The relevance of the vitamin D endocrine system (VDES) for tumorigenesis, prevention, and treatment of non-melanoma skin cancer (NMSC)

Present concepts and future perspectives

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Introduction

The important function of the skin as the site of photosynthesis of vitamin D₃ is well known.¹⁴ The presence of the vitamin D receptor (VDR) in most cell types of the skin, including keratinocytes, hair follicle cells, melanocytes, fibroblasts and others identifies the human skin as a target of biologically active vitamin D compounds and strongly indicates a major relevance of vitamin D for skin physiology including signaling pathways that are of relevance for tumorigenesis of non-melanoma skin cancer [NMSC; most importantly basal (BCC) and squamous (SCC) cell carcinomas].¹⁻⁸ It has been demonstrated that 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃, calcitriol] the biologically active natural metabolite of vitamin D, has great impact on keratinocyte growth and differentiation and consequently is, together with calcipotriol and other analogs, successfully used for the treatment of the hyperproliferative skin disorder psoriasis.⁹,¹⁰ Solar and artificial UV (UV)-B-radiation (280–320 nm) exerts both beneficial and adverse effects on human health.¹⁻⁶ On the one hand, UV-B-radiation is the most important environmental risk factor for the development of NMSC, that represent the most common malignancies in Caucasian populations.¹⁻⁶ On the other hand, the human body's requirements of vitamin D are mainly achieved by UV-B-induced cutaneous photosynthesis. This dilemma represents a serious problem in many populations, for an association of vitamin D-deficiency and multiple independent diseases including various types of cancer has been convincingly demonstrated. In line with these findings, epidemiologic and laboratory investigations now indicate that vitamin D and its metabolites have a risk reducing effect for NMSC. Potential mechanisms of action include inhibition of the hedgehog signaling pathway (BCC) and modulation of p53-mediated DNA damage response (SCC). As a consequence of these new findings it can be concluded that UV-B-radiation exerts both beneficial and adverse effects on risk and prognosis of NMSC. It can be assumed that many independent factors, including frequency and dose of UV-B exposure, skin area exposed, and individual factors (such as skin type and genetic determinants of the skin’s vitamin D status and of signaling pathways that are involved in the tumorigenesis of NMSC) determine whether UV-B exposure promotes or inhibits tumorigenesis of NMSC. Moreover, these findings may help to explain many of the differential effects of UV-B radiation on risk of NMSC, including variation in the dose-dependent risk for development of SCC in situ (actinic keratosis, AK), invasive SCC, and BCC. In this review, we analyze the relevance of the vitamin D endocrine system (VDES) for tumorigenesis, prevention, and treatment of NMSC and give an overview of present concepts and future perspectives.
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**Epidemiology and Photocarcinogenesis of Basal Cell Carcinoma (BCC) and Cutaneous Squamous Cell Carcinoma (SCC): The Two Most Predominant Types of Non-Melanoma Skin Cancer (NMSC)**

Epidemiology of basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (SCC). Cutaneous squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) represent the two types of non-melanoma skin cancer (NMSC) with the highest incidence and prevalence rates worldwide. While the incidence of skin cancer has dramatically increased during the last decades, it is now accepted that the reasons for this development are multifactorial. It has been speculated that besides the age pyramid and other factors, cultural changes that result in increased UV-exposure, may be of particular importance.

BCCs and SCCs show a locally aggressive and invasive growth pattern, but in comparison with SCCs (metastatic potential in about 5% of all cases), BCCs only very rarely metastasize. Actinic keratoses (AK) are precursors of SCC and are now classified as SCC in situ. Epidemiological studies have convincingly shown that living in parts of the world with increased erythemal UV or high average annual bright sun results in increased risks of SCC and BCC, with the greatest increased risk for SCC. These investigations are in line with studies of personal exposure, demonstrating that higher levels of occupational and total UV exposure increase the risk for NMSC, with greater correlation for SCC than for BCC. “Intermittent” sun exposure, such as high exposure only at weekends or holidays tends to be associated to some extent with increased risk of BCC. Sunburn at any age increases the risk of BCCs and SCCs, with greater correlation for SCC than for BCC. The age at which high UV exposures occur may also be of importance, since there is epidemiological evidence 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 y, as compared with people who live since birth in a high solar UV environment. Pale skin increases the risk of SCC and BCC. SCCs and BCCs tend to occur in constantly sun-exposed skin areas like the face, ears, neck and back of the hands, with greater association for SCC than for BCC. Standard therapy for both skin cancers is surgical excision. Due to a high percentage of local and systemic recurrence, dermatologists have been looking for chemopreventive and/or chemotherapeutic agents for years. Interestingly, it has been shown that vitamin D compounds have chemopreventive effects at least for BCCs. The combination of retinoids and calcitriol has been reported to be effective in the chemotherapy of cutaneous malignancies, including BCCs and SCCs.

**Vitamin D status in BCC and SCC.** Vitamin D can be absorbed from the diet or synthesized from 7-dehydrocholesterol (7-DHC) in the skin by the action of sunlight (UV-B). It is metabolized in the liver by CYP2R1 or CYP27A1 to 25-hydroxyvitamin D [25(OH)D] and then in the kidney or in other tissues by CYP27B1 to the biologically active metabolite 1,25-dihydroxyvitamin D [1,25(OH)2D]. In the blood, vitamin D metabolites are mostly bound to a specific transport protein, the vitamin D binding protein (VDBP, GC). 1,25(OH)2D is metabolized in target cells at least in part by 24-hydroxylase (CYP24A1), resulting in a specific C-24 oxidation pathway to yield the biliary excretory product calcitroic acid. It is well accepted that the serum 25(OH)D concentration represents the best parameter to determine a person’s vitamin D status. Individual factors that predispose for a person’s vitamin D status, such as skin type and UV exposure, have been identified. Vitamin D deficiency is common in many populations and low serum 25(OH)D concentrations are associated with an increased incidence and an unfavorable outcome of multiple diseases, such as various types of cancer, infectious, cardio-vascular, and autoimmune diseases. At present, epidemiologic studies do not show a clear relationship between serum 25(OH)D concentration and risk of NMSC. However, the interpretation of these epidemiologic studies is difficult due to many limitations that include low case numbers and potentially confounding factors such as UV-B radiation. In many of these investigations, that are difficult to compare due to differences in the study design (including use of different parameters to determine vitamin D status, e.g., analysis of 25(OH)D and/or 1,25(OH)2D serum concentrations; use of different assays to measure 25(OH)D serum concentrations; different location/latitude of study populations) the positive relationship of UV exposure with both vitamin D status and NMSC risk makes it difficult to interpret the findings. A nested case-control study (Osteoporotic fractures in men, MrOS) in ambulatory elderly men with or without NMSC showed that individuals with the highest baseline serum 25(OH)D concentrations (> 30 ng/ml) had 47% lower odds ratios for NMSC (95% confidence interval, 0.3–0.93; p = 0.026), compared with those with the lowest 25(OH)D concentrations. The authors concluded that high 25(OH)D levels may be associated with a reduced risk for NMSC, and that a diagnosis of NMSC does not indicate an adequate vitamin D status. However, some epidemiologic studies do not support the hypothesis that an adequate vitamin D status reduces the risk of NMSC. A prospective investigation evaluated the association between baseline plasma 25(OH)D levels and the risk of incident SCC and BCC among 4,641 women from the Nurses’ Health Study (NHS) and the NHS II with 510 incident BCC cases and 75 incident SCC cases. In that study, plasma 25(OH)D levels were positively associated with risk of BCC after adjusting for age at blood draw, season of blood draw, lab batch, hair color, burning tendency, the number of sunburns, and UV B flux of residence at blood collection. Women in the highest quartile of 25(OH)D had more than 2-fold increased risk of BCC compared with women in the lowest quartile (OR = 2.07, 95% CI = 1.52–2.80, P for trend < 0.0001). The authors also found a significantly positive association between plasma 25(OH)D levels and SCC risk after adjusting for the same covariates (OR, highest vs. lowest quartile = 3.77, 95% CI = 1.70–8.36, P for trend = 0.0002).
In this prospective study of women, plasma 25(OH)D levels were positively associated with NMSC risk. The authors concluded that, considering that most circulating vitamin D is due to sun exposure, the positive association between plasma 25(OH)D and NMSC is confounded by sun exposure and that their data suggest that one-time measurement of plasma vitamin D levels may reasonably reflect long-term sun exposure and predict the risk of NMSC.

Results of a case control study (Kaiser Permanente Northern California population) indicate that higher prediagnostic 25(OH)D levels may be associated with a small increased risk of BCC.21 Prospective cohort studies in women published in 199222 and in men published in 200023 using dietary questionnaires found no association between intake of vitamin D and risk of BCC. A prospective investigation analyzing white individuals of a Health maintenance organization cohort who sought care for actinic keratoses found no association between intake of vitamin D and risk of BCC.24 Vitamin D-binding protein (VDBP) single nucleotide polymorphisms (SNP) affect 25(OH)D levels and thereby may influence skin carcinogenesis.25 One study tested the association between two functional VDBP SNPs and the susceptibility to (multiple) BCCs.25 Of the 7983 participants, 5790 (72.5%) and 5823 (72.9%) participants were genotyped for rs7041 and rs4588, respectively, and three haplotypes (Gc1s, Gc2 and Gc1f) were analyzed. Two hundred and 33 persons developed a BCC of whom 122 (52.4%) developed multiple BCCs during a mean follow-up of 11.6 y. In that study, the VDBP genotype was not associated with (multiple) BCC development using Cox proportional hazards and Andersen-Gill analyses, respectively. Stratifying age groups demonstrated that in the youngest age-group, the A/T variant of rs7041 and rs4588, respectively, and three haplotypes (Gc1s, Gc2 and Gc1f) were analyzed. Two hundred and 33 persons developed a BCC of whom 122 (52.4%) developed multiple BCCs during a mean follow-up of 11.6 y. In that study, the VDBP genotype was not associated with (multiple) BCC development using Cox proportional hazards and Andersen-Gill analyses, respectively. Stratifying age groups demonstrated that in the youngest age-group, the A/T variant of rs7041 and rs4588, respectively, and three haplotypes (Gc1s, Gc2 and Gc1f) were analyzed. Two hundred and 33 persons developed a BCC of whom 122 (52.4%) developed multiple BCCs during a mean follow-up of 11.6 y. In that study, the VDBP genotype was not associated with (multiple) BCCs, but that age-gene interactions were observed.25

Some pilot studies indicate that patients with basal cell nevus syndrome (BCNS; Goltz-Gorlin syndrome) or xeroderma pigmentosum, that are prone to develop BCCs and/or SCCs due to mutations in genes of the hedgehog signaling pathway or in DNA repair genes, and who therefore have to protect themselves consequently from UV radiation have relatively low serum concentrations of 25(OH)D.26,27 In a retrospective cohort study (41 ambulatory patients with BCNS who participated in a 2-y chemoprevention clinical trial vs. 360 population-based controls), 23 patients with BCNS (56%) were vitamin D deficient (defined as a 25(OH)D level of ≤ 20 ng/mL).27 Patients with BCNS had mean 25(OH)D levels below those of the general population (−3 ng/mL; p = 0.02) and were 3 times more likely to be vitamin D deficient (56% vs. 18%; p < 0.001). It can be concluded that patients with BCNS are at increased risk for vitamin D deficiency, depending on their adherence to photoprotection.27

Expression of vitamin D receptor (VDR) in BCC and SCC. Effects of 1,25(OH)D3 on target cells are at least in part mediated via its corresponding intranuclear receptor (VDR), which belongs to the superfamily of transacting transcriptional regulatory factors, including the steroid and thyroid hormone receptors as well as the retinoid-X receptors (RXRs) and retinoic acid receptors (RARs).3,4,28 Different classes of vitamin D response elements (VDRE) have been characterized in target genes that are activated either by VDR-homodimers or by heterodimers of VDR and RXRs.3,4,28 It was demonstrated that ligands of RXRs (e.g., 9-cis retinoic acid) can enhance the transcriptional activity in 1,25(OH)D3-mediated nuclear signaling pathways. Thus, there are different vitamin D signaling pathways that are determined by the VDR, its dimerization-partner, corresponding ligands, and the nature of the VDRE.28 Increasing evidence now indicates that the VDR protects against the development of NMSC.29 It has been shown that mice lacking the VDR are sensitive to epidermal tumor formation induced by the carcinogen DMBA or following UV radiation (UVR).29,31 The epidermis of VDR null mice shows hyperproliferation of keratinocyte cell layers, and distortion of hair follicles, structures from which these carcinogen-induced skin tumors may develop.29,31

Strong VDR immunoreactivity and mRNA expression has been reported both in BCCs32,34 (Fig. 1) and SCCs.34,35 An in vitro investigation using real-Time RT-PCR technology showed a statistically highly significantly increased ratio of VDR/GAPDH gene expression in BCCs (median: 16.54) and SCCs (median, 37.00) as compared with normal human skin (median, 0.00021 for the BCC study and 0.000006 for the SCC study; both p < 0.005).33,35 Immunohistochemical in vitro investigations demonstrate that almost every tumor cell of BCCs and SCCs reveals nuclear immunoreactivity for VDR.32,35 In these studies, VDR staining intensity was markedly stronger in both skin tumor types as compared with adjacent epidermis or to distant unaffected epidermis of the same sections. In BCCs, VDR immunoreactivity was pronounced in the palisaded array of peripheral tumor cells.32 There was no visual difference comparing VDR staining pattern in the different variants of BCCs (nodular type, superficial type, fibrosing type) or of SCCs (poorly, moderately, well differentiated).32,35 Analyzing immunohistochemically the expression of nuclear VDR cofactors in BCCs, strong staining for RXR-α has been reported while in contrast, RXR-β and RXR-γ were not or only weakly detectable in BCCs.34 Analysis of the VDR heterodimerization partners suggests that selective vitamin D analogs activating exclusively the predominant VDR-RXR-α heterodimer may be most effective in the treatment of BCC with little risk of side effects.

When the immunohistochemical staining of VDR was analyzed in BCC and SCC for correlation with the proliferation marker Ki-67, no visual correlation of labeling patterns was found.32-35 Heterogeneous Ki-67 immunoreactivity with no visual differences between central and peripheral areas was found in most SCC specimens (11 of 15), although some SCCs revealed pronounced labeling for Ki-67 antigen in peripheral tumor cells (4 of 15). Confocal laser scanning microscopy confirmed these results, showing that double-stained sections for VDR and Ki-67
revealed no visual correlation of labeling patterns in both tumor (Fig. 2). Double-stained sections for VDR and the differentiation markers transglutaminase K or cytokeratin 10 also revealed no visual correlation of labeling patterns. When the immunohistochemical staining of VDR was analyzed for correlation with apoptotic cells, no visual correlation of labeling patterns was found. In that study, all BCC and SCC specimens revealed single scattered terminal UTP nucleotide end-labeled apoptotic cells with considerable variation in their number. Distribution of apoptotic cells within the tumors was heterogeneous. There were markedly fewer apoptotic cells than VDR-positive cells. In summary, comparison of staining patterns revealed no evidence for strong correlation of VDR expression and apoptosis and/or cell proliferation/differentiation in BCC or SCC.

**Vitamin D receptor (VDR) polymorphisms (SNPs) in BCC and SCC.** As outlined above, vitamin D deficiency is associated with various types of cancer. Functional polymorphisms (most importantly single nucleotide polymorphisms, SNPs) along the 105 kilobase VDR gene have important implications for mediating actions of 1,25(OH)2D. The VDR gene encompasses two promoter regions, eight protein-coding exons namely 2–9 and in the 3' UTR region. Most of the VDR polymorphisms, that may represent restriction fragment lengths polymorphisms (RFLP), have an unknown functional effect. In some cases, it has been indicated that they may be linked to truly functional polymorphisms elsewhere in the VDR gene (or in a nearby gene). Consequently, it has been shown that VDR polymorphisms are associated with various malignancies, including cancers of the breast, colon, and prostate. There is a significant increase in breast cancer risk for carriers of FokI ff compared with FF genotype, and a significant decrease of prostate cancer risk for BsmI Bb in comparison with bb carriers. Little is known about VDR polymorphisms and the occurrence of skin cancer. In a study analyzing SCC, the BB genotype of VDR was significantly associated with increased cancer risk (OR = 1.51). Moreover, an interaction between the BsmI polymorphism and total vitamin D intake was observed in SCC patients with > 2-fold higher risk seen in women with the BB genotype and high vitamin D intake (OR = 2.38, p interaction = 0.08). Another study suggested that the TaqI polymorphism TT was associated with an increased number of BCCs that developed per year, particularly in combination with skin type I and male sex. In 2009, a review and meta-analysis of 67 independent studies on the association between the two most studied VDR polymorphisms (FokI and BsmI) and cancer risk was reported. When comparing FokI ff with FF carriers, a significant increase in skin cancer risk [SOR; 95% confidence intervals (CIs): 1.30; 1.04–1.61].

Analyzing the BsmI genotypes in Caucasian populations, both Bb and BB carriers had a significant reduced risk of skin cancer. In conclusion, this meta-analysis strongly supports the concept that VDR FokI and BsmI polymorphisms modulate the risk of NMSC. In a pilot study in the German population, we have analyzed the presence of several VDR polymorphisms (Apa1, Taq1, Bgl1) in BCC and cutaneous SCC as compared with healthy controls. Variations were observed in the distribution of VDR polymorphisms in the tumor groups compared with the healthy controls (Apa1 Aa genotype: 56.1% in BCC, 51.1% in SCC, 45.1% in healthy controls; Taq1 Tt genotype: 58.6% in BCC, 50.0% in SCC, 48.0% in healthy controls; Bgl1 Bb genotype: 54.5% in BCC, 50.0% in SCC, 43.1%). Interestingly, there were associations indicating that Apa1 and Taq1 genotypes may be of importance for photocarcinogenesis of BCCs, but not of SCCs. These data indicate that VDR polymorphisms are of importance for the tumorigenesis of BCC and SCC, and may contribute to the differential role of UV-B radiation for the development of these malignancies.

**Expression of CYP27A1, CYP27B1 and CYP24A1 in BCC and SCC.** The formation of 1,25(OH)2D, the biologically most active natural ligand of the VDR, is mediated through several main enzymes that facilitate hydroxylations of vitamin D at position 25 in the liver (CYP2R1, CYP27A1) and of resulting 25(OH)D at position 1 in the kidneys (CYP27B1). These hydroxylases belong to a class of cytochrome P450 mixed function enzymes. After activation of vitamin D in the kidneys, the active 1,25(OH)2D is transported to all tissues where it binds to the VDR, either in the cytosol or nucleus, thus initiating transcription of specific genes. To date, only the transcriptional effect of 1,25(OH)2D on the expression of CYP27A1 has been established. Interestingly, the expression of CYP27A1 in BCC tumors is significantly increased compared with SCC tumors and healthy controls. In a recent study, a significant correlation between the expression of CYP27A1 and total serum 25(OH)D was found. A positive correlation between CYP27A1 expression and 25(OH)D levels was also observed in SCC tumors. These findings support the concept that CYP27A1 expression is directly regulated by vitamin D. The expression of CYP27B1 in BCC and SCC was found to be significantly increased compared with healthy controls. This finding is consistent with previous studies showing that the expression of CYP27B1 is increased in a variety of cancer types. In contrast, the expression of CYP24A1, which catalyzes the conversion of 1,25(OH)2D to 24,25(OH)2D, was found to be significantly decreased in BCC and SCC tumors compared with healthy controls. These findings suggest a potential role of CYP24A1 in the regulation of vitamin D levels in cancer tissues. In conclusion, the expression of CYP27A1, CYP27B1 and CYP24A1 in BCC and SCC is altered compared with healthy controls, with increased expression of CYP27A1 and CYP27B1 and decreased expression of CYP24A1. This suggests a possible role of these enzymes in the pathogenesis of BCC and SCC.
monooxidases. Extrarenal activity of CYP27B1 has been reported in various cell types, including macrophages, keratinocytes, as well as prostate and colon cancer cells. It has been found that modulation of these enzymes influences the proliferation and differentiation status of 1,25(OH)\(_2\)D\(_3\)-sensitive cells, benign or neoplastic.\(^{3,5,6}\) Using array-competitive genomic hybridization (CGH), amplification of CYP24A1 was detected as a likely target oncogene of the amplification unit 20q13.2 in breast cancer cell lines and tumors.\(^{42}\) It has been speculated that increased expression of CYP24A1 due to gene amplification may abrogate vitamin D\(_3\)-mediated growth control. Moreover, amplification of the CYP27B1 gene has been demonstrated in glioblastomas.\(^{43}\)

In in vitro investigations, real-time PCR analysis showed mRNA ratios of CYP27B1/GAPDH (median: 0.739 in BCCs and 2.02 in SCCs) and CYP24A1/GAPDH (median, 0.0058 in BCCs and 0.382 in SCCs) gene expression in BCCs and SCCs, significantly increased as compared with normal human skin (median, 0.0008; 0.0000004, respectively).\(^{3,5,6}\) The ratio of CYP27A1/GAPDH was only in SCCs significantly increased to normal skin (median SCC, 33.00 vs. NS, 0.000004; p < 0.02), whereas in BCCs ratio of CYP27A1/GAPDH were not significantly altered (median BCCs, 0.17 vs. NS, 0.0166; p = 0.62).\(^{3,5,6}\)

It is not known whether increased expression of the CYP27A1, CYP27B1 or CYP24A1 mRNA in NMSC is a result of gene amplification or of other mechanisms such as transcriptional regulation.\(^{3,5,6}\) If the increased expression of CYP27B1 results in an increased production of biologically active 1,25(OH)\(_2\)D\(_3\), the accumulation of this metabolite should inhibit growth, invasion and metastasis of NMSC.\(^{3,5,6}\) It can be speculated whether increased expression of CYP27B1 and CYP27B1 in NMSC may represent a physiological feed-back loop coupled to the increased proliferative activity in these tumors. However, one has to keep in mind that 1,25(OH)\(_2\)D\(_3\) may be rapidly metabolized via CYP24A1, whose expression is increased in BCCs and SCCs as well.\(^{3,5,6}\) It is not known whether increased expression of CYP27A1, CYP27B1 and CYP24A1 genes results in increased or reduced levels of 1,25(OH)\(_2\)D\(_3\) in NMSC.\(^{3,5,6}\) Therefore, the question of cellular and systemic consequences of the increased expression of CYP27A1, CYP27B1 and CYP24A1 in skin tumors remains to be clarified. Nevertheless, it can be speculated whether precursors of biologically active 1,25(OH)\(_2\)D\(_3\) or inhibitors of CYP24A1 may be of benefit for the prevention or treatment of BCCs and SCCs.\(^{3,5,6}\)

**Figure 2.** Schematic illustration of the theory suggesting that Ptc1 regulates Smo by removing oxysterols. Activation of the Hedgehog (Hh)-signaling pathway due to deficiency in the Hh receptor Patched1 (Ptc1) is the crucial molecular defect that causes the formation of BCCs in human skin. Ptc1 possesses a sterol sensing domain (SSD), which is important for suppression of the activity of Smoothened (Smo), the signal transduction partner of Ptc1. A current theory suggests that Ptc1 regulates Smo by removing oxysterols from Smo. Ptc acts like a sterol pump and removes oxysterols that have been created by 7-dehydrocholesterol reductase. Upon binding of a Hh protein or a mutation in the SSD of Ptc the pump is turned off allowing oxysterols to accumulate around Smo. This accumulation of sterols allows Smo to become active via GL signaling or to remain on the cell membrane for a longer period of time.

**Carcinogenesis of Basal Cell Carcinoma (BCC) and Cutaneous Squamous Cell Carcinoma (SCC): Convincing Evidence for Suppression of Skin Carcinogenesis by the Vitamin D Endocrine System**

In mouse skin, the abnormal activation of two interacting pathways critical for epidermal and hair follicle function, β-catenin and hedgehog (Hh), leads to epidermal tumors.\(^{29-31}\) The canonical hedgehog signaling pathway represents a key regulator of development in humans and in animals and is present in every bilaterian.\(^{5,6,30}\) The pathway is called after its polypeptide ligand Hedgehog (Hh), an intercellular signaling molecule discovered in fruit flies of the genus Drosophila.\(^{5,6,30}\) Hh is one of Drosophila’s segment polarity gene products, involved in creating the molecular and structural basis of the fly body plan.\(^{5,6,30}\) The molecule remains important during many stages of embryogenesis and metamorphosis. Sonic hedgehog (SHH) is the best investigated...
ligand of the vertebrate pathway.\textsuperscript{5,6,30} It is now accepted that activation of the Hh-signaling pathway due to deficiency in the Hh receptor Patched1 (Ptc) is the crucial molecular defect that causes the formation of BCCs in human skin.\textsuperscript{5,6,30} Ptc1 possesses a sterol sensing domain (SSD), which has been shown to be important for suppression of the activity of Smoothened (Smo), the signal transduction partner of Ptc.\textsuperscript{5,6,30} A current theory suggests that Ptc regulates Smo by removing oxysterols from Smo. Ptc acts like a sterol pump and removes oxysterols that have been created by 7-dehydrocholesterol reductase.\textsuperscript{5,6,30} Upon binding of a Hh protein or a mutation in the SSD of Ptc the pump is turned off allowing oxysterols to accumulate around Smo.\textsuperscript{5,6,30} This accumulation of sterols allows Smo to become active via GLI signaling or to stay on the cell membrane for a longer period of time.\textsuperscript{5,6,30} This hypothesis is supported by the presence of a number of small molecule agonists and antagonists of the pathway that act on Smo.\textsuperscript{5,6,30} The binding of SHH relieves Smo inhibition, leading to activation of the GLI transcription factors: the activators Gli1 and Gli2 and the repressor Gli3.\textsuperscript{5,6,30} It has been shown that all elements of the Hh signaling pathway are elevated in the epidermis and uroticles of VDR null mice, and that 1,25(OH)\textsubscript{2}D3 blocks expression of these elements in normal mouse skin.\textsuperscript{25} In addition the transcriptional activity of β-catenin is increased in keratinocytes lacking the VDR.\textsuperscript{31} Using primary cultured human keratinocytes, it was demonstrated that 1,25(OH)\textsubscript{2}D\textsubscript{3} suppresses cyclin D1 and Gli1 which are regulated by β-catenin/TCF signaling and have a critical role in epidermal carcinogenesis.\textsuperscript{31} Blockage of VDR by siRNA resulted in hyperproliferation of keratinocytes, and increased expression of cyclin D1 and Gli1.\textsuperscript{31} Moreover, it was demonstrated that 1,25(OH)\textsubscript{2}D\textsubscript{3}/VDR directly regulates transcriptional activity of β-catenin/TCF signaling using the -catenin reporter TopGlow.\textsuperscript{31} Using K14 driven tamoxifen-induced cre recombinase to delete both VDR and β-catenin in keratinocytes of mice following the first hair follicle cycle, it was found that ablation of epidermal specific β-catenin cannot rescue VDR null mice from UVB-induced skin tumor formation.\textsuperscript{31} Moreover, convincing evidence indicates the Ptc-dependent secretion of a vitamin D\textsubscript{3}-related compound, which acts as an endogenous inhibitor of Hh signaling by blocking the activity of Smo, the signal transduction partner of Ptc.\textsuperscript{30} It has been suggested that this substance is lacking in Ptc-deficient tumor cells, which in turn may result in activation of Hh-signaling.\textsuperscript{30} It has been demonstrated that the application of 1,25(OH)\textsubscript{2}D\textsubscript{3} inhibits proliferation and growth of BCC in Ptc mutant mice in vitro and in vivo.\textsuperscript{50} This effect is associated with activation of VDR and induction of BCC differentiation.\textsuperscript{50} In addition, it was shown that 1,25(OH)\textsubscript{2}D\textsubscript{3} inhibits Hh signaling at the level of Smo in a VDR-independent manner.\textsuperscript{30} The concomitant antiproliferative effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} on BCC growth were shown to be stronger than those of the Hh-specific inhibitor cyclopamine, even though the latter more efficiently inhibits Hh signaling.\textsuperscript{30} In conclusion, there is convincing evidence that exogenous supply of 1,25(OH)\textsubscript{2}D\textsubscript{3} controls the activity of 3 independent pathways, Hh, β-catenin/TCF and VDR signaling, which are relevant for tumorigenesis and treatment of BCC. These data strongly support the concept that vitamin D compounds may represent promising options for prevention and treatment of BCC and that the VDR acts as a tumor suppressor in skin.

For SCC development, UV-induced DNA damage is the most important environmental risk factor.\textsuperscript{5,6} UV-R often causes gene mutations that may lead to cellular transformation and malignancy.\textsuperscript{1,6,44-46} DNA damage also initiates and promotes mechanisms that suppress immune surveillance responsible for detecting and eliminating transformed cells.\textsuperscript{3,49,50} UV exposure causes different types of DNA lesions that are produced either photochemically and directly or indirectly by UV activation of various photoreceptors that are able to alter the cellular redox equilibrium, thereby generating reactive oxygen species (ROS).\textsuperscript{5} ROS induced by UV radiation are able to cause oxidative damage to DNA, and lipid peroxidation.\textsuperscript{5} Moreover, it has been shown that excess levels of nitric oxide (NO) are induced by UV-mediated upregulation of nitric oxide synthase,\textsuperscript{5,51-53} and also by UV-A (320–400 nm) mediated decomposition of NO stores in nitrosothiols and nitric.\textsuperscript{5,54,55} Pathophysiologically elevated levels of NO and ROS have been demonstrated to combine to form genotoxic NO derivatives such as peroxynitrite that cause oxidative and nitrosative modifications to the sugar-phosphate scaffold and bases of DNA.\textsuperscript{5}

It is generally acknowledged that mutagenic effects of UV radiation represent a hallmark in the carcinogenesis of SCC, and that promutagenic pyrimidine dimers are the major forms of DNA damage produced directly by UV.\textsuperscript{5,6,56} The predominant type of pyrimidine dimer detected 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.\textsuperscript{5,6,57-60} CPDs are generated by the perturbation of the 5–6 double bonds in two adjacent pyrimidines, followed by abnormal covalent binding that connects the 2 pyrimidines by a stable ring configuration forming a bipyrimidine product.\textsuperscript{5,6,61,62} It is well accepted that CPD production requires the wavelengths of UV-B (290–320 nm).\textsuperscript{5,6} However there is some evidence for generation of thymine dimer by UV-A wavelengths below 330 nm.\textsuperscript{5,6,60,63-66} Wavelengths of UV-A are less energetic but considerably more abundant (20-fold higher) than UV-B in sunlight, and can penetrate to deeper skin levels.\textsuperscript{5,67-69} The shorter, more highly energetic UV-C wavelengths below 290 nm can also induce CPDs.\textsuperscript{5} However, they are completely absorbed by the stratosphere and are therefore not present at the earth’s surface.\textsuperscript{5} They may only become hazardous in the future if stratospheric ozone levels should be depleted.\textsuperscript{5} CPDs can also be produced chemically in isolated DNA, probably be via a triplet energy transfer mechanism.\textsuperscript{5,70,71} This mechanism may explain the production of CPDs in skin cells by a nitric oxide donor in the absence of UV,\textsuperscript{5,72} and a decrease in UV-induced CPDs in skin cells treated with inhibitors of nitric oxide synthase.\textsuperscript{5,73}

Another important promutagenic photolesion detected in human skin is 8-hydroxy-2′-deoxyguanosine, which is produced indirectly by oxidation of the base guanine by peroxynitrite.\textsuperscript{5,74,75} Peroxynitrite is also able to cause nitrosative damage to form 8-nitroguanine,\textsuperscript{5,76-79} which is converted to a promutagenic abasic
site within a few hours. DNA strand breaks have also been shown to be induced by nitrosation of primary amines by another NO derivative, nitrous anhydride.5,80 Photosensitizers resistant to DNA repair are able to cause deleterious gene mutations that form either by deletion, base mispairing, or substitutions during DNA replication when adenine is inserted as the default base.5 Mutations that affect cellular function can promote skin carcinogenesis.5,16 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.5,6,81-84 G to T transversions are associated with 8-hydroxy-2'-deoxyguanosine5,85 and occur in isolated DNA exposed to peroxynitrite.5,86

**UV-Induced DNA Damage Response: Modulation by Vitamin D Signaling**

In order to protect genome integrity, cells respond to DNA damage by inducing signal transduction pathways that cause cell cycle arrest before the affected cells can replicate.5 This enables either DNA-repair or the elimination of severely damaged cells by apoptosis.5,6,87,88

Apoptosis, representing a mode of programmed cell death, is induced following UV-B-irradiation when cellular damage is too severe to be repaired.5,6,89-93 It has convincingly been shown that the biologically active vitamin D metabolite 1,25(OH)₂D protects human skin cells from UV-induced cell death and apoptosis.5,6,89-93 In these studies, cytotoxic effects of 1,25(OH)₂D on UV-B-irradiated keratinocytes were seen morphologically and using a colorimetric cell survival assay.5,6,89-93 Moreover, using an ELISA that detects DNA-fragmentation, it was shown that pretreatment with 1,25(OH)₂D suppresses UV-B-induced apoptosis by 55–70%.5,6,89-93 This suppression requires pharmacological concentrations of 1,25(OH)₂D and a preincubation period of several hours.5,6,89-93 In addition, it was demonstrated that pretreatment with 1,25(OH)₂D also inhibits mitochondrial cytochrome C release, a hallmark event of UV-B-induced apoptosis.5,6,89-93

Furthermore, it was demonstrated that 1,25(OH)₂D reduces two important mediators of the UV-response, namely, c-Jun-NH₂-terminals kinase (JNK) activation and interleukin-6 (IL-6) production.5,6,89-93 As shown by western blotting, pretreatment of keratinocytes with 1,25(OH)₂D diminishes UV-B-stimulated JNK activation by more than 30%. Furthermore, 1,25(OH)₂D treatment reduces the UV-B-induced IL-6 mRNA expression and protein secretion by 75–90%. Analyzing the cleavage of PARP further supported these observations. Pretreatment of keratinocytes with 1,25(OH)₂D inhibits efficiently, but not completely, this UV-B-induced PARP-cleavage.5,6,89-93

Metallothionein (MT)-induction may be relevant for the photoprotective effects of 1,25(OH)₂D. MT acts as a radical scavenger in oxygen-mediated UV-B-injury.5,6,89-93 MTs are a class of small cysteine-rich proteins that bind and exchange heavy metal ions but also have clear scavenging properties for ROS.5,6,89-93 Part of the UVB-induced damage to cells occurs through the formation of ROS and antioxidative agents such as MT have been demonstrated to be photoprotective.5,6,89-93 In these studies, MT mRNA expression was shown to be clearly induced by 1,25(OH)₂D.6

The anti-apoptotic effect of 1,25(OH)₂D in keratinocytes was confirmed, using cisplatin and doxorubicin as apoptotic triggers.6,89-93 In that study, it was demonstrated that 1,25(OH)₂D activated two independent survival pathways in keratinocytes: the MEK/extracellular signal regulated kinase (ERK) and the phosphatidylinositol 3-kinase (PI-3K)/Akt pathway.5,89-93 Activation of ERK and Akt by 1,25(OH)₂D was transient, required a minimal dose of 10⁻⁹ mol/L and could be blocked by actinomycin D and cycloheximide.6 Moreover, inhibition of Akt or ERK activity with a PI-3K inhibitor (LY294002) or MEK inhibitors (PD98059, U0126) respectively, partially or totally suppressed the anti-apoptotic capacity of 1,25(OH)₂D.6 Finally, 1,25(OH)₂D modulates the expression of different apoptosis regulators belonging to the Bcl-2 family.6 It has been shown that 1,25(OH)₂D treatment increases levels of the anti-apoptotic protein Bcl-2 and decreases levels of the pro-apoptotic proteins Bax and Bad in a time- and dose-dependent way.6,89-93 The authors of these investigations concluded that 1,25(OH)₂D protects keratinocytes against apoptosis by activating the MEK/ERK and the PI-3K/Akt survival pathways and by increasing the Bcl-2 to Bax and Bad ratio.5,6,89-93

Moreover, it has been demonstrated that 1,25(OH)₂D protects primary human keratinocytes against the UV-B-induced generation of CPDs.5,6,89-95 In some studies, this protection required pharmacologic doses of 1,25(OH)₂D and an incubation period of at least 8 h before UV-B-irradiation.5,6 CPDs are primarily eliminated by the nucleotide excision repair (NER) pathway that has a relatively long half-life of 7–12 h.5,6,94-97 Individuals with the inherited disorder xeroderma pigmentosum bear a defect in one of the key enzymes of NER pathway and are highly prone to UV-induced skin carcinogenesis.5,6,98,99 Oxidative DNA damage is repaired by the more rapid alternate base excision repair (BER) pathway.5,6,100-104 However, the repair enzyme human 8-oxoguanine-DNA glycosylase 1 is less abundant in the basal layers than the upper layers of the human epidermis, which indicates that repair of oxidative damage in the dividing keratinocytes of the epidermis is less efficient.5,104

The tumor suppressor protein p53, representing a key regulator of the DNA damage response as mentioned above, is activated by DNA damage.5,6,66,75,103,106 Physiological doses of UV-A and UV-B can induce inactivating mutations in the p53 gene. Mutations in the tumor suppressor p53 in engineered human skin were found to be predominantly UV-A finger print mutations induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7 These mutations are induced by oxidative damage located in the basal layer of the epidermis.5,7
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-R. This was demonstrated by the progressive growth of tumors transplanted into irradiated mice, while the tumors were rejected in unirradiated mice. Pyrimidine dimers are important mediators of photoimmune suppression. 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. Photommun suppression was also reduced in irradiated mice after reduction of CPDs by application of encapsulated T4 endonuclease, the specific repair enzyme for pyrimidine dimers.

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. Cis-urocanic acid formed by UV isomerisation of the photoreceptor trans-urocanic acid located in the outermost layers of skin also inhibits the antigen propensity of Langerhan’s cells. Depletion of Langerhan’s cells in the skin by DNA damage and oxidative stress reduces their antigen-presenting propensity. 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. 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 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. UV activation of Src, located on the inner surface of the keratinocyte plasma membrane, triggers signaling cascades that activate the transcription factors AP-1 and NF-κB that regulate immune regulatory cytokines, which is also blocked by antioxidant treatment. Activation of an alternate complement pathway has also been implicated in inflammatory and immune modulating activities.

Both UVB and UVA components of sunlight are immunosuppressive in mice and humans, while certain wavelengths of UVA have been shown to have a protective effect against UVB-induced immunosuppression in mice. However there is some conflicting data from studies in mice and humans regarding the particular wavelengths of UVA and their immunomodulatory effects.

Vitamin D compounds exert potent effects on the immune system and modulate the UV-induced immune response. The cytokine IL-6 represents an important mediator of the sunburn reaction, of UV-B-dependent immune suppression, and has been implicated in the tumorigenesis of BCCs. UV-B-irradiation strongly induces IL-6 mRNA and release of IL-6 protein by human keratinocytes. In cultured human keratinocytes, 1,25(OH)D treatment reduces the UV-B-induced IL-6 mRNA expression and protein secretion by 75–90%. Moreover, vitamin D compounds exert potent effects on the UV-induced immune response via many other mechanisms that include modulation of expression and function of regulatory T-cells.

Summary

The data presented in this review provide convincing evidence that the VDES is of high importance for prevention and treatment of NMSC. Besides a sufficient vitamin D status, the molecular basis that underlies these preventive effects of vitamin D compounds is the expression and functional integrity of the VDR and other key components of the
VDES. While some of the complex interactions of the VDES with other signaling pathways that contribute to skin carcinogenesis (e.g., hedgehog signaling) have been identified, future laboratory investigations will address unanswered questions and will increase our knowledge about the impact of the VDES on skin carcinogenesis. Most importantly, additional well-designed observational and interventional studies are needed to define the efficacy and safety of vitamin D compounds in the prevention and treatment of NMSC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Table 1. The role of the VDES for BCC prevention

| 25(OH)D serum concentration | evidence | convincing | presumable | possible | insufficient |
|-----------------------------|----------|------------|------------|----------|-------------|
| Clinical studies            |          |            |            |          | (results difficult to interpret due to UV radiation as confounder) |
| Animal studies              |          |            |            |          |             |
| In vitro investigations     |          |            | (via regulation of hedgehog signaling) |          |             |
| VDR polymorphisms (SNPs)   |          |            |            |          |             |
| Clinical studies            |          |            |            |          | @ or ↓      |
| Animal studies              |          |            |            |          | Ø (lack of data) |
| In vitro investigations     |          |            |            |          | Ø (lack of data) |

- Reduction of BCC risk resulting from substitution or treatment with vitamin D compounds (e.g., in interventional studies), from increasing 25(OH)D serum concentration, from association with SNPs (in observational studies), or from in vitro investigations; @ Increase of BCC risk resulting from substitution or treatment with vitamin D compounds (e.g., in interventional studies), from increasing 25(OH)D serum concentration, or from association with SNPs (in observational studies); Ø no association; Ø insufficient evidence.

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Table 2. The role of the VDES for SCC prevention

| SCC risk | 25(OH)D serum concentration | evidence |
|----------|-----------------------------|----------|
| Clinical studies | convincing | presumable | possible | insufficient |
|Animal studies | ↓ or ↑ | ↓ (results difficult to interpret due to UV radiation as con-founder) |
|In vitro investigation | ↓ (via regulation of apoptosis, reduction of CPDs following UVR) |

VDR polymorphisms (SNPs)

| Clinical studies | convincing | presumable | possible | insufficient |
|Animal studies | ↓ or ↑ | Ø (lack of data) |
|In vitro investigation | Ø (lack of data) |

Polymorphisms (SNPs) in other VDES-related genes (CYP27A1, CYP27B1, CYP24A1, CYP2R1, GC)

| Clinical studies | convincing | presumable | possible | insufficient |
|Animal studies | Ø (lack of data) |
|In vitro investigation | Ø (lack of data) |

- Reduction of SCC risk resulting from substitution or treatment with vitamin D compounds (e.g., in interventional studies), from increasing 25(OH)D serum concentration (in observational studies), from association with SNPs (in observational studies), or from in vitro investigations; Ø Increase of SCC risk resulting from substitution or treatment with vitamin D compounds (e.g., in interventional studies), from increasing 25(OH)D serum concentration (in observational studies), from association with SNPs (in observational studies), or from in vitro investigations; ○ no association; Ø insufficient evidence.

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