry*, **2004**, *43* (25), 8169-8177.

[63] Murata, M.; Kawanishi, S. Oxidative DNA damage induced by nitrotyrosine, a biomarker of inflammation. *Biochem. Bioph. Res. Co.*, **2004**, *316* (1), 123-128.

[64] Ouhtit, A.; Gorny, A.; Muller, H. K.; Hill, L. L.; Owen-Schaub, L.; Ananthaswamy, H. N. Loss of Fas-ligand expression in mouse

keratinocytes during UV carcinogenesis. *Am. J. Pathol.*, **2000**, *157* (6), 1975-1981.

[65] Matsumura, Y.; Ananthaswamy, H. N. Toxic effects of ultraviolet radiation on the skin. *Toxicol. Appl. Pharm.*, **2004**, *195* (3), 298-308.

[66] Mitchell, D. L.; Cleaver, J. E.; Epstein, J. H. Repair of pyrimidine(6-4)pyrimidone photoproducts in mouse skin. *J. Invest. Dermatol.*, **1990**, *95* (1), 55-59.

[67] Katiyar, S. K. Kinetics of UV light-induced cyclobutane pyrimidine dimers in human skin *in vivo*: an immunohistochemical analysis of both epidermis and dermis. *Clin. Cancer Res.*, **2000**, *6* (10), 3864-3869.

[68] Matsumura, Y.; Ananthaswamy, H. N. Short-term and long-term cellular and molecular events following UV irradiation of skin: implications for molecular medicine. *Expert Reviews in Molecular Medicine*, **2002**, *4*, 1-22.

[69] Schul, W.; Jans, J.; Rijksen, Y. M.; Klemann, K. H.; Eker, A. P.; de Wit, J.; Nikaido, O.; Nakajima, S.; Yasui, A.; Hoeijmakers, J. H.; van der Horst, G. T. Enhanced repair of cyclobutane pyrimidine dimers and improved UV resistance in photolyase transgenic mice. *EMBO J.*, **2002**, *21* (17), 4719-4729.

[70] Cleaver, J. E. Defective repair replication of DNA in xeroderma pigmentosum. *Nature* **1968**, *218* (142), 652-656.

[71] McGregor, J. M.; Hawk, J. L. M. Acute Effects of Ultraviolet Radiation on the Skin. In *Fitzpatrick's Dermatology in General Medicine*, 5th ed.; Freedberg, I. M., Eisen,A, Wolff K, Austen, K.F., Goldsmith, L, Katz S and Fitzpatrick, T.B., Eds. McGraw- Hill: 1999; Vol. 1, pp 1555-1561.

[72] Mitra, S.; Boldogh, I.; Izumi, T.; Hazra, T. K. Complexities of the DNA base excision repair pathway for repair of oxidative DNA damage. *Environ. Mol. Mutagen.*, **2001**, *38* (2-3), 180-190.

[73] Nilsen, H.; Krokan, H. E. Base excision repair in a network of defence and tolerance. *Carcinogenesis*, **2001**, *22* (7), 987-998.

[74] Zurer, I.; Hofseth, L. J.; Cohen, Y.; Xu-Welliver, M.; Hussain, S. P.; Harris, C. C.; Rotter, V. The role of p53 in base excision repair following genotoxic stress. *Carcinogenesis*, **2004**, *25* (1), 11-19.

[75] David, S. S.; O'Shea, V. L.; Kundu, S. Base-excision repair of oxidative DNA damage. *Nature*, **2007**, *447* (7147), 941-950.

[76] Javeri, A.; Huang, X. X.; Bernerd, F.; Mason, R. S.; Halliday, G. M. Human 8-oxoguanine-DNA glycosylase 1 protein and gene are expressed more abundantly in the superficial than basal layer of human epidermis. *DNA Repair*, **2008**, *7* (9), 1542-1550.

[77] Hall, P. A.; McKee, P. H.; Menage, H. D.; Dover, R.; Lane, D. P. High levels of p53 protein in UV-irradiated normal human skin. *Oncogene*, **1993,** *8 (1), 203-7.*

[78] Eller, M. S.; Maeda, T.; Magnoni, C.; Atwal, D.; Gilchrest, B. A. Enhancement of DNA repair in human skin cells by thymidine dinucleotides: evidence for a p53-mediated mammalian SOS response. *Proc. Natl. Acad. Sci. U. S. A.*, **1997**, *94* (23), 12627-32.

[79] Saito, S.; Yamaguchi, H.; Higashimoto, Y.; Chao, C.; Xu, Y.; Fornace, A. J., Jr.; Appella, E.; Anderson, C. W. Phosphorylation site interdependence of human p53 post-translational modifications in response to stress. *J. Biol.Chem.*, **2003**, *278* (39), 37536-44.

[80] Zhu, Q.; Wani, M. A.; El-Mahdy, M.; Wani, A. A. Decreased DNA repair efficiency by loss or disruption of p53 function preferentially affects removal of cyclobutane pyrimidine dimers from non-transcribed strand and slow repair sites in transcribed strand. *J. Biol.Chem.*, **2000**, *275* (15), 11492-7.

[81] Amundson, S. A.; Patterson, A.; Do, K. T.; Fornace, A. J., Jr., A nucleotide excision repair master-switch: p53 regulated coordinate induction of global genomic repair genes. *Cancer Biol. Ther.*, **2002**, *1* (2), 145-9.

[82] Mullauer, L.; Gruber, P.; Sebinger, D.; Buch, J.; Wohlfart, S.; Chott, A. Mutations in apoptosis genes: a pathogenetic factor for human disease. *Mutat, Res.*, **2001**, *488* (3), 211-31.

[83] Carrier, F.; Georgel, P. T.; Pourquier, P.; Blake, M.; Kontny, H. U.; Antinore, M. J.; Gariboldi, M.; Myers, T. G.; Weinstein, J. N.; Pommier, Y.; Fornace, A. J., Jr. Gadd45, a p53-Responsive Stress Protein, Modifies DNA Accessibility on Damaged Chromatin. *Mol. Cell. Biol.*, **1999**, *19* (3), 1673-1685.

[84] McKay, B. C.; Francis, M. A.; Rainbow, A. J. Wildtype p53 is required for heat shock and ultraviolet light enhanced repair of a UV-damaged reporter gene. *Carcinogenesis*, **1997**, *18* (2), 245-9.

[85] Donehower, L. A.; Harvey, M.; Slagle, B. L.; McArthur, M. J.; Montgomery, C. A., Jr.; Butel, J. S.; Bradley, A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*, **1992**, *356* (6366), 215-21.

[86] Siliciano, J. D.; Canman, C. E.; Taya, Y.; Sakaguchi, K.; Appella, E.; Kastan, M. B. DNA damage induces phosphorylation of the amino terminus of p53. *Gene. Dev.*, **1997**, *11* (24), 3471-81.

[87] Banin, S.; Moyal, L.; Shieh, S.; Taya, Y.; Anderson, C. W.; Chessa, L.; Smorodinsky, N. I.; Prives, C.; Reiss, Y.; Shiloh, Y.; Ziv, Y. Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science*, **1998**, *281* (5383), 1674-7.

[88] Maclaine, N. J.; Hupp, T. R. The regulation of p53 by phosphorylation: a model for how distinct signals integrate into the p53 pathway. *Aging*, **2009**, *1* (5), 490-502.

[89] Bruins, W.; Zwart, E.; Attardi, L. D.; Iwakuma, T.; Hoogervorst, E. M.; Beems, R. B.; Miranda, B.; van Oostrom, C. T.; van den Berg, J.; van den Aardweg, G. J.; Lozano, G.; van Steeg, H.; Jacks, T.; de Vries, A. Increased sensitivity to UV radiation in mice with a p53 point mutation at Ser389. *Mol. Cell. Biol.*, **2004**, *24* (20), 8884-94.

[90] Kripke, M. L. Antigenicity of murine skin tumors induced by ultraviolet light. *J. Natl. Cancer I.*, **1974**, *53* (5), 1333-6.

[91] Ullrich, S. E. Mechanisms underlying UV-induced immune suppression. *Mutat. Res.*, **2005**, *571* (1-2), 185-205.

[92] Schwarz, A. UVR-Induced regulatory T cells switch antigen-presenting cells from a stimulatory to a regulatory phenotype. *J. Invest. Dermatol.*, **2010**, *130*.

[93] De Fabo, E. C. Mechanism of immune suppression by ultraviolet irradiation *in vivo*. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. *J. Exp. Med.*, **1983**, *158*, 84-98.

[94] Beissert, S. IL-12 prevents the inhibitory effects of cis-urocanic acid on tumor antigen presentation by Langerhans cells: Implication for photocarcinogenesis. *J. Immunol.*, **2001**, 6232-6238.

[95] Vink, A. A.; Strickland, F. M.; Bucana, C.; Cox, P. A.; Roza, L.; Yarosh, D. B.; Kripke, M. L. Localization of DNA damage and its role in altered antigen-presenting cell function in ultraviolet-irradiated mice. *J. Exp. Med.*, **1996**, *183* (4), 1491-500.

[96] Halliday, G. M.; Muller, H. K. Sensitization through carcinogen-induced Langerhans cell-deficient skin activates specific long-lived suppressor cells for both cellular and humoral immunity. *Cell. Immunol.*, **1987,** *109*(1), 206-221.

[97] Caceres-Dittmar, G.; Ariizumi, K.; Xu, S.; Tapia, F. J.; Bergstresser, P. R.; Takashima, A. Hydrogen peroxide mediates UV-induced impairment of antigen presentation in a murine epidermal-derived dendritic cell line. *Photochem. Photobiol.*, **1995**, *62* (1), 176-183.

[98] Devary, Y.; Gottlieb, R. A.; Smeal, T.; Karin, M. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell*, **1992**, *71* (7), 1081-1091.

[99] Tobin, D.; van Hogerlinden, M.; Toftgard, R. UVB-induced association of tumor necrosis factor (TNF) receptor 1/TNF receptor-associated factor-2 mediates activation of Rel proteins. *Proc. Natl. Acad. Sci. U. S. A.*, **1998**, *95* (2), 565-569.

[100] Stapelberg, M. P.; Williams, R. B.; Byrne, S. N.; Halliday, G. M., The alternative complement pathway seems to be a UVA sensor that leads to systemic immunosuppression. *J. Invest. Dermatol.*, **2009**, *129* (11), 2694-2701.

[101] Halliday, G. M. Common links among the pathways leading to UV-induced immunosuppression. *J. Invest. Dermatol.*, **2010**, *130* (5), 1209-1212.

[102] Halliday, G. M.; Byrne, S. N.; Kuchel, J. M.; Poon, T. S.; Barnetson, R. S. The suppression of immunity by ultraviolet radiation: UVA, nitric oxide and DNA damage. *Photochem. Photobiol. Sc.*, **2004**, *3* (8), 736-740.

[103] Damian, D. L.; Barnetson, R. S.; Halliday, G. M. Low-dose UVA and UVB have different time courses for suppression of contact hypersensitivity to a recall antigen in humans. *J. Invest. Dermatol.*, **1999**, *112* (6), 939-944.

[104] Poon, T. S.; Barnetson, R. S.; Halliday, G. M. Sunlight-induced immunosuppression in humans is initially because of UVB, then UVA, followed by interactive effects. *J. Invest. Dermatol.*, **2005**, *125* (4), 840-846.

[105] Damian, D. L.; Matthews, Y. L.; Phan, T. A.; Halliday, G. M. An action spectrum for ultraviolet radiation-induced immunosuppression in humans. *Brit. J. Dermatol.*, **2011**, *164*, 657-659.

[106] Tyrrell, R. M.; Reeve, V. E. Potential protection of skin by acute UVA irradiation--from cellular to animal models. *Prog.Biophys.Mol.Bio.*, **2006**, *92* (1), 86-91.

[107] Matthews, Y. J.; Halliday, G. M.; Phan, T. A.; Damian, D. L. Wavelength dependency for UVA-induced suppression of recall immunity in humans. *J. Dermatol. Sci.*, **2010**, *59* (3), 192-197.

[108] Gilchrest, B. A. Mechanisms of Disease: The Pathogenesis of Melanoma Induced by Ultraviolet Radiation. *New Engl. J. Med.*, **1999**, *340* (1), 1341-1348.

[109] von Thaler, A. K.; Kamenisch, Y.; Berneburg, M. The role of ultraviolet radiation in melanomagenesis. *Exp. Dermatol.*, **2010**, *19* (2), 81-88.

[110] Dillon, P.; Thomas, N.; Sharpless, N.; Collichio, F. Regression of advanced melanoma upon withdrawal of immunosuppression: case series and literature review. *Med. Oncol.*, **2010**, *27* (4), 1127-1132.

[111] Kong, Y.; Kumar, S. M.; Xu, X. Molecular pathogenesis of sporadic melanoma and melanoma-initiating cells. *Arch. Pathol. Lab. Med.*, **2010**, *134* (12), 1740-1749.

[112] Wang, H. T.; Choi, B.; Tang, M. S. Melanocytes are deficient in repair of oxidative DNA damage and UV-induced photoproducts. *Proc. Natl. Acad. Sci. U. S. A.*, **2010**, *107* (27), 12180-12185.

[113] Bikle, D. D. The vitamin D receptor: a tumor suppressor in skin. *Discovery Medicine* **2011**, *11* (56), 7-17.

[114] Reichrath, J.; Kamradt, J.; Zhu, X. H.; Kong, X. F.; Tilgen, W.; Holick, M. F. Analysis of 1,25-dihydroxyvitamin D(3) receptors (VDR) in basal cell carcinomas. *Am. J. Pathol.*, **1999**, *155* (2), 583-589.

[115] Lehmann, B.; Querings, K.; Reichrath, J. [New relevance of vitamin D3 metabolism in the skin]. *Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete* **2004**, *55* (5), 446-452.

[116] Reichrath, J.; Rafi, L.; Rech, M.; Meineke, V.; Tilgen, W.; Seifert, M. No evidence for amplification of 25-hydroxyvitamin D-1 alpha-OHase (1 alpha-OHase) or 1,25-dihydroxyvitamin D-24-OHase (24-OHase) genes in malignant melanoma (MM). *J. Steroid Biochem.Mol. Biol.*, **2004**, *89-90* (1-5), 163-166.

[117] Uhmann, A.; Niemann, H.; Lammering, B.; Henkel, C.; Hess, I.; Nitzki, F.; Fritsch, A.; Prufer, N.; Rosenberger, A.; Dullin, C.; Schraepler, A.; Reifenberger, J.; Schweyer, S.; Pietsch, T.; Strutz, F.; Schulz-Schaeffer, W.; Hahn, H. Antitumoral effects of calcitriol in basal cell carcinomas involve inhibition of hedgehog signaling and induction of vitamin D receptor signaling and differentiation. *Mol. Cancer Ther.*, **2011**, *10* (11), 2179-88.

[118] Reichrath, J.; Rafi, L.; Rech, M.; Mitschele, T.; Meineke, V.; Gartner, B. C.; Tilgen, W.; Holick, M. F. Analysis of the vitamin D system in cutaneous squamous cell carcinomas. *J. Cutan. Pathol.*, **2004**, *31* (3), 224-31.

[119] Biijlsma MF, S. C. Repression of smoothened by patched independent (pro)vitamin D3 secretion. *PLoS Biol.*, **2006**.

[120] Denzer, N.; Vogt, T.; Reichrath, J. Vitamin D receptor (VDR) polymorphisms and skin cancer: A systematic review. *Dermato-endocrinology*, **2011**, *3* (3), 205-10.

[121] Kostner, K.; Denzer, N.; Koreng, M.; Reichrath, S.; Graber, S.; Klein, R.; Tilgen, W.; Vogt, T.; Reichrath, J. Association of genetic variants of the vitamin D receptor (VDR) with cutaneous squamous cell carcinomas (SCC) and basal cell carcinomas (BCC): a pilot study in a German population. *Anticancer Res.*, **2012**, *32* (1), 327-33.

[122] Carless, M. A.; Kraska, T.; Lintell, N.; Neale, R. E.; Green, A. C.; Griffiths, L. R. Polymorphisms of the VDR gene are associated with presence of solar keratoses on the skin. *Br. J. Dermatol.*, **2008**, *159* (4), 804-10.

[123] Ramachandran, S.; Fryer, A. A.; Lovatt, T. J.; Smith, A. G.; Lear, J. T.; Jones, P. W.; Strange, R. C. Combined effects of gender, skin type and polymorphic genes on clinical phenotype: use of rate of increase in numbers of basal cell carcinomas as a model system. *Cancer Lett.*, **2003**, *189* (2), 175-81.

[124] Han, J.; Colditz, G. A.; Hunter, D. J., Polymorphisms in the MTHFR and VDR genes and skin cancer risk. *Carcinogenesis*, **2007**, *28* (2), 390-7.

[125] Osborne, J. E.; Hutchinson, P. E. Vitamin D and systemic cancer: is this relevant to malignant melanoma? *Br. J. Dermatol.*, **2002**, *147* (2), 197-213.

[126] Reichrath, J.; Rech, M.; Moeini, M.; Meese, E.; Tilgen, W.; Seifert, M. *In vitro* comparison of the vitamin D endocrine system in 1,25(OH)2D3-responsive and -resistant melanoma cells. *Cancer Biol. Ther.*, **2007**, *6* (1), 48-55.

[127] Frankel, T. L.; Mason, R. S.; Hersey, P.; Murray, E.; Posen, S. The synthesis of vitamin D metabolites by human melanoma cells. *J. Clin. Endocrinol. Metab.*, **1983**, *57* (3), 627-31.

[128] Mason, R. S.; Pryke, A. M.; Ranson, M.; Thomas, H. E.; Posen, S. Human melanoma cells: functional modulation by calciotropic hormones. *J. Invest. Dermatol.*, **1988**, *90* (6), 834-40.

[129] Ranson, M.; Posen, S.; Mason, R. S. Extracellular matrix modulates the function of human melanocytes but not melanoma cells. *J. Cell. Physiol.*, **1988**, *136* (2), 281-8.

[130] Albertson, D. G.; Ylstra, B.; Segraves, R.; Collins, C.; Dairkee, S. H.; Kowbel, D.; Kuo, W. L.; Gray, J. W.; Pinkel, D. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. *Nature Genetics*, **2000**, *25* (2), 144-6.

[131] Diesel, B.; Radermacher, J.; Bureik, M.; Bernhardt, R.; Seifert, M.; Reichrath, J.; Fischer, U.; Meese, E. Vitamin D(3) metabolism in human glioblastoma multiforme: functionality of CYP27B1 splice variants, metabolism of calcidiol, and effect of calcitriol. *Clin. Cancer Res.*, **2005**, *11* (15), 5370-80.

[132] Essa, S.; Reichrath, S.; Mahlknecht, U.; Montenarh, M.; Vogt, T.; Reichrath, J. Signature of VDR miRNAs and epigenetic modulation of vitamin D signaling in melanoma cell lines. *Anticancer Res.*, **2012**, *32* (1), 383-9.

[133] Hutchinson, P. E.; Osborne, J. E.; Lear, J. T.; Smith, A. G.; Bowers, P. W.; Morris, P. N.; Jones, P. W.; York, C.; Strange, R. C.; Fryer, A. A. Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. *Clin. Cancer Res.*, **2000**, *6* (2), 498-504.

[134] Halsall, J. A.; Osborne, J. E.; Potter, L.; Pringle, J. H.; Hutchinson, P. E. A novel polymorphism in the 1A promoter region of the vitamin D receptor is associated with altered susceptibilty and prognosis in malignant melanoma. *Br. J. Cancer*, **2004**, *91* (4), 765-70.

[135] Mandelcorn-Monson, R.; Marrett, L.; Kricker, A.; Armstrong, B. K.; Orlow, I.; Goumas, C.; Paine, S.; Rosso, S.; Thomas, N.; Millikan, R. C.; Pole, J. D.; Cotignola, J.; Rosen, C.; Kanetsky, P. A.; Lee-Taylor, J.; Begg, C. B.; Berwick, M. Sun exposure, vitamin D receptor polymorphisms FokI and BsmI and risk of multiple primary melanoma. *Cancer Epidemiology*, **2011**, *35* (6), e105-10.

[136] Mocellin, S.; Nitti, D. Vitamin D receptor polymorphisms and the risk of cutaneous melanoma: a systematic review and meta-analysis. *Cancer*, **2008**, *113* (9), 2398-2407.

[137] Nurnberg, B.; Graber, S.; Gartner, B.; Geisel, J.; Pfohler, C.; Schadendorf, D.; Tilgen, W.; Reichrath, J. Reduced serum 25-hydroxyvitamin D levels in stage IV melanoma patients. *Anticancer Res.*, **2009**, *29* (9), 3669-3674.

[138] Newton-Bishop, J. A.; Beswick, S.; Randerson-Moor, J.; Chang, Y. M.; Affleck, P.; Elliott, F.; Chan, M.; Leake, S.; Karpavicius, B.; Haynes, S.; Kukalizch, K.; Whitaker, L.; Jackson, S.; Gerry, E.; Nolan, C.; Bertram, C.; Marsden, J.; Elder, D. E.; Barrett, J. H.; Bishop, D. T. Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. *J. Clin. Oncol.*, **2009**, *27* (32), 5439-5444.

[139] Bade B.; Gräber, S.; Geisel, J.; Hafner, C.; Vogt, Th.; Reichrath, J. Low serum 25-hydroxyvitamin D concentrations are associated with increased risk for melanoma and unfavourable prognosis. *submitted for publication*.

[140] Berwick, M.; Armstrong, B. K.; Ben-Porat, L.; Fine, J.; Kricker, A.; Eberle, C.; Barnhill, R. Sun exposure and mortality from melanoma. *J Natl Cancer Inst* **2005**, *97* (3), 195-199.

[141] Boniol, M.; Armstrong, B. K.; Dore, J. F. Variation in incidence and fatality of melanoma by season of diagnosis in new South Wales, Australia. *Cancer Epidem. Biomar.*, **2006**, *15* (3), 524-526.

[142] (http://www.melanoma.org.au/research/clinical-trials/current-clinical-trials.html).

[143] Sanders, K. M.; Stuart, A. L.; Williamson, E. J.; Simpson, J. A.; Kotowicz, M. A.; Young, D.; Nicholson, G. C. Annual high-dose

oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA*, **2010**, *303* (18), 1815-1822.

[144] Tang, J. Y.; Fu, T.; Leblanc, E.; Manson, J. E.; Feldman, D.; Linos, E.; Vitolins, M. Z.; Zeitouni, N. C.; Larson, J.; Stefanick, M. L. Calcium plus vitamin D supplementation and the risk of nonmelanoma and melanoma skin cancer: post hoc analyses of the women's health initiative randomized controlled trial. *J. Clin. Oncol.*, **2011**, *29* (22), 3078-3084.

[145] Wong, G.; Gupta, R.; Dixon, K. M.; Deo, S. S.; Choong, S. M.; Halliday, G. M.; Bishop, J. E.; Ishizuka, S.; Norman, A. W.; Posner, G. H.; Mason, R. S. 1,25-Dihydroxyvitamin D and three low-calcemic analogs decrease UV-induced DNA damage *via* the rapid response pathway. *J. Steroid Biochem. Mol. Biol.*, **2004**, *89-90* (1-5), 567-570.

[146] De Haes, P.; Garmyn, M.; Verstuyf, A.; De Clercq, P.; Vandewalle, M.; Degreef, H.; Vantieghem, K.; Bouillon, R.; Segaert, S. 1,25-Dihydroxyvitamin D3 and analogues protect primary human keratinocytes against UVB-induced DNA damage. *J. Photochem. Photobiol. B.*, **2005**, *78* (2), 141-148.

[147] Dixon, K. M.; Deo, S. S.; Wong, G.; Slater, M.; Norman, A. W.; Bishop, J. E.; Posner, G. H.; Ishizuka, S.; Halliday, G. M.; Reeve, V. E.; Mason, R. S. Skin cancer prevention: a possible role of 1,25dihydroxyvitamin D3 and its analogs. *J. Steroid Biochem. Mol. Biol.*, **2005**, *97* (1-2), 137-143.

[148] Dixon, K. M.; Deo, S. S.; Norman, A. W.; Bishop, J. E.; Halliday, G. M.; Reeve, V. E.; Mason, R. S. *In vivo* relevance for photoprotection by the vitamin D rapid response pathway. *J Steroid Biochem. Mol. Biol.*, **2007**, *103* (3-5), 451-456.

[149] Lee, J.; Youn, J. I. The photoprotective effect of 1,25-dihydroxyvitamin D3 on ultraviolet light B-induced damage in keratinocyte and its mechanism of action. *J. Dermatol. Sci.*, **1998**, *18* (1), 11-18.

[150] Damian, D. L. Photoprotective effects of nicotinamide. *Photochem. Photobiol. Sci*., **2010**, *9* (4), 578-585.

[151] Dixon, K. M.; Norman, A. W.; Sequeira, V. B.; Mohan, R.; Rybchyn, M. S.; Reeve, V. E.; Halliday, G. M.; Mason, R. S. 1 alpha, 25$(OH)_2$-vitamin D and a nongenomic vitamin D analogue inhibit ultraviolet radiation-induced skin carcinogenesis. *Cancer Prevent.Res.*, **2011**, *4* (9), 1485-1494.

[152] Dhanalakshmi, S.; Mallikarjuna, G. U.; Singh, R. P.; Agarwal, R. Silibinin prevents ultraviolet radiation-caused skin damages in SKH-1 hairless mice via a decrease in thymine dimer positive cells and an up-regulation of p53-p21/Cip1 in epidermis. *Carcinogenesis*, **2004**, *25* (8), 1459-1465.

[153] Ouhtit, A.; Muller, H. K.; Davis, D. W.; Ullrich, S. E.; McConkey, D.; Ananthaswamy, H. N. Temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. *Am. J. Pathol*., **2000,** *156(1)*, 201-207

[154] Mason, R. S.; Holliday, C. J. 1,25 Dihydroxyvitamin D contributes to photoprotection in skin cells. In *Vitamin D Endocrine system: Structural, Biological, Genetic and Clinical Aspects*, Norman, A.; Bouillon, R; Thomasset, M. Eds. University of California: Riverside, **2000**; pp 605-608.

[155] Manggau, M.; Kim, D.S.; Ruwisch, L.; Vogler, R.; Korting, H.C.; Schafer-Korting, M.; Kleuser, B. 1alpha,25-dihydroxyvitamin D_3 protects human keratinocytes from apoptosis by the formation of sphingosine-1-phosphate. *J. Invest. Dermatol.*, **2001**, *117* (5), 1241-9.

[156] Mason, R. S.; Holliday, C. .J.; Gupta, R. 1,25 Dihydroxyvitamin D and Photoprotection in Skin Cells. In *Modern Trends in Skin Pharmacology*, 1st ed.; Tsambos, D.; Merk, H., Eds. Parissianos Medical Publications S.A. Athens: Athens, Greece, **2002**; pp 59-66.

[157] (a) De Haes, P.; Garmyn, M.; Degreef, H.; Vantieghem, K.; Bouillon, R.; Segaert, S. 1,25-Dihydroxyvitamin D_3 inhibits ultraviolet B-induced apoptosis, Jun kinase activation, and interleukin-6 production in primary human keratinocytes. *J. Cell. Biochem.*, **2003**, *89* (4), 663-73; (b) De Haes, P.; Garmyn, M.; Carmeliet, G.; Degreef, H.; Vantieghem, K.; Bouillon, R.; Segaert, S. Molecular pathways involved in the anti-apoptotic effect of 1,25-dihydroxyvitamin D_3 in primary human keratinocytes. *J. Cell. Biochem.*, **2004**, *93* (5), 951-967.

[158] Youn, J. I. Photoprotective effect of calcipotriol upon skin photoreaction to UVA and UVB. *Photodermatol. Photo*., **1997**, *13* (3), 109-114.

[159] Sheehan, J. M.; Cragg, N.; Chadwick, C. A.; Potten, C. S.; Young, A. R. Repeated ultraviolet exposure affords the same protection against DNA photodamage and erythema in human skin types II and IV but is associated with faster DNA repair in skin type IV. *J. Invest. Dermatol.*, **2002**, *118* (5), 825-829.

[160] Hanada, K.; Sawamura, D.; Nakano, H.; Hashimoto, I. Possible role of 1,25-dihydroxyvitamin D3-induced metallothionein in photoprotection against UVB injury in mouse skin and cultured rat keratinocytes. *J. Dermatol. Sci.*, **1995**, *9* (3), 203-208.

[161] Mitani, H.; Naru, E.; Yamashita, M.; Arakane, K.; Suzuki, T.; Imanari, T. Ergocalciferol promotes *in vivo* differentiation of keratinocytes and reduces photodamage caused by ultraviolet irradiation in hairless mice. *Photodermatol. Photo.*, **2004**, *20* (5), 215-223.

[162] Dixon, K. M.; Sequeira, V. B.; Camp, A. J.; Mason, R. S. Vitamin D-fence. *Photochem. Photobiol. Sci.*, **2010**, *9* (4), 564-570.

[163] Hanneman, K. K.; Scull, H. M.; Cooper, K. D.; Baron, E. D. Effect of topical vitamin D analogue on *in vivo* contact sensitization. *Arch. Dermatol.*, **2006**, *142* (10), 1332-1334.

[164] Gorman, S. Topically applied 1,25-dihydroxyvitamin D-3 enhances the suppressive activity of CD4(+)CD(25+), cells in the draining lymph nodes. *J. Immunol.*, **2007**, *179*, 6273-6283.

[165] Singh, S.; Aiba, S.; Manome, H.; Tagami, H. The effects of dexamethasone, cyclosporine, and vitamin D(3) on the activation of dendritic cells stimulated by haptens. *Arch. Dermatol. Res.*, **1999**, *291* (10), 548-554.

[166] (a) Griffin, M. D.; Lutz, W.; Phan, V. A.; Bachman, L. A.; McKean, D. J.; Kumar, R. Dendritic cell modulation by 1alpha,25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.*, **2001**, *98* (12), 6800-5; (b) Mathieu, C.; Adorini, L., The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. *Trends Mol. Med.*, **2002**, *8* (4), 174-179.

[167] Dong, X.; Lutz, W.; Schroeder, T. M.; Bachman, L. A.; Westendorf, J. J.; Kumar, R.; Griffin, M. D. Regulation of relB in dendritic cells by means of modulated association of vitamin D receptor and histone deacetylase 3 with the promoter. *Proc. Natl. Acad. Sci. U. S. A.*, **2005**, *102* (44), 16007-16012.

[168] (a) Janjetovic, Z.; Zmijewski, M. A.; Tuckey, R. C.; DeLeon, D. A.; Nguyen, M. N.; Pfeffer, L. M.; Slominski, A. T. 20-Hydroxycholecalciferol, product of vitamin D3 hydroxylation by P450scc, decreases NF-kappaB activity by increasing IkappaB alpha levels in human keratinocytes. *PLoS One*, **2009**, *4* (6), e5988; (b) Janjetovic, Z.; Tuckey, R. C.; Nguyen, M. N.; Thorpe, E. M., Jr.; Slominski, A. T. 20,23-dihydroxyvitamin D3, novel P450scc product, stimulates differentiation and inhibits proliferation and NF-kappaB activity in human keratinocytes. *J. Cell. Physiol.*, **2010**, *223* (1), 36-48.

[169] Biggs, L.; Yu, C.; Fedoric, B.; Lopez, A. F.; Galli, S. J.; Grimbaldeston, M. A. Evidence that vitamin D(3) promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice. *J. Exp. Med.*, **2010**, *207* (3), 455-463.

[170] Yang, S.; Smith, C.; Prahl, J. M.; Luo, X.; DeLuca, H. F. Vitamin D deficiency suppresses cell-mediated immunity *in vivo*. *Arch. Biochem. Biophys.*, **1993**, *303* (1), 98-106.

[171] Liu, P. T.; Stenger, S.; Li, H.; Wenzel, L.; Tan, B. H.; Krutzik, S. R.; Ochoa, M. T.; Schauber, J.; Wu, K.; Meinken, C.; Kamen, D. L.; Wagner, M.; Bals, R.; Steinmeyer, A.; Zugel, U.; Gallo, R. L.; Eisenberg, D.; Hewison, M.; Hollis, B. W.; Adams, J. S.; Bloom, B. R.; Modlin, R. L. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*, **2006**, *311* (5768), 1770-1773.

[172] Veldman, C. M.; Cantorna, M. T.; DeLuca, H. F., Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. *Arch. Biochem. Biophys.*, **2000**, *374* (2), 334-338.

[173] Wood, A. W.; Chang, R. L.; Huang, M. T.; Uskokovic, M.; Conney, A. H. 1 alpha, 25-Dihydroxyvitamin D3 inhibits phorbol ester-dependent chemical carcinogenesis in mouse skin. *Biochem. Bioph. Res. Co.*, **1983**, *116* (2), 605-611.

[174] Kensler, T. W.; Dolan, P. M.; Gange, S. J.; Lee, J. K.; Wang, Q.; Posner, G. H. Conceptually new deltanoids (vitamin D analogs) inhibit multistage skin tumorigenesis. *Carcinogenesis*, **2000**, *21* (7), 1341-1345.

[175] Zinser, G. M.; Sundberg, J. P.; Welsh, J. Vitamin D(3) receptor ablation sensitizes skin to chemically induced tumorigenesis. *Carcinogenesis.* **2002**, *23* (12), 2103-2109.

[176] Ellison TI, S. M., Gilliam AC, McDonald PN. Inactivation of the vitamin D receptor enhances susceptibility of murine skin to UV-induced tumorigenesis. J. *Invest. Dermatol.*, **2008**, *128*, 2508-2517.

[177] Norman, A. W.; Manchand, P. S.; Uskokovic, M. R.; Okamura, W. H.; Takeuchi, J. A.; Bishop, J. E.; Hisatake, J. I.; Koeffler, H. P.; Peleg, S. Characterization of a novel analogue of 1alpha,25(OH)(2)-vitamin D(3) with two side chains: interaction with its nuclear receptor and cellular actions. *J. M. Chem.*, **2000**, *43* (14), 2719-2730.

[178] Menegaz, D.; Mizwicki, M. T.; Barrientos-Duran, A.; Chen, N.; Henry, H. L.; Norman, A. W. Vitamin D receptor (VDR) regulation of voltage-gated chloride channels by ligands preferring a VDR-alternative pocket (VDR-AP). *Mol. Endocrinol.*, **2011**, *25* (8), 1289-1300.

[179] Norman, A. W.; Mizwicki, M. T.; Norman, D. P. Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nature Reviews*, **2004**, *3* (1), 27-41.

[180] Dixon, K. M.; Mason, R. S. Vitamin D. *Int. J. Biochem. Cell B.*, **2009**, *41* (5), 982-985.

[181] Norman, A. W.; Ishizuka, S.; Okamura, W. H. Ligands for the vitamin D endocrine system: different shapes function as agonists and antagonists for genomic and rapid response receptors or as a ligand for the plasma vitamin D binding protein. *J. Steroid Biochem. Mol. Biol.*, **2001**, *76* (1-5), 49-59.

[182] Zanello, L. P.; Norman, A. W. Rapid modulation of osteoblast ion channel responses by 1alpha,25(OH)2-vitamin D3 requires the presence of a functional vitamin D nuclear receptor. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101* (6), 1589-1594.

[183] Nemere, I.; Farach-Carson, M. C.; Rohe, B.; Sterling, T. M.; Norman, A. W.; Boyan, B. D.; Safford, S. E. Ribozyme knockdown functionally links a 1,25(OH)2D3 membrane binding protein (1,25D3-MARRS) and phosphate uptake in intestinal cells. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101* (19), 7392-7397.

[184] Sequeira, V. B.; Rybchyn, M. S.; Tongkao-On, W.; Gordon-Thomson, C.; Malloy, P. J.; Nemere, I.; Norman, A. W.; Reeve, V. E.; Halliday, G. M.; Feldman, D.; Mason, R. S. The role of the vitamin D receptor and ERp57 in photoprotection by 1alpha,25-dihydroxyvitamin D_3. *Mol. Endocrinol.*, **2012**, *26* (4), 574-582.

[185] Flores, O.; Burnstein, K. L. GADD45gamma: a new vitamin D-regulated gene that is antiproliferative in prostate cancer cells. *Endocrinology*, **2010**, *151* (10), 4654-4664.

[186] Halliday, G. M. Inflammation, gene mutation and photoimmunosuppression in response to UVR-induced oxidative damage contributes to photocarcinogenesis. *Mutat. Res.*, **2005**, *571* (1-2), 107-120.

[187] Jaiswal, M.; LaRusso, N. F.; Burgart, L. J.; Gores, G. J. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res.*, **2000**, *60* (1), 184-190.

[188] Bau, D. T.; Gurr, J. R.; Jan, K. Y. Nitric oxide is involved in arsenite inhibition of pyrimidine dimer excision. *Carcinogenesis*, **2001**, *22* (5), 709-716.

[189] Kuchel, J. M.; Barnetson, R. S.; Halliday, G. M. Cyclobutane pyrimidine dimer formation is a molecular trigger for solar-simulated ultraviolet radiation-induced suppression of memory immunity in humans. *Photochem. Photobiol. Sc.*, **2005**, *4* (8), 577-582.

[190] Leccia, M. T.; Yaar, M.; Allen, N.; Gleason, M.; Gilchrest, B. A. Solar simulated irradiation modulates gene expression and activity of antioxidant enzymes in cultured human dermal fibroblasts. *Exp. Dermatol.*, **2001**, *10* (4), 272-279.

[191] Ravid, A.; Rubinstein, E.; Gamady, A.; Rotem, C.; Liberman, U. A.; Koren, R. Vitamin D inhibits the activation of stress-activated protein kinases by physiological and environmental stresses in keratinocytes. *J. Endocrinol.*, **2002**, 173(3):525-532.

[192] Hanada, K.; Gange, R. W.; Siebert, E.; Hasan, T. Protective effects of cadmium chloride against UVB injury in mouse skin and in cultured human cells: a possible role of cadmium-induced metallothionein. *Photodermatol. Photo.*, **1991**, *8* (3), 111-115.

[193] Hanada, K.; Baba, T.; Hashimoto, I.; Fukui, R.; Watanabe, S. Possible role of cutaneous metallothionein in protection against photo-oxidative stress--epidermal localization and scavenging activity for superoxide and hydroxyl radicals. *Photodermatol. Photo.*, **1992**, *9* (5), 209-213.

[194] Reeve, V. E.; Nishimura, N.; Bosnic, M.; Michalska, A. E.; Choo, K. H. Lack of metallothionein-I and -II exacerbates the immunosuppressive effect of ultraviolet B radiation and cis-urocanic acid in mice. *Immunology*, **2000**, *100* (3), 399-404.

[195] Widyarini, S.; Allanson, M.; Gallagher, N. L.; Pedley, J.; Boyle, G. M.; Parsons, P. G.; Whiteman, D. C.; Walker, C.; Reeve, V. E. Isoflavonoid photoprotection in mouse and human skin is dependent on metallothionein. *J. Invest. Dermatol.*, **2006**, *126* (1), 198-204.

[196] Karasawa, M.; Hosoi, J.; Hashiba, H.; Nose, K.; Tohyama, C.; Abe, E.; Suda, T.; Kuroki, T. Regulation of metallothionein gene expression by 1 alpha,25-dihydroxyvitamin D3 in cultured cells and in mice. *Proc. Natl. Acad. Sci. U. S. A.*, **1987**, 84 (24), 8810-8813.

[197] Mason, R. S.; Sequeira, V. B.; Gordon-Thomson, C. Vitamin D: the light side of sunshine. *Eur. J. Clin. Nutr.*, **2011**, *65* (9), 986-993.

[198] Vieth, R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am. J. Clin. Nutr.*, **1999**, 69 (5), 842-856.

[199] (a) Bischoff, H. A.; Stahelin, H. B.; Urscheler, N.; Ehrsam, R.; Vonthein, R.; Perrig-Chiello, P.; Tyndall, A.; Theiler, R. Muscle strength in the elderly: its relation to vitamin D metabolites. [comment]. *Arch. Phys. Med. Rehab.*, **1999**, *80* (1), 54-58; (b) Bischoff-Ferrari, H. A. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. *Adv. Exp. Med. Biol.*, **2008**, *624*, 55-71.

[200] Gallagher, J. C.; Sai, A.; Templin, T., 2nd; Smith, L. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. *Ann. Intern. Med.*, **2012**, *156* (6), 425-437.

[201] Zittermann, A.; Iodice, S.; Pilz, S.; Grant, W. B.; Bagnardi, V.; Gandini, S. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am. J. Clin. Nutr.*, **2012**, *95* (1), 91-100.

[202] Ross, A. C.; Manson, J. E.; Abrams, S. A.; Aloia, J. F.; Brannon, P. M.; Clinton, S. K.; Durazo-Arvizu, R. A.; Gallagher, J. C.; Gallo, R. L.; Jones, G.; Kovacs, C. S.; Mayne, S. T.; Rosen, C. J.; Shapses, S. A. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J. Clin. Endocrinol. Metab.*, **2011**, *96* (1), 53-58.

[203] Holick, M. F.; Binkley, N. C.; Bischoff-Ferrari, H. A.; Gordon, C. M.; Hanley, D. A.; Heaney, R. P.; Murad, M. H.; Weaver, C. M. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab.*, **2011**, *96* (7), 1911-1930.

[204] Gorham, E. D.; Garland, C. F.; Garland, F. C.; Grant, W. B.; Mohr, S. B.; Lipkin, M.; Newmark, H. L.; Giovannucci, E.; Wei, M.; Holick, M. F. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. *Am. J. Prev. Med.*, **2007**, *32* (3), 210-216.

[205] Giovannucci, E.; Liu, Y.; Rimm, E. B.; Hollis, B. W.; Fuchs, C. S.; Stampfer, M. J.; Willett, W. C. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst.*, **2006**, *98* (7), 451-459.

[206] Grant, W. B.; Moan, J.; Reichrath, J. Comment on "the effects on human health from stratospheric ozone depletion and its interactions with climate change" by M. Norval, A. P. Cullen, F. R. de Gruijl, J. Longstreth, Y. Takizawa, R. M. Lucas, F. P. Noonan and J. C. van der Leun, Photochem. Photobiol. Sci., 2007, 6, 232. *Photochem. Photobiol. Sc.* **2007**, *6* (8), 912-5; discussion 916-918.

[207] Garland, C. F.; Gorham, E. D.; Mohr, S. B.; Grant, W. B.; Giovannucci, E. L.; Lipkin, M.; Newmark, H.; Holick, M. F.; Garland, F. C., Vitamin D and prevention of breast cancer: pooled analysis. *J. Steroid Biochem. Mol. Biol.*, **2007**, *103* (3-5), 708-711.

[208] Kunitomo, M.; Kinoshita, K.; Bando, Y. Experimental atherosclerosis in rats fed a vitamin D, cholesterol-rich diet. *J. Pharmacobio-Dynam.*, **1981**, *4* (9), 718-723.

[209] Toda, T.; Leszczynski, D. E.; Kummerow, F. A. The role of 25-hydroxy-vitamin D3 in the induction of atherosclerosis in swine and rabbit by hypervitaminosis D. *Acta Pathol. Japon.*, **1983**, *33* (1), 37-44.

[210] Peng, S. K.; Taylor, C. B.; Tham, P.; Mikkelson, B. Role of mild excesses of vitamin D3 in arteriosclerosis. A study in squirrel monkeys. *Paroi Arteriel.*, **1978**, *4* (4), 229-243.

[211] Hathcock, J. N.; Shao, A. Risk assessment for glucosamine and chondroitin sulfate. *Regul. Toxicol. Pharmacol.*, **2007**, *47* (1), 78-83.

[212] Grant, W. B.; Cross, H. S.; Garland, C. F.; Gorham, E. D.; Moan, J.; Peterlik, M.; Porojnicu, A. C.; Reichrath, J.; Zittermann, A. Estimated benefit of increased vitamin D status in reducing the economic burden of disease in western Europe. *Progress in biophysics and molecular biology* **2009**, *99* (2-3), 104-113.

[213] Grant, W. B. Solar ultraviolet irradiance and cancer incidence and mortality. *Adv. Exp. Med.Biol.*, **2008**, *624*, 16-30.

[214] Grant, W. B.; Garland, C. F.; Holick, M. F. Comparisons of estimated economic burdens due to insufficient solar ultraviolet irradiance and vitamin D and excess solar UV irradiance for the United States. *Photochem. Photobiol.*, **2005**, *81* (6), 1276-1286.

[215] Holick, M. F. Sunlight "D"ilemma: risk of skin cancer or bone disease and muscle weakness. *Lancet*, **2001**, *357* (9249), 4-6.

[216] Eller, M. S.; Asarch, A.; Gilchrest, B. A. Photoprotection in human skin--a multifaceted SOS response. *Photochem. Photobiol.*, **2008**, *84* (2), 339-49.

Received: April 18, 2012 **Revised: April 21, 2012** **Accepted: May 07, 2012**