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Review

Vitamin D in the skin physiology and pathology

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Vitamin D plays important, pleiotropic role in the maintenance of global homeostasis. Its influence goes far beyond the regulation of calcium and phosphorus balance, as diverse activities of vitamin D and its natural metabolites assure proper functioning of major human organs, including skin. Recently, we reviewed the current understanding of vitamin D impact on human health from historical perspective (Wierzbicka *et al.* (2014) The renaissance of vitamin D. *Acta Biochim Pol* 61: 679–686). This article focuses on its functions in the skin. The skin and its appendages, creates a platform connecting and protecting internal organs against, usually harmful, external environment. It uppermost layer — epidermis in order to maintain a protective barrier undergoes a constant exchange of cornified keratinocytes layer. Its disturbance leads to development of serious skin disorders including psoriasis, vitiligo, atopic dermatitis and skin cancer. All of those dermatopathologies have a huge impact on modern societies, affecting not only the physical, but also mental state of patients as well as their social status. Furthermore, multiple human systemic diseases (autoimmune, blood and digestive diseases) have skin manifestation, thus "condition of the skin" often reflects the condition and pathological changes within the internal organs. In humans, the skin is the natural source of vitamin D, which is produced locally from 7-dehydrocholesterol in photoreaction induced by ultraviolet B (UVB) radiation from the sun. It is also well established, that the process of proliferation and differentiation of keratinocytes is tightly regulated by calcium and the active form of vitamin D ($1,25(OH)_2D_3$). Thus, the skin physiology is inseparably connected with vitamin D production and activity. Unfortunately, UVB, which is required for vitamin D production, is also known as the main cause of a skin cancer, including melanoma. Here, we are going to review benefits of vitamin D and its analogues in the maintenance of epidermal barrier and its potential use in the treatment of common skin diseases.

Key words: vitamin D, vitamin D , cancer, skin, keratinocytes, vitamin D analogues

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INTRODUCTION

Vitamin D is considered as the oldest hormone on the earth, and there is no doubt, that this ancient molecule is inherently connected with well-being of almost every form of life, from phytoplankton to humans (Holick *et al.*, 2007). Even though the vitamin D physiological role, most commonly, is attributed to maintenance of musculoskeletal system, the biological properties of this relatively simple compound goes far beyond the regulation of calcium-phosphorus homeostasis (Wacker & Holick, 2013).

The major source of vitamin D is the exposure of epidermis to solar irradiation. In the photochemical reaction 7-dehydrocholesterol (7-DHC) is converted to vitamin D_3 under UVB light (280–320 nm) in keratinocytes of the basal layer of epidermis (Bikle, 2012). After its release to the extracellular space, vitamin D_3 is captured in the capillary bed by vitamin D binding protein (DBP) (Holick *et al.*, 2007). Vitamin D produced in the skin or obtained with food is biologically inactive and requires two subsequent hydroxylations to gain its full hormonal activity (Holick *et al.*, 2007; Holick, 2011). Initially, vitamin D_3 is converted to 25-hydroxyvitamin D_3 ($25(OH)D_3$) in hepatocytes by the key vitamin D 25-hydroxylase — CYP2R1 (Cheng *et al.*, 2004; Strushkevich *et al.*, 2008). $25(OH)D_3$ is the major metabolite of vitamin D, thus its serum level is widely used in clinic as the representation of vitamin D status (Holick, 2011). The second requisite hydroxylation occurs in the kidney, due to the action of another hydroxylase — CYP27B1 which results in formation of $1,25(OH)_2D_3$, calcitriol (Takeyama *et al.*, 1997). The level of vitamin D is tightly regulated by CYP24A1, which is 24-hydroxylase. Its activity leads to deactivation of $1,25(OH)_2D_3$ or $25(OH)D_3$ and their subsequent removal with urine (Bikle, 2011). Finally, recently revealed alternative metabolic pathway for 7-DHC and vitamin D with major contribution of CYP450scc (CYP11A1) broadens the spectrum of naturally occurring vitamin D derivatives (see recent reviews for discussion: Slominski *et al.*, 2013a–c; 2014b).

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Abbreviations: $1\alpha,25(OH)_2D_3$, calcitriol (1α,25-dihydroxyvitamin D_3); 20-OH D_3, 20-hydroxyvitamin D_3; 25-OH D_3, calcifediol (25-hydroxyvitamin D_3); 7-DHC, 7-dehydrocholesterol (provitamin D_3, cholesta-5,7-dien-3β-ol); AD, atopic dermatitis; AMPs, antimicrobial peptides; BAK, Bcl-2 homologous antagonist/killer, a pro-apoptotic member of the Bcl-2 gene family; BAX, Bcl-2-associated X protein, a pro-apoptotic member of the Bcl-2 gene family; BCC, basal cell carcinoma; CaR, calcium receptor; CDKN1A, cyclin-dependent kinase inhibitor 1A; CMM, cutaneous malignant melanoma; CYP24 or 24OHase, 24-hydroxylase; CYP27A1 or 25OHase, 25-hydroxylase; CYP27B1 or 1αOHase, 1α-hydroxylase; CYP450scc, cytochrome P450scc, also known as CYP11A1; DBP, vitamin D-binding protein; DRIP/mediator-vitamin D receptor interacting protein; GADD45A, growth arrest and DNA-damage-inducible protein, alpha; IFN, interferon; IL, interleukin; JLS, juvenile localized scleroderma; MARRS receptor, membrane-associated rapid response to steroid binding protein (other names: ERp57, GRp58, Pdia3); MED, minimal erythemal dose; NFkappaB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDIA3, protein disulfide-isomerase A3; PKC, protein kinase C; PLC, phospholipases C; PUVA, psoralen and ultraviolet A radiation; SCC, squamous cell carcinoma; SNP, single-nucleotide polymorphism; SRC, *steroid* receptor coactivator; SSc, systemic sclerosis; TNFα, tumour necrosis factor; UVA/B, ultraviolet radiation A and B; VDR, vitamin D receptor; VDRE, vitamin D response elements

$1,25(OH)_2D_3$, a hormonal form of vitamin D_3, may elicit rapid responses, which rely on nuclear receptor — VDR. Upon binding of $1,25(OH)_2D_3$, VDR with its co-receptor RXR form a powerful transcription factor, that regulates expression of more than 3000 target genes in human genome (Haussler *et al*., 2011). On the other hand, $1,25(OH)_2D_3$ can bind to ER membrane-bound protein — PDIA3. As a result, multiple signal transduction pathways are activated, leading to changes in intracellular calcium concentration (Haussler *et al*., 2011; Nemere *et al*., 2012).

Beyond the beneficial role of vitamin D_3 in the regulation of calcium homeostasis, it is already known, that the potential of activated $1,25(OH)_2D_3$ is much more diverse. The VDR is present not only in enterocytes or osteoblasts, but in multiple immune cells, cells of parathyroid gland, keratinocytes and ovarian cells as well (DeLuca, 2004). It should be also emphasized, that vitamin D_3 supplementation improves muscle performance and reduces falls in vitamin D-deficient older adults (Garcia *et al*., 2011). Heath and Elovic reported, that nearly 90% of patients, who suffered from musculoskeletal pain, were vitamin D_3 deficient (Heath & Elovic, 2006). It is already suggested that vitamin D_3 might have an anabolic effect on skeletal muscles (Okuno *et al*., 2012). Furthermore, the growing body of evidence suggests also neuroprotective role of vitamin D_3. A few years ago Eyles and coworkers reported, that vitamin D receptor and 25(OH) D_3-1α-hydroxylase (CYP27B1), are both present in the brain, mainly in the hypothalamus and the dopaminergic neurons of the substantia nigra (Eyles *et al*., 2005). It was also shown, that higher concentration of vitamin D_3 in the bloodstream is associated with better performance on neuropsychiatric tests in the non-demented subset of patients suffering from Parkinson's disease, especially concerning verbal fluency and verbal memory (Peterson *et al*., 2013). Recent study also suggests potential use of high-doses of vitamin D in prevention and treatment of preeclampsia in pregnancy, broadening the spectrum of its medical uses (Zabul *et al*., 2015).

Although the beneficial effect of vitamin D on human health is already well established, vitamin D deficiencies have become a global problem (Holick, 2006; Hosseinnezhad & Holick, 2013). Recent studies conducted in Poland also underlined this issue (Kmieć *et al*., 2014; Pludowski *et al*., 2014, Kmieć *et al*., 2015). Furthermore, seasonal changes in vitamin D level that were observed, pointed out the necessity of its proper supplementation, especially in the winter season (Pludowski *et al*., 2013, Kmieć *et al*., 2015).

The active form of vitamin D_3 — calcitriol, was also shown to play an important role in cancer prevention. An antitumor activity of calcitriol is reflected in the inhibition of growth, induction of differentiation and apoptosis of cancer cells (Ylikomi *et al*., 2002; Deeb *et al*., 2007; Szyszka *et al*., 2012). Inhibition of growth due to vitamin D or its new analogues was reported for many cancer cell lines (Yuan *et al*., 2012; Chiang *et al*., 2013; Guo *et al*., 2013; Lundqvist *et al*., 2014; Thill *et al*., 2015; Wierzbicka *et al*., 2015). Indeed, the inverse association between the concentration of 25(OH)D in the circulating serum and total cancer incidence and mortality was recently described (Yin *et al*, 2013), emphasizing the vital role of vitamin D in cancer prophylaxis.

Skin production is the efficient and natural way to compensate deficiency of vitamin D in the body (Holick, 2007). Having in mind global precaution of the sun overexposure, as a known factor for the melanoma development, alternative sources of vitamin D are usually considered. Vitamin D may be introduced in diet, but only limited forms of food naturally contain it. The list is relatively short and includes fatty fish, cod liver oil and some mushrooms (Holick, 2011). Therefore in some countries, for instance in Great Britain or United States, due to the appreciation of vitamin D pleiotropic benefits for human health, a fortification of food with vitamin D is a strategy rather than an exception (Wacker & Holick, 2013). Unfortunately, this is still not a case in Poland and other countries, where proper food fortification with vitamin D in uncommon (Wierzbicka *et al*., 2014). In Poland, however, unequivocal and significantly extended legal regulations in this field are needed. At the moment, existing national legislations concerning public health issue require vitamin D fortification of margarine, butter and oils. Companies practice also fortification of milk and dairy products.

Human skin seems to be inseparably connected with vitamin D by being its source and a target as well. Here we are presenting a comprehensive overview of current knowledge concerning the impact of vitamin D and its analogues on skin physiology and pathology with potential clinical implications.

VITAMIN D IN SKIN PHYSIOLOGY

Differentiation of keratinocytes

Keratinocytes forming epidermal layer of the skin are highly specialized cells designated to protect the organism from the environmental factors. In order to accomplish this goal keratinocytes produce keratin intermediate filaments and secrete elements of cornified envelope (Eckert & Rorke, 1989). The epidermis is composed of keratinocytes arranged in four layers, which represent various stages of differentiation. The innermost *stratum basale,* which rests on the basal lamina, is composed of highly proliferating cells as well as epidermal progenitor cells that are designed to provide constituents for upper differentiating layers. These basal cells are interconnected by an extensive, intracellular network of keratin filaments, mainly keratins K5 and K14 (Eichner *et al*., 1986). The cells of the spinous layer (*stratum spinosum*), which is situated above the basal layer, initiate the production of keratins K1 and K10, considered as indicators of more differentiated layers of the epidermis (Moll *et al*., 1982). Moreover, synthesis of involucrin (Warhol *et al*., 1985), which is one of the cornified envelope precursors, and the transglutaminases (Thacher & Rice, 1985; Eckert *et al*., 2014), responsible for cross-linking of these precursors, also begins in the *stratum spinosum*. The process of keratinocytes' differentiation escalates in the granular layer (*stratum granulosum*). The structural changes of the cells in this particular layer involve accumulation of specific keratohyalin granules in the cytoplasm, filled with loricrin, involucrin and profilaggrin. The latter is the precursor of filaggrin, which is believed to be involved in the process of keratin filaments aggregation (Dale *et al*., 1985). What is more, the cytoplasm of granular cells is enriched in lamellar bodies, glycolipids-filled structures, which contribute to the formation of water-impermeable barrier (Elias *et al*., 1988). Simultaneously, the nuclei of the cells undergo atrophy and eventually newly formed superficial cornified layer (*stratum corneum*) is composed of tightly packed, akaryotic cells that provide highly insoluble barrier against water loss and invasion of pathogens as well (Hennings & Holbrook, 1983).

Skin as a target of vitamin D

Vitamin D is produced endogenously in the skin. It was also demonstrated that keratinocytes produce abundant quantities of $1\alpha,25(OH)_2D_3$ from 25-OH D_3 under the regulation of exogenous $1\alpha,25(OH)_2D_3$. Importantly, biologically active vitamin D_3 production varies with the degree of the keratinocytes differentiation (Pillai *et al.*, 1988). What is more, since keratinocytes express VDR, they are therefore able to respond to the fully active form of vitamin D_3 — $1\alpha,25(OH)_2D_3$, which, together with calcium, is one of the most potent regulators of the epidermal differentiation (Bikle, 2004). *In vivo* calcium forms gradient in the epidermis (Fig. 1), with the lowest concentration in *stratum basale* and the highest in the *stratum granulosum* (Menon *et al.*, 1985). $1,25(OH)_2D_3$ increases the expression of involucrin, transglutaminase, loricrin and filaggrin and potentiates calcium-induced differentiation of the keratinocytes at the level of both gene expression and mRNA stability (Su *et al.*, 1994). It also enhances formation of the cornified envelope (Bikle & Pillai, 1993). This phenomenon occurs, at least partially, due to the ability of the hormonal form of vitamin D_3 to increase intracellular calcium levels via induction of the calcium receptor (CaR) and phospholipase C (PLC) (Pillai *et al.*, 1995; Ratnam *et al.*, 1999). On the other hand, $1\alpha,25(OH)_2D_3$ inhibits proliferation of keratinocytes (Bikle, 2011). During the differentiation process of epidermal cells specific genes are sequentially turned on and off, due to the collective work of $1\alpha,25(OH)_2D_3$ and calcium, to fulfil the inherent specialization of keratinocytes (Bikle, 2004). For instance, keratinocytes of the basal layer express cytokeratins 5 and 14, which are replaced by cytokeratins 1, 10 and involucrin in the spinous layer (Bikle, 2004). Two distinct coactivators are involved in VDR transactivation during keratinocytes differentiation: interacting proteins (DRIP/mediator) and the p160 steroid receptor coactivator family (SRC/p160) (Bikle *et al.*, 2003; Bikle *et al.*, 2004). Interestingly, in proliferating keratinocytes, the VDR binds selectively to the DRIP/mediator complex of coactivators. During differentiation DRIP's expression is decreasing and VDR switches partners in favour of SRC/p160 (Fig. 1). Both of discussed coactivators effectively potentiate vitamin D-induced transcription in proliferating cells (Oda *et al.*, 2004). Knockdown of DRIP205 in epithelium causes increased keratinocytes proliferation (Oda, *et al.*, 2007) and disturbs expression of keratins 1, 10, and involucrin (Hawker, *et al.*, 2007). On the contrary, SRC3 knockdown decreases the production of glucosylceramide and the lamellar body formation (Oda, *et al.*, 2009). These studies indicated, that both coactivators are differentially involved in vitamin D-induced differentiation of epidermal keratinocytes — SRC in regulation of terminal differentiation, whereas DRIP in regulation of proliferation and early keratinocytes differentiation. Interestingly, it was also shown, that apart from coactivators, liganded VDR interacts reciprocally with β-catenin, which itself promotes proliferation rather than differentiation. This interaction results in enhanced expression of VDR-stimulated genes involved in the differentiation process, while genes involved in proliferation stimulated solely by β-catenin are repressed (Hu *et al.*, 2014).

Another aspect of keratinocytes growth and differentiation is the formation of cell-cell junctions, which are essential for intercellular communication and therefore for regulation of epithelial morphogenesis, growth, and differentiation (Klymkowsky & Parr, 1995). Now it is well known, that any impairment of intracellular junctions is closely associated with carcinogenesis, tumour progression, and metastasis (Frixen *et al.*, 1991; Mbalaviele *et al.*, 1996). Expression of E-cadherin and β-catenin (proteins forming intracellular junctions) is also decreased in skin malignant tumours, including basal cell carcinoma, squamous cell carcinoma and melanoma (Fuller *et al.*, 1996; Seline *et al.*, 1996; Takayama *et al.*, 1996). One of the studies revealed, that 4-day incubation of human keratinocytes with $1\alpha,25(OH)_2D_3$ caused the assembly of adherens junctions, but not of desmosomes. The same study demonstrated that $1\alpha,25(OH)_2D_3$ may induce formation of intracellular junctions by protein kinase C (PKC) activation (Gniadecki *et al.*, 1997). Thus, it is speculated that $1\alpha,25(OH)_2D_3$-induction of cell–cell junctions formation may be a novel, promising mechanism of the anti-neoplastic and anti-proliferative cancer treatment.

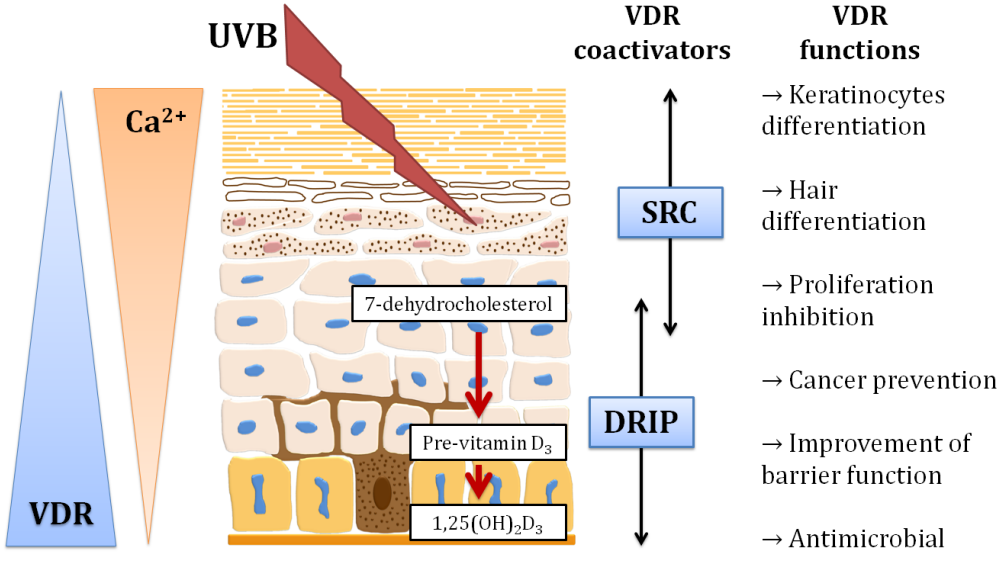

Figure 1. Regulation of keratinocytes functions, proliferation and differentiation by calcium and VDR with its co-activators .

UV LIGHT — FRIEND OF FOE

It is well established that sunlight (mainly ultraviolet type B (UVB)) is required for the efficient production of vitamin D. Paradoxically, the same solar radiation is considered as one of the most harmful factors for the skin. The UVB (280–320 nm) causes direct DNA and cell damage, thus contributes to the development of skin neoplasia. On the other hand, the UVA (320–400 nm) is mainly responsible for the skin aging. Thus, for the last decades physicians and scientists are warning against the potential danger concerning sunbathing (O'Leary *et al.*, 2014). As a result, people all over the world avoid the sun, but meanwhile we are facing the global vitamin D deficiency (Ben-Shoshan, 2012), with noticeable outburst of rickets (Misra *et al.*, 2008). Surprisingly, the number of new cases of melanoma and none melanoma skin cancer have constantly increased over the last years. The latter could be attributed to better diagnostic methodology, but it seems also, that simply avoiding the sun is not the solution. Moreover, it is well established, that melanoma may develop in the unexposed area of the skin, such as soles and palms which is observed especially in individuals with dark complexion (Bataille, 2013). Recent studies also suggested that occupational exposure to the solar radiation is actually a protecting factor (Field *et al.*, 2013), but one also has to take under consideration, that the history of sunburns dramatically increases the probability of melanoma (Cust *et al.*, 2011). It has to be underlined, that optimal skin photoproduction of vitamin D does not require extensive sunbathing. As little as approximately 15 minutes long exposure of arms and legs in a sunny day (0.25–0.50 minimal erythemal dose (MED)) is sufficient to generate equivalent of about 2000–4000 IU of vitamin D (Pludowski *et al.*, 2013). Moreover, it was estimated, that exposure to 1 MED results in production of around 20000 units of vitamin D (Holick, 2004). Nevertheless, it is recommended to expose the skin to the sun, but without exceeding 1 MED, or just simply provide an equivalent of vitamin D by supplementation. The detailed recommendations concerning the supplementation for different groups were published recently (Pludowski *et al.*, 2013).

Interestingly, it is not possible to overdose vitamin D by sunbathing, because the excessive exposition to UV light leads to structural rearrangements of vitamin D and its subsequent photodegradation. The main products are 5,6-transvitamin D_3, and suprasterols I and II (Webb *et al.*, 1989). Moreover, irradiation of 7-DHC and its analogues, called 5,7-dienes, may result in formation of 5,7,9(11)-trienes, which were described as a photosensitizing agents, thought to be responsible for generation of reactive oxygen species (Chignell *et al.*, 2006; Zmijewski *et al.*, 2009). It is still unknown, whether vitamin D has any anti-oxidative or pro-oxidative properties, because several groups presented contradictory results and concepts. For instance, recent studies showed protective effects of $1\alpha,25(OH)_2D_3$ and its analogues against UVB-induced DNA damage (Gordon-Thomson *et al.*, 2012; Slominski *et al.,* 2015b), while previously described results suggested otherwise. It seems that several factors have to be taken under consideration. First, one has to differentiate between the effects of $1\alpha,25(OH)_2D_3$ on cellular homeostasis and the process of vitamin D production. The best studied effects of $1\alpha,25(OH)_2D_3$ require VDR activation and lead to alteration of gene expression, including several genes involved in reactive oxygen response and DNA repair (Moukayed & Grant, 2013). Vitamin D is also considered as a stimulator of melanogenesis, thus contributing to the skin protection against UV irradiation (see for discussion: Szyszka *et al.*, 2012). Meanwhile, the photolysis of 7-DHC provides surprisingly large variety of by-products and alternative routes, including formation of 5,7,9(11)-trienes and oxidized derivatives (for instance as reported recently for short-side chain analogues of 7-DHC) (Zmijewski *et al.*, 2009; Zmijewski *et al.*, 2011; Slominski *et al.*, 2013c–d). Although it requires further investigation, all of those compounds may not only possess similar activity to vitamin D, but also unique properties, including direct interaction with reactive oxygen species generated as a result of UVB irradiation. Secondly, genomic and non-genomic effects has to be distinguished (see our recent review for discussion: Wierzbicka *et al.*, 2014). Finally, the effect of vitamin D might be strongly dependent on cell type and

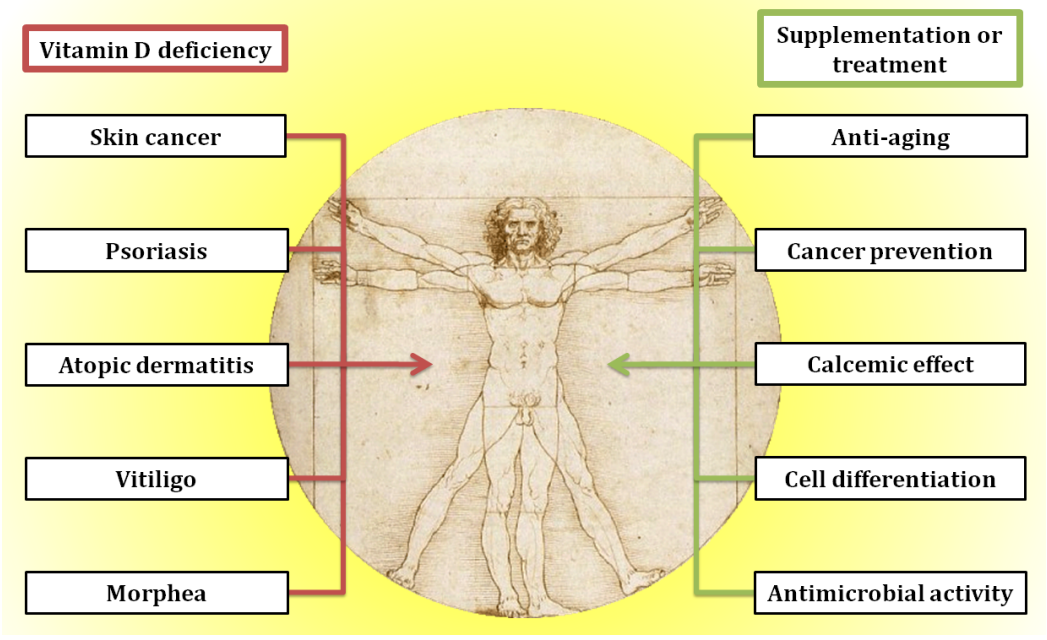

Figure 2. Effect of vitamin D deficiency on development of skin diseases and potential advantages of proper supplementation.

modulated by internal and external factors, pathological conditions or genetic background. Thus, it seems that in order to understand the complexity of vitamin D function in the human skin, we have to go far beyond classical pathways and structures, especially in relation to solar radiation (Slominski *et al.*, 2013c, d; Slominski *et al.*, 2014a; Slominski *et al.*, 2014b). The possible interactions between UV radiation, skin carcinogenesis and vitamin D are summarised in Fig. 2.

UV light is not only the main factor in skin carcinogenesis, but also strongly contributes to skin aging, which could be defined as a slow process of flattening of both epidermis and dermis which results in deterioration of skin elasticity and its barrier function. It is caused by the decrease in the number of and the increase of heterogeneity of keratinocytes and melanocytes as well as by decreased mitotic activity, migration and differentiation of keratinocytes. Skin aging is associated with decline in DNA repair capability, mitochondrial dysfunction, destabilization of extracellular matrix, including disintegration of dermal collagen and elastic fibers, and overall deregulation of cellular metabolism. Interestingly, all of those could be associated with hormonal dysfunction (Zouboulis & Makrantonaki, 2011). It has to be stressed out that the vitamin D production capacity of the skin decreases with age, thus the supplementation in the elderly is especially recommended (Zouboulis & Makrantonaki, 2011). Several factors are considered to explain this phenomenon besides limited sun exposure and insufficient dietary supplementation. For instance, the decreased level of vitamin D precursor — 7-DHC was observed in elderly people, which also coincides with lower level of previtamin D in the skin. Thus, elderly population is more prone to vitamin D deficiency (Zouboulis & Makrantonaki, 2011).

Summarizing, sun is a friend, but it has to be used wisely. Its beneficial effects, exemplified by epidermal vitamin D production, may be overcome by melanoma risk, especially in individuals with very light complexion, freckles and red hair, which, among others, are very strong contraindications for direct solar exposure.

VITAMIN D IN SKIN PATHOLOGY

Skin cancer

It has to be underlined that the incidence of various types of cancer is constantly increasing. According to World Health Organisation one in every three cancers diagnosed is a skin cancer (Ferlay *et al.*, 2015) and in the white population melanoma and non-melanoma skin cancer are now the most common types of cancer (Leiter *et al.*, 2014). Skin cancers, depending on their source, can be divided into two groups: melanomas and non-melanoma skin cancer. Less than 5% of all cases of skin cancer is diagnosed as cutaneous malignant melanoma (CMM), but it is characterized by the highest mortality (according to American Cancer Society, *Skin Cancer — Melanoma*). The latter group in turn includes squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), which are the most common types of skin cancer. The classification of skin malignancies is based on the origin of the cells — BCC and SCC develop from the epidermis (basal and squamous layer, respectively), while melanoma originates from the epidermal melanocytes (Egan, 2009).

During the past years the frequency of melanoma occurrence and mortality due to this disease have increased dramatically, especially in developed countries (Bataille & de Vries, 2008). The risk of developing skin cancer is strongly influenced by UV radiation and skin colour (Narayanan *et al.*, 2010). Approximately, 35–45% of all diagnosed neoplasms in Caucasians are skin cancers, while their occurrence in other ethnic groups is relatively low: Hispanics (4–5%), Asians (2–4%) and Blacks (1–2%) (Bradford, 2009). The incidence of melanoma is also associated with latitude — the closer to the equator, the higher is the risk (Berwick *et al.*, 2005). There are numerous epidemiological studies that describe the effect of UV radiation on the skin cancers (Linos *et al.*, 2009). It was also estimated that UV radiation is responsible for about 65% of melanoma cases and 90% of all skin cancers (Armstrong & Kricker, 1993), but for melanoma the relationship is more complex and less direct

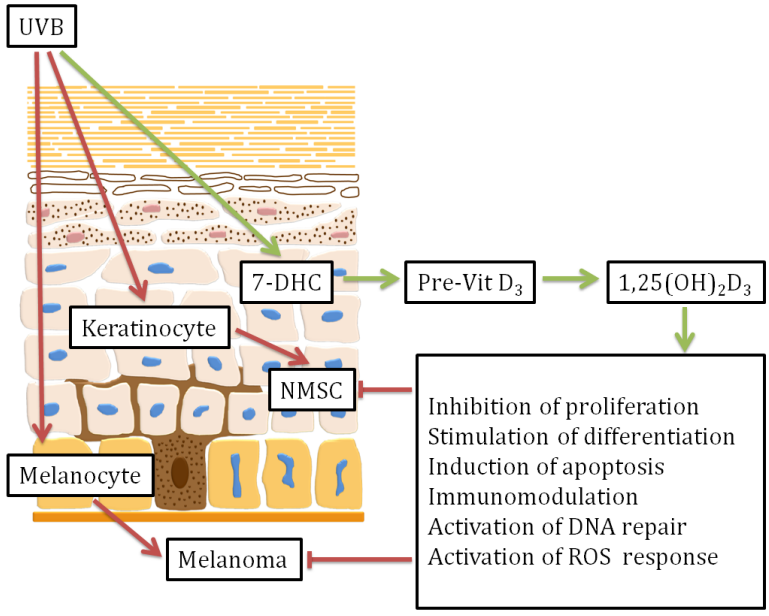

Figure 3. Vitamin D and its metabolites show an inhibitory effects on non-melanoma and melanoma skin cancer.

than for non-melanoma skin cancer. The BCC and SCC are proved to arise from skin chronically exposed to sunlight, while melanoma can develop in regions of the body (palms, armpits and soles) with very limited contact with UV radiation (Bataille & de Vries, 2008; Egan, 2009), or even in internal organs such as oesophagus, trachea, bronchial tree, cervix, colon, urinary tract and central nervous system (Cummins *et al*., 2006; Brozyna *et al*., 2007).

Having in mind antiproliferative and immunosuppressive activity of vitamin D and its analogues, their use in treatment of several dermatopathies is currently under investigation (Fig. 3).

Melanoma

So far the most effective treatment for melanoma is surgical removal of the neoplastic lesions (Garbe *et al*., 2010). Therefore, early detection of melanoma in patient increases the chances for successful curing of this disease. The significant mortality is caused by the ineffectiveness of standard treatment methods, particularly during the stage of metastasis (Dummer *et al*., 2008). Notable advance in the treatment of this type of cancer was the introduction of targeted therapy with inhibitors for tyrosine kinase receptor KIT (Carvajal *et al*., 2011). Recent developments, including BRAF-inhibitors, as well as drugs targeting the MAPK pathway, bring new perspectives to the field, especially when used as a combined immunotherapy (Aris & Barrio, 2015). Nevertheless, seeking for new drugs is still required, since during the treatment many tumours acquire resistance to the therapies.

Studies conducted in the 70's showed, that vitamin D can stimulate the activity of tyrosinase, the principal enzyme involved in melanin synthesis in cultured melanoma cell line (Oikawa & Nakayasu, 1974). Following studies confirmed the presence of the vitamin D receptor (VDR) in the primary melanoma tissue (Colston *et al*., 1981). Having in mind its antiproliferative properties, vitamin D and its analogues are potential candidates for melanoma treatment. In fact, it was shown that the metabolites of vitamin D inhibit proliferation and induce differentiation of melanoma cells expressing VDR (recent reviews: Szyszka *et al*., 2012; Slominski *et al*., 2014; Burns *et al*., 2015). Interestingly, it seems that not transformed melanocytes and keratinocytes are protected by $1,25(OH)_2D_3$ and its analogues (Slominski *et al*., 2012). There is also evidence, that $1\alpha,25(OH)_2D_3$ promotes survival of the cells and inhibits the tumour invasion and angiogenesis (Osborne & Hutchinson, 2002; De Haes *et al*., 2005). In the Gupta's study, keratinocytes after the treatment with $1\alpha,25(OH)_2D_3$ and exposure to UV radiation were characterized by the increased survival and reduced amount of DNA damage (Gupta *et al*., 2007). Similar protective effect was observed in melanoma cells. Furthermore, VDR was shown to take part in activation of DNA repair mechanism. Thus, it seems that vitamin D is an important, physiologically relevant factor preventing UV-induced carcinogenesis (Reichrath & Rass, 2014). It is not surprising, that a number of recent studies pointed out that the development of melanoma can be linked with vitamin D deficiency or defects in vitamin D signalling pathway (Reichrath & Nurnberg, 2009; Pinczewski & Slominski, 2010; Newton-Bishop *et al*., 2011). In humans, VDR gene has multiple splicing variants, which are likely to affect protein expression and activity of the VDR. In addition, over 1 000 polymorphic sites have been detected in VDR gene, some of which have been correlated with increased risk of melanoma occurrence, its aggressiveness and prognosis (Gapska *et al*., 2009; Randerson-Moor *et al*., 2009), as well as with other pathologies (Malodobra-Mazur *et al*., 2012).

The best studied SNPs linked with melanoma development are: rs10735810 (T > A nucleotide change in the restriction site FokI), rs1544410 (G > A in the BsmI) and rs731236 (T > C TaqI). For instance, FokI f and BsmI b alleles have been identified as high-risk alleles (Hutchinson *et al*., 2000; Li *et al*., 2007), while TaqI t allele as a protective one (Li *et al*., 2007). In addition, rs4516035 allele (change A > G in the EcoRV restriction site) has been identified as an exclusive risk allele for melanoma (Halsall *et al*., 2009). Similar conclusions were drawn about the influence of defects in the vitamin D signalling pathway on the development and progression of melanoma, mainly of the decrease in vitamin D receptor expression (Pinczewski & Slominski, 2010; Brozyna *et al*., 2011). It should be stressed that the analysis of VDR expression and polymorphism may serve as important prognostic factor for melanoma treatment with vitamin D and its analogues (see discussion in: Szyszka *et al*., 2012). What is more, recent studies proved that melanogenesis reduces the overall survival and disease-free survival in patients with melanoma at III and IV stage (Brozyna *et al*., 2013). In the light of these studies, it is also important to draw the attention to two other reports. The first of them demonstrated that reduced level or absence of VDR is associated with melanoma progression (melanogenesis can suppress the expression of the receptor), resulting in deteriorated survival of melanoma patients (Brozyna *et al*., 2011). Subsequent study showed that the expression of CYP27B1 was inversely proportional to the level of melanin in the melanoma cells in cultures, both *in vivo* and *in vitro*. Thus, reduced level of the enzyme involved in the synthesis of the active form of vitamin D (CYP27B1), correlates with melanoma phenotype, but has no effect on the survival of patients (Brozyna *et al*., 2013). Recent studies also provided evidence that vitamin D and its analogues may be effective in melanoma treatment (Wasiewicz *et al.,* 2015), especially recently tested low-calcemic analogues such as 20-OH D_3 (Slominski *et al*., 2012) or analogues with pregnenolone-like side chain (21-hydroxypregnacalciferol) (Zmijewski *et al*., 2009; Zmijewski *et al*., 2011; Kim *et al*., 2012; Slominski *et al*., 2012; Tang *et al*., 2013; Slominski *et al*., 2015a).

Non-melanoma skin cancers

Similarly as in case of melanomas, there are number of studies concerning the role of vitamin D in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Just like keratinocytes, BCC cells also express VDR, and furthermore, peripheral cells forming BCC tumours show even higher expression of VDR than neighbouring, unaffected epidermal cells (Kamradt *et al*., 2003; Mitschele *et al*., 2004). It was demonstrated that vitamin D suppresses a key tumour pathway in BCCs development — hedgehog signalling pathway (Bijlsma *et al*., 2006; Tang *et al*., 2007). It was shown that VDR-knockout mice, after the exposure to a carcinogen, were more prone to BCCs skin tumours development than the wild type animals (Zinser *et al*., 2005). Another studies on mice showed that topical application of vitamin D_3 reduces BCC cell proliferation and also inhibits the hedgehog signalling pathway, both *in vitro* and *in vivo* (Tang *et al*., 2007). $1\alpha,25(OH)_2D_3$ also inhibits the growth of SCCs *in vivo* as well as *in vitro* (Hershberger *et al*., 1999). Similar studies

on animals showed that mice lacking VDR and exposed to high and prolonged doses of UVB are predisposed to SCC tumour formation (Ellison *et al.*, 2008). In addition, as in BCCs, topically applied 1α,25$(OH)_2D_3$ inhibits formation of chemically induced tumour in a dose-dependent manner (Wood *et al.*, 1983; Hershberger *et al.*, 1999). What is more, 20$(OH)D_3$, 20,22$(OH)D_3$ and 20,23$(OH)D_3$, novel vitamin D_3 analogues produced by P450scc, show pro-differentiation, anti-proliferative and anticancer properties (Zbytek *et al.*, 2008; Janjetovic *et al.*, 2009; Janjetovic *et al.*, 2010; Slominski & Zmijewski *et al.*, 2014a). Although the *in vitro* and animal studies suggested that vitamin D may prevent development of BCCs and SCCs, additional studies on humans are needed to assess the suitability of topical or oral vitamin D_3 supplementation in chemoprevention of non-melanoma skin cancers.

Psoriasis

Psoriasis is a chronic, inflammatory skin disease, affecting approximately 2% of the Western population (Burfield & Burden, 2013). The affliction in the most common form is characterized by well-demarcated red plaques, covered in scales, symmetrically affecting the extensor surfaces of the limbs and with a preference for the scalp (Slominski & Zbytek *et al.*, 2013d; Nedoszytko *et al.*, 2014; Stawczyk-Macieja *et al.*, 2015).

It has to be emphasized, however, that the development of psoriasis may go far beyond the skin, resulting in the increased risk of psoriatic arthritis, inflammatory bowel disease, cardiovascular disease, depression and metabolic syndrome (Burfield & Burden, 2013). First-line therapy in psoriasis is the topical treatment with corticosteroids and vitamin D analogues (Hsu *et al.*, 2012; Slominski & Kim *et al.*, 2013). Efficiency of the therapy is based on the fact, that keratinocytes as well as the lymphocytes infiltrating the lesions do express the vitamin D receptor (Provvedini *et al.*, 1983; Burfield & Burden, 2013).

Interestingly, the first effect of vitamin D analogues treatment of psoriatic patient was noticed accidentally, when during 1α-hydroxyvitamin D_3 therapy of osteoporosis the unexpected remission of psoriatic lesions was observed (Morimoto & Kumahara, 1985). Over the following years the oral or topical administration of 1α,25$(OH)_2D_3$ as well as the topical treatment with 1,24$(OH)_2D_3$ were used, showing a significant improvement of the skin status in 70–80% of patients. What is more, the complete disappearance of any signs of psoriasis was noticed in 20–25% of suffering patients (Nagpal *et al.*, 2001). The topical administration of calcipotriol, another vitamin D analogue, is also practiced in psoriasis (Karthaus *et al.*, 2014). It has to be emphasized, however, that the treatment of the disease depends on its severity and location. When topical therapies fail or especially when psoriasis is extensive, the phototherapy should be managed. The best studied combination of psoralen and ultraviolet A radiation (PUVA) treatment has been used for decades to treat severe psoriasis, allowing patients to resume normal life activities. Unfortunately, PUVA was shown to increase the risk of skin cancer. Since then, the phototherapy using narrow-band UVB is the preferred option, highly effective at clearing psoriasis plaques (Burfield & Burden, 2013). Interestingly, such treatment has to stimulate epidermal production of vitamin D, but this phenomenon and its implication to the healing process have to be studied in details, yet.

Although the pathogenesis of psoriasis is still not fully understood, it seems that development of psoriatic plaques is mediated by Th1 cells and connected with hyperproliferation of keratinocytes. Thus, having in mind immunosuppressive and antiproliferative properties of vitamin D-like compounds, such as calcipotriol, their successful introduction in the therapy of psoriasis is fully justified (Brown & Slatopolsky, 2008). Deregulation of the keratinocytes differentiation process observed in psoriasis also prevents the formation of the functional impermeable epidermal barrier. The application of 1α,25$(OH)_2D_3$ induces, among others, expression of involucrin and transglutaminase and therefore stimulates the formation of cornified envelope (Pillai & Bikle, 1991). Moreover, VDR ligands inhibit expression of pro-inflammatory cytokines produced by T lymphocytes, such as IL-2, IFN-γ, IL-6 and IL-8, which are responsible for the exacerbation of the skin inflammation (Manolagas *et al.*, 1985). Apart from that, 1α,25$(OH)_2D_3$ enhances expression of anti-inflammatory cytokine, IL-10, within the psoriatic lesions (Kang *et al.*, 1998), as well as the expression of its receptor in keratinocytes (Michel *et al.*, 1997). It seems that antigen presenting cells and dendritic cells in particular, also remain under modulatory influence of 1α,25$(OH)_2D_3$ and its analogues, as they inhibit differentiation, maturation, activation and the survival of these cells. Consequently, biological activity of vitamin D_3 analogues leads also to suppression of the T cell-mediated immune response (Penna & Adorini, 2000). Furthermore, it was also shown that calcitriol as well as novel vitamin D_3 derivatives, 20$(OH)D_3$ and 20,23$(OH)_2D_3$, produced by Cyp450scc, inhibit the transcriptional activity of NFkappaB, a major inducer of inflammation, and therefore they may be effective therapeutic agents for inflammatory and hyperproliferative skin disorders (Janjetovic *et al.*, 2009; Janjetovic *et al.*, 2010). Finally, it seems that epidermal production of vitamin D may be at least partially impaired in psoriatic skin, which may contribute to the aggravation of symptoms. Recently, several single nucleotide polymorphisms (SNPs) in VDR and CYP24A1 genes were found to correlate with occurrence and severity of psoriasis, although it seems that effect of SNPs may be limited to the specific ethnic groups, as it was suggested recently (Kostner *et al.*, 2009).

Atopic dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory disease of the skin, clinically represented by pruritus and eczematous plaques. Furthermore, its occurrence not infrequently is a prelude to asthma or other allergic disorders (Boguniewicz & Leung, 2011; Mesquita Kde *et al.*, 2013). Patients with advanced or persistent form of this disorder experience significant impairment of the quality of life (Beattie & Lewis-Jones, 2006). It should be emphasized that the incidence of AD has tripled since 1960, making this affliction a growing problem in developing as well as in developed countries (Mesquita Kde *et al.*, 2013). In the United States the occurrence is up to 20% in children and up to 3% in adults (Amestejani *et al.*, 2012). Although the ethiopathogenesis of AD is very complex and therefore still not fully elucidated, it is connected to genetic predisposition, structural abnormalities in the epidermis together with changes in the immune system and its interaction with environmental factors (Nedoszytko *et al.*, 2014). It is worth pointing out that the incidence of the disease varies not only between countries, but surprisingly even between regions in the same country, showing the noticeable impact of factors, such as sun exposure or dietary habits, on manifestation

of AD (Mesquita Kde *et al*., 2013). In addition, patients suffering from AD are prone to colonization by microorganisms, especially *Staphylococcus aureus* and *Herpes simplex virus* (Mesquita Kde *et al*., 2013). It is estimated, that *S. aureus* in particular, is found in as many as 90% of patients with AD, leading to exacerbated, persistent skin inflammation. What is more, this pathogen can colonize healthy appearing skin in AD patients (Cho *et al*., 2001). It was shown, that vitamin D deficiency is a risk factor of methicillin-resistant *S. aureus* infection (Matheson *et al*., 2010). Vitamin D is also recognized to be involved in the regulation of cutaneous production of antimicrobial peptides (AMPs) in keratinocytes (Searing & Leung, 2010), which constitute the crucial component of the innate immune system for the defence against invading microorganisms (Gschwandtner *et al*., 2014). It was already shown, that after oral supplementation with 4000 IU/d vitamin D for 21 days, lesional skin from AD patients showed a statistically significant increase in an expression of classic AMP — cathelicidin (Hata *et al*., 2008). It is not surprising, because the cathelicidin promoter possesses putative vitamin D response element (Wang *et al*., 2004). Similar positive effect of vitamin D supplementation versus placebo was observed in AD children (Sidbury *et al*., 2008) and adults (age 14 and older) (Amestejani *et al*., 2012). In fact, the inverse relationship between the severity of atopic dermatitis and vitamin D levels was already shown (Peroni *et al*., 2011). VDR is considered in turn as a potent regulator of proinflammatory cytokines expression in keratinocytes, such as IL-6 or TNF-α. It is also involved in the inhibition of dendritic cells maturation. Polymorphism of VDR gene may therefore result in diminished responsiveness to vitamin D in inflammatory conditions (Heine *et al*., 2013). As it was stated previously, in addition to AMPs expression, vitamin D also improves epidermal barrier function by modulation of the filaggrin and involucrin expression. Thus, proper vitamin D supplementation results in the improvement of AD patients skin status and subsequently in alleviation of the inflammatory cascade (Mutgi & Koo, 2013). This statement is supported by the observation that specific VDR haplotype is more frequent in patients with severe AD indeed (Heine *et al*., 2013). These encouraging results point towards avoiding, at least, vitamin D hypovitaminosis, which constitutes a risk factor for infections and chronic disorders.

Vitiligo

Vitiligo is an autoimmune pigmentary disorder, characterized by the destruction of melanocytes, cells responsible for pigment production, and clinically described by smooth, white patches within normally pigmented skin (Abu Tahir *et al*., 2010). The designation 'vitiligo' presumably comes from Latin word 'Vitilus', that denotes 'calf', and was introduced in the first century A.D. by Roman physician, Celsus (Abu Tahir *et al*., 2010). The disorder tends to be associated with systemic autoimmune conditions, such as hypo- and hyperthyroidism (Niepomniszcze & Amad, 2001), type I diabetes mellitus, pernicious anaemia, rheumatoid arthritis and systemic lupus erythematosus (Sehgal *et al*., 1976; Montes *et al*., 2003; Adorini & Penna, 2008). Connection between vitamin D deficiency and some autoimmunological diseases was already reported, indicating this condition as an environmental trigger for the induction of autoimmunity (Saleh *et al*., 2013). It was shown recently, that serum concentration of 25-OH D_3 was significantly lower in patients with vitiligo than in the healthy controls ($p < 0.001$). What is more, 70.5% of the patients with vitiligo were vitamin D deficient (25-OH D_3<20ng.mL) (Aksu Cerman *et al*., 2014). Therefore, apart from topical treatment with steroids, which are still first-line of therapy in most cases (Abu Tahir *et al*., 2010), combination of PUVA (psoralen-UVA therapy) and vitamin D analogue calcipotriol is highly effective (Ameen *et al*., 2001). Vitamin D analogues as a monotherapy are not as effective as the topical corticosteroids, nevertheless their co-administration increases effectiveness of the treatment (Hossani-Madani & Halder, 2011). It was also reported that patients suffering from vitiligo, who display low levels of vitamin D, constitute a risk group for secondary forms of autoimmunity. Furthermore, insufficient vitamin D levels were associated with increasing Fitzpatrick phototype (Silverberg *et al*., 2010). Hence, monitoring of the serum levels of 25-OH D_3 among patients with vitiligo, may be considered as a screening tool for the presence of coexisting autoimmunity. Still, further investigations are required for the evaluation of vitamin D potential in vitiligo treatment.

Morphea

Scleroderma is a rare condition among children and adults. At least two typical categories of scleroderma could be distinguished: juvenile systemic sclerosis (SSc) and juvenile localized scleroderma (JLS). JLS, also referred to as 'morphea' in the literature, is characterized by inflammation and skin thickening with increased collagen deposition and sometimes also by subcutaneous tissue sclerosis (Nelson, 2001; Zulian *et al*., 2005; Zulian *et al*., 2006). Unfortunately, treatment of localized scleroderma is still very difficult and there is no accepted or proved therapeutic procedure for this disease. Initial studies showed that vitamin D may inhibit the proliferation of fibroblasts derived from skin of scleroderma patients, or even regulate the collagen gene expression (Boelsma *et al*., 1995). Yet, in clinical trials oral administration of $1,25(OH)_2D_3$ showed mixed results. In adult patients with generalized morphea after 3 to 7 months of treatment with 0.50 to 0.75 μg of $1,25(OH)_2D_3$ daily, improvement of joint mobility and skin extensibility was observed (Hulshof *et al*., 1994). However, in more recent studies oral $1,25(OH)_2D_3$ did not demonstrate effectiveness among 20 adult patients with localized scleroderma (Hulshof *et al*., 2000). On the other hand, in a study of seven children with morphea treated with oral $1,25(OH)_2D_3$, five of them showed a good to excellent reduction of their skin lesions without adverse effects. One of the patients showed an excellent response to the secondary therapy and one patient failed to respond to therapy with $1,25(OH)_2D_3$ (Elst *et al*., 1999). Other studies focused on vitamin D analogue — calcipotriol — administered topically (0.005%). After 3-months trial, all 12 patients aged 12 to 38 years with active morphea or linear scleroderma showed significant skin improvement. No adverse effects were observed (Cunningham *et al*., 1998). Recently, novel vitamin D derivatives like $20(OH)D_3$ were tested for their antifibrotic activities. Both *in vitro* and *in vivo* studies showed that they may be a potential candidates for therapeutic agents to treat diseases like scleroderma or fibrosis (Slominski *et al.,* 2011; Slominski *et al.,* 2013a). Abovementioned studies indicate, that $1,25(OH)_2D_3$ and its analogues can be effective agents for treating localized scleroderma, but further clinical trials have to be conducted.

CONCLUSIONS

There is cumulative evidence that human epidermis is not only the natural source of vitamin D, but also the main regulator of skin physiology.

Overall status of vitamin D, as measured by serum concentration of 25-hydroxyvitamin D, or alternation in vitamin D response and metabolism, have strong impact on skin physiology, thus vitamin D analogues are currently used or tested for potential therapy of multiple skin diseases. What is more, it seems that new vitamin D analogues with little or none impact on calcium level are promising candidates not only for topical treatment but for general applications in prophylaxis and treatment of multiple skin or systemic human disorders.

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