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Review

1α,25-Dihydroxyvitamin D_3; its role for homeostasis of keratinocytes

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Epidermal homeostasis is influenced by a number of hormones and regulative growth factors that are maintained by a tightly regulated balance between cell proliferation, cell differentiation, and cell death. Vitamin D is one of those regulatory factors. It has recently been demonstrated that 1α,25-dihydroxyvitamin D_3 (1α,25(OH)$_2$D$_3$) takes part in the regulation of the cell cycle by multiple and complex functions. This review discusses 1α,25(OH)$_2$D$_3$ and its analogues in connection with the renewal of epidermal keratinocytes as well as the molecular mechanisms underlying terminal differentiation or rather programmed cell death. Furthermore, interest is focused on the possible clinical application of vitamin D_3 analogues. © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:642–649, 1996.)

Keywords: vitamin D_3; keratinocyte; signal transduction; terminal differentiation; apoptosis

Introduction

Epidermal homeostasis is dependent on the fine-tuned interaction of a number of hormones and other agents which ultimately regulate the balance between cell proliferation, cell differentiation, and apoptosis. Vitamin D_3 is one of these regulatory factors. The active form 1α,25-dihydroxyvitamin D_3 (1α,25-(OH)$_2$D$_3$, calcitriol) and the synthetic vitamin D_3 analogue calcipotriol (MC903) (*Figure 1*) have been studied extensively with respect to their capacity to inhibit proliferation and induce terminal differentiation of epidermal keratinocytes. A specific receptor for 1α,25-(OH)$_2$D$_3$ (Vitamin D Receptor, VDR) has been detected in human skin[1,2] as well as in cultures of human epidermal keratinocytes.[3] VDR antigens were found to be expressed by all keratinocytes of the epidermis, except those of the stratum corneum, and by other cells of epidermal appendages, as shown by immunohistochemical studies of normal skin.[4] This has provided new concepts and raised many important questions concerning the possible physiological and pathophysiological role of vitamin D_3, especially on the physiological function in terminal differentiation or apoptosis. 1α,25-(OH)$_2$D$_3$ plays an important role in the conversion of epidermal keratinocytes from proliferation to terminal differentiation.[5,6] Investigations have suggested that 1α,25-(OH)$_2$D$_3$ regulates the expression of several cell-cycle-associated genes.[7] These new findings establish the basis for molecular biology and biochemistry to analyse how this vitamin and its analogues take part in maintaining homeostasis of the epidermal keratinocyte pool.

1α,25-(OH)$_2$D$_3$ and signal transduction

Although over 20 metabolites of vitamin D are known, 1α,25-(OH)$_2$D$_3$ is the most potent biologically active metabolite.[8] Two basic mechanisms responsible for the biological activity of 1α,25-(OH)$_2$D$_3$ are postulated: genomic responses and non-genomic responses (*Figure 2*). The genomic responses are mediated by regulating gene expression whereas the non-genomic responses are mediated by influencing intracellular signalling pathways.

It is well established that 1α,25-(OH)$_2$D$_3$ regulates gene expression via binding to the cytosolic VDR distributed in the cytosol and in the nuclear, which belongs to the nuclear steroid-hormone-receptor superfamily. The differences in the localization of extra- and intranuclear VDRs were suggested to be associated with 1α,25(OH)$_2$D$_3$ function, because its effects on VDRs were rapid. On the one hand, the

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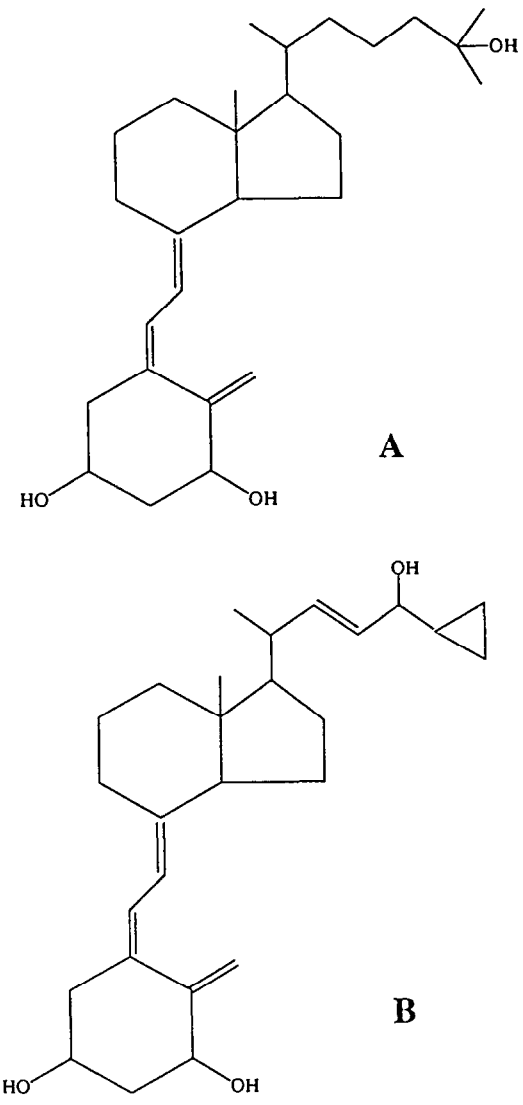

Figure 1 Chemical structure of 1α,25-$(OH)_2D_3$ (A) and the analogue, calcipotriol (B).

earliest phase of VDR change was observed to be clumping of VDRs after 1α,25$(OH)_2D_3$ addition. It was considered that this could be an anatomic counterpart for the steroid receptor homodimerization process. The next rapid step was observed to be alignment of VDR clumps along cytoplasmic fibrils, directed rapidly toward the nucleus.[9] The rapid accumulation and reorganization of VDRs in the nucleus may be an extention of previous biochemical studies showing interaction of steroid receptors with the nucleus.[10,11] Then the complex of 1α,25-$(OH)_2D_3$ and VDR modulates gene transcription by binding to specific DNA binding sites so-called VD-response-elements (VDRE). These VDREs are parts of the promotor regions of target genes of 1α,25-$(OH)_2D_3$.[12,13] On the other hand 1α,25-$(OH)_2D_3$ has been shown to increase the intracellular Ca^{++} concentration within minutes, which could not based on nuclear mechanisms like gene expression.[5,14–16] Therefore, 1α,25-$(OH)_2D_3$-induced signal *trans*-duction exerts its control by both genomic and nongenomic effects.

Very recently, in HL-60 cells, the sphingomyelin cycle was initially identified as a new cell signal pathway in response to vitamin D_3.[17] It was shown that early and reversible hydrolysis of sphingomyelin occurs through activation of a sphingomyelinase, which increases the intracellular ceramide pool.[18] Several other agonists of the sphingomyelin cycle have already been published, including tumor necrosis factor α (TNF-α), γ-interferon, dexamethasone, interleukin-1 (IL-1), nerve growth factor (NGF), complement and brefeldin A.[19,20] It was shown that activation of the sphingomyelin cycle ultimately leads to terminal differentiation and apoptosis.[21,22] Using cell-permeable ceramide analogues, it was possible to mimic the effect of several inducers of sphingomyelin hydrolysis on cell proliferation and differentiation.[23] Recently, the existence of the sphingomyelin cycle could be demonstrated in human keratinocytes and in the immortalized human keratinocyte cell line HaCaT.[24] In contrast with other agonists of sphingomyelin hydrolysis, e.g., IL-1, NGF or TNFα, which act after 10 to 15 min, 1α,25-$(OH)_2D_3$ increases the breakdown of sphingomyelin after 3 to 4 hr. This suggests that 1α,25-$(OH)_2D_3$ activates sphingomyelin hydrolysis indirectly via an autocrine mechanism. Therefore, a genomic effect of 1α,25-$(OH)_2D_3$ influencing gene expression has to be taken into consideration. This shows the tight interaction of genomic and non-genomic, cell signalling mechanisms.

A nongenomic effect of 1α,25-$(OH)_2D_3$ correlates to its influence on Ca^{++}, which is known to regulate cellular proliferation and differentiation. In vitro, a dose-dependent earlier rise of the free intracellular Ca^{++} concentration has been shown to be induced in cultured keratinocytes by 1α,25-$(OH)_2D_3$ in response to the differentiation stimulus of high intracellular Ca^{++}.[5,14,15,25] Several studies in which single human keratinocytes were observed for up to 20 min detected an immediate effect of 1α,25-$(OH)_2D_3$ or its analogues on cytosolic Ca^{++}.[16] The later significant changes observed occurred 4 h after the addition of 1α,25-$(OH)_2D_3$, but elevated Ca^{++} levels were measured for up to 3 days.[15] These periods of elevated Ca^{++} levels are timed according to the stimulus, but it occurs before any change in the expression of differentiation markers.[26] In keratinocytes, the high intracellular Ca^{++} level has been suggested as one possible second messenger signal for terminal differentiation.[25]

The activity and synthesis of Ca^{++}-dependent enzymes play an important role in apoptotic cells. Tissue type II transglutaminase, which is normally also present in other cell types, catalyzes the formation of cornified envelopes, highly insoluble rigid structures that prevent leakage of cytoplasmic content. These envelopes also serve as an apoptotic index for measuring the apoptosis rate. This enzyme is also thought to take part in membrane remodelling during apoptosis.[27,28] It has been shown that 1α,25-$(OH)_2D_3$ and its analogues influence the expression of epidermal transglutaminase at the transcriptional level, an effect apparently mediated via the VDR.[29]

Induction of epidermal transglutaminase activity and cornified-envelope formation by 12-O-tetradecanoylphorbol-13-acetate (TPA) both in vitro and in vivo[30] suggested that protein kinase C (PKC) probably plays a decisive regulatory role in keratinocyte transglutaminase (k-TG) gene

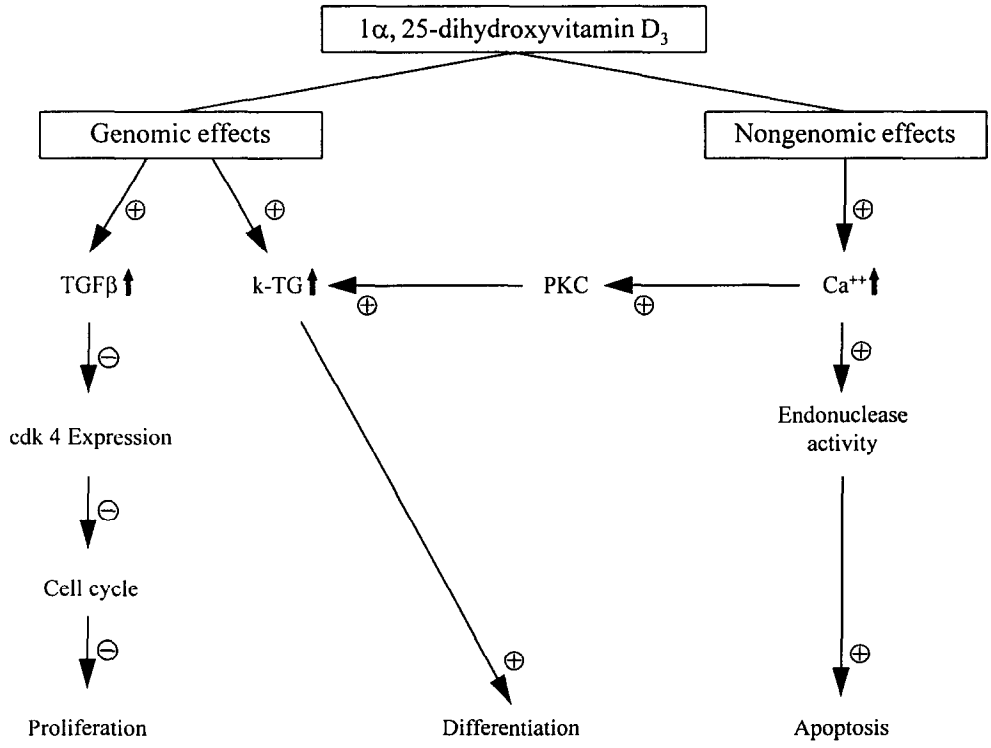

Figure 2 *Main effects of 1α,25-$(OH)_2D_3$ on cellular mechanisms leading to inhibition of cell proliferation and induction of differentiation and apoptosis.* (Abbreviations: TGFβ, transforming growth factor β; k-TG, keratinocyte transglutaminase; PKC, protein kinase C; cdk 4, cyclin-dependent kinase 4)

expression in vitro and in the terminal stage of keratinocyte differentiation.[31] These results provide evidence that late stages of epidermal differentiation are regulated through Ca^{++}-dependent activation of the PKC pathway. The observation that increased extracellular Ca^{++} was associated with elevated cellular diacylglycerol levels[25,32] represents a biochemical link coupling the Ca^{++} signal for keratinocyte differentiation to the PKC signaling pathway. Furthermore, the ability to block Ca^{++}-mediated keratinocyte transglutaminase (k-TG) gene expression by inactivating PKC in mouse[31] as well as in human[33] keratinocytes provide additional evidence that high intracellular Ca^{++} induces this differentiation marker through the PKC signaling pathway. Along with the upregulation of k-TG gene expression by PKC, the influence of other factors is likely to be important in determining the final level of expression. The elevated k-TG levels and transglutaminase activity as a secondary change[34,35] have been reported in psoriatic skin. Therefore, identification of PKC as a key regulator of k-TG gene expression suggests that selective inhibitors of this signaling pathway may be useful in treating dermatoses characterized by hyperkeratosis. Although the role of Ca^{++} in triggering keratinocyte-specific gene expression is well established, the biochemical pathways regulating this complex process have not been fully elucidated.

Transforming growth factor-β (TGF-β) is thought to affect cyclin and cyclin-dependent kinases (cdk), each of which has subtypes that regulate the cell cycle. It suppresses cdk4 expression in the G1 phase, an inhibition linked to cell-cycle arrest,[36] and inhibits the assembly of cyclin E to cdk2.[37] However, TGF-β indirectly affects phosphorylation of retinoblastoma (Rb) protein, a tumor-suppressor gene protein. In keratinocytes, TGF-β has also been reported to directly suppress phosphorylation of Rb.[38] Activity and suppression of phosphorylated Rb protein affects the expression of genes of DNA polymerase, myc, etc., that are involved in DNA synthesis. It has recently been reported that the active form of vitamin D_3 suppresses phosphorylation of Rb protein in cultured human keratinocytes comparable to the action of TGF-β.[39] A recent study has also been shown that MC903 induces the increase of dephosphorylated Rb and the decrease of phosphorylated Rb. These findings suggested that the increase of dephosphorylated Rb by 1α,25 $(OH)_2D_3$ or MC903 occurred at an early stage in G1/G0 phase.[40]

One of the strongest correlations of a specific biochemical event with apoptosis is thought to be the activation or increased synthesis of a Ca^{++}/Mg^{++}-dependent endonuclease. Endonuclease activity has been demonstrated to be involved in the induction of apoptosis in keratinocytes. The enzyme activity during apoptosis results in the production of oligonucleosome-length DNA fragments that can be resolved by agarose gel electrophoresis. In vitro, a sustained increase in the cytosolic Ca^{++} level has been shown to stimulate endonuclease activation.[41] But the supposition that increased cytosolic Ca^{++} mediates the activation of the endogenous endonuclease is problematic, inasmuch as the Ca^{++} levels of 0.1–5 mM required by the nuclease for activity levels that cannot be reached physiologically.[28] Whether the endonuclease is a cause or a consequence is still under discussion.

In conclusion, the mechanism of 1α,25-$(OH)_2D_3$ and

Ca^{++} in promoting keratinocyte differentiation remains unclear. Although in vitro 1α,25-$(OH)_2D_3$ does not increase the expression of keratin,[42] a number of investigations have suggested that, unlike retinoic acid, it promotes dose-dependent Ca^{++}-induced differentiation, as shown by transglutaminase and involucrin gene expression on mRNA levels and by cornified envelope formation.[5,12] Further studies are required to elucidate the interaction between 1α,25-$(OH)_2D_3$ and Ca^{++}-induced differentiation signals, and between 1α,25-$(OH)_2D_3$ and ceramide-induced differentiation signals.[43]

Cellular responses to 1α,25-$(OH)_2D_3$: cell proliferation, cell differentiation and apoptosis

In vitro, 1α,25-$(OH)_2D_3$ was found to regulate a dose-dependent decrease in the cell proliferation and an increase in the morphological differentiation of cultured human keratinocytes.[5,7,29] However, the receptor expression for 1α,25-$(OH)_2D_3$ differed in various tissues depending on the degree of cell differentiation. The number of receptor molecules is higher in preconfluent and confluent than in postconfluent keratinocytes.[43] The evidence for a role of 1α,25-$(OH)_2D_3$ in keratinocytes is based on the presence of receptors specific for 1α,25-$(OH)_2D_3$, and the ability of 1α,25-$(OH)_2D_3$ to inhibit proliferation and stimulate differentiation. Regnier et al. reported that 1α,25-$(OH)_2D_3$ specifically stimulated the last steps of epidermal differentiation with no effect on basal cells.[6] Exogenous 1α,25-$(OH)_2D_3$ inhibited 1-hydroxylase at all stages of keratinocyte growth.[43]

However, the skin is both a synthesis and a target organ of vitamin D_3. For a detailed review on vitamin D_3 synthesis, see Holick et al.[44] The synthesis of 1α,25-$(OH)_2D_3$ in the epidermis correlated with the early differentiation events such as expression of transglutaminase and involucrin. 1α-hydroxylase and 24-hydroxylase activity is also involved in keratinocyte differentiation, because 24,25-$(OH)_2D_3$ synthesis runs parallel with the decrease of 1α,25-$(OH)_2D_3$ and with a decrase of terminal differentiation markers, especially cornified-envelope formation. Thus, both the activity of and the responses to the enzymes regulate vitamin D_3 and lead to complete and harmonious epidermal differentiation.[29] The switch from 1α,25-$(OH)_2D_3$ to 24,25-$(OH)_2D_3$ production, coupled with the reduction in 1α,25-$(OH)_2D_3$ receptors on terminally differentiated keratinocytes, provides mechanisms to reduce the influence of 1α,25-$(OH)_2D_3$ on these cells. It is still unclear whether the increase in the conversion of 25-$(OH)D_3$ to 24,25-$(OH)_2D_3$ or its decrease in the later stages of differentiation is a mechanism for reducing the cellular 1α,25-$(OH)_2D_3$ level or a requirement for terminal differentiation.

Skin evidences three variants of cell death: necrosis, terminal differentiation, and apoptosis. The latter two are designated as programmed cell death. In the epidermis, characteristic morphological changes of programmed cell death have been demonstrated by ultrastructural,[45] histochemical,[46] and especially immunohistochemical[47,48] studies. The terms "Civatte bodies,"[49] "dyskeratotic cell,"[50] "dark cell,"[51] "sunburn cell,"[52,53] "apoptosis bodies," or "keratin bodies"[54] have now been applied for recognizing and identifying apoptosis in the epidermis. Terminal differentiation of cells is a specialized form of the apoptotic program that has evolved to serve tissue-specific functions or rather to generate the specialized differentiation products in the suprabasal layer. This finally leads to apoptosis.[27] Apoptosis is an active, genetically controlled process of cell deletion displaying characteristic morphological and biochemical features. It is unclear whether terminal differentiation and apoptosis are two entirely different phenomena of regulation not only in their morphology but also in their biochemistry. In the skin, however, it has been shown that some important regulatory mechanisms of apoptosis are also significant for epidermal keratinocyte differentiation. The two forms of programmed cell death lead to identical final functional results, i.e., corneocyte formation and keratinocyte loss by desquamation.

1α,25$(OH)_2D_3$ and regulation of gene expression

As mentioned above 1α,25-$(OH)_2D_3$ appears to be a typical steroid hormone with regulation of gene transcription through ligand binding to and activation of intracellular VDR and subsequent interaction of these complexes with specific sequences in the target gene. These target sequences (VDRE) have been identified in rats and humans.[8,12,13,55] Recently, two classes of response elements were demonstrated for vitamin D: one activated by VDR homodimers and the other by VDR/RXR, retinoid X receptor, and possibly VDR/RAR, retinoic acid receptor, heterodimers.[56] The endogenous ligand of RXRs serves as a stereoisomer of retinoic acid, 9-*cis* retinoic acid, which directly binds to and activates RXRα. RXRα greatly enhances the activity of RXRs, VDRs, and thyroid hormone receptors (THRs). Their ligands influence epidermal differentiation in the skin, *i.e.*, keratin gene expression, which differs between basal cells and differentiated keratinocytes, and are under the direct control of RARs and THRs, but not of VDR.[42] This means that VDR does not act on the transcript level. But some authors suggest that other proteins, such as transcription factors, may be involved in the interaction between VDR and VDRE.

Cytokines induced by 1α,25-$(OH)_2D_3$ at the mRNA level in keratinocytes are shown in *Table 1*. It has been shown that vitamin D_3 and its analogues are immunosup-

Table 1 Examples of genes regulated by 1α,25-$(OH)_2D_3$ at the level of mRNA accumulation in keratinocytes

Cell type	Vitamin D_3 effect on gene expression (↑/↓*)
Keratinocytes	↓ c-myc ↓ c-fos ↓ HLA-DR ↑ p53 ↓ IL-1α, IL-1β, IL-6 and IL-8 ↓ parathormon-like peptide ↑ TGF-β ↑ NGF

Adapted from Refs.[5,7]
*Expression increased/decreased.

pressors. There is no question concerning the fact that a major function of kertinocytes is to provide a physically tough, relatively impermeable barrier between the host and his environment. This is achieved by differentiation and cornification. p53, c-myc, c-fos, HLA-DR, TGF-β, IL-1, IL-6, and IL-8 gene expression modulated by 1α,25-$(OH)_2D_3$ are known to affect cell proliferation and can also stimulate apoptosis in epidermal keratinocytes. In vitro studies have shown that IL-1 is expressed when keratinocytes are proliferating in low-calcium medium, whereas mRNA of this cytokine is not detectable if keratinocytes terminally differentiate in high-calcium medium. These results can be explained by the way in which the production of IL-1 is confined to the less differentiated actively proliferating basal keratinocytes in the epidermis.[57] IL-6 has a variety of biological activities in different target cells and systems. It stimulates the proliferation of normal human keratinocytes. It is interesting to note that the high-affinity IL-6 receptor has been detected in normal human cultured keratinocytes.[58] The production of IL-6 is decreased in cultured human keratinocytes treated with 1α,25-$(OH)_2D_3$ in vitro and in lesional and unaffected psoriatic skin after topical treatment with calcipotriol in vivo.[58a] IL-8 was expressed exclusively in the upper layers of lesional psoriatic epidermis, but not in uninvolved skin from psoriatic patients or normal skin from nonpsoriatic patients, suggesting that IL-8 is an important disease-promoting cytokine in psoriasis.[59,60]

TGF-β is expressed in a wide variety of normal cells and tissues, including human keratinocytes and is regarded as a multifunctional growth regulator. It was found to inhibit lymphocyte proliferation. This means that the balance of cutaneous immunohomeostasis may involve several different keratinocyte-derived factors that may be either lymphocyte-activating like IL-1 or lymphocyte-inhibiting like TGF-β.[61] TGF-β has no effect on k-TG,[62] and it blocks the shift from the G1 to the S phase. TGF-β was also reported to enhance urokinase-type plasminogen activator (u-PA) activity in association with growth inhibition. Vitamin D_3 and MC903 can cause decreased expression of u-PA and tissue-type plasminogen activator (t-PA) activity in human keratinocytes and mouse keratinocyte cell lines. The t-PA/u-PA ratio increases with keratinocyte differentiation.[63]

It seems to be important that the pattern of expression of the same protooncogenes/tumor suppressor genes that control the cell cycle and proliferation are changed, suggesting that they play some regulatory role in the cascade of events leading to cell death. Activation of c-myc is associated with cell proliferation, whereas activation of c-fos is thought to occur in association with cellular differentiation. C-myc plays a key role in cell proliferation and cell apoptosis. There has been recent evidence that the growth inhibition of normal human keratinocytes by 1α,25-$(OH)_2D_3$ is preceded by marked inhibition of c-myc mRNA synthesis.[64] 1α,25-$(OH)_2D_3$ decreases c-fos gene expression in normal human keratinocytes. In contrast, addition of 1α,25-$(OH)_2D_3$ to epidermal-growth-factor-stimulated keratinocytes increases c-fos expression.[7] Besides protooncogenes, the tumor suppressor gene p53 is likewise expressed in normal cells and can also alter cell growth. There is evidence that the p53 gene product mediate the suppression of c-fos mRNA, possibly acting as a transcriptional regulator of the c-fos promoter.[65] The subcellular localization of p53 varies throughout the cell cycle with accumulation in the nucleus following the initial step of DNA synthesis around the beginning of the S phase and reaccumulation in the cytoplasm during the resting phase.[7] The intracellular level of p53 dramatically increases in response to a variety of DNA-damaging agents.[27]

In conclusion, the inhibition of cell growth appears to be a prerequisite for apoptosis and is perhaps initiated by either TGF-β or p53 under physiological conditions. The cells can either die rapidly by apoptosis or slowly by terminal differentiation. It is thought that the basic regulatory concept of apoptosis is active under physiological conditions and controlled by signal-transduction pathways. However, the active stimulation of apoptosis programs is supported under pathological conditions. 1α,25$(OH)_2D_3$ probably plays an important role in influencing apoptosis.

Clinical perspectives

The epidermal balance between keratinocyte proliferation and differentiation is interpreted on the basis of processes in which keratinocyte apoptosis plays an essential role. But how are the mechanisms of cell proliferation and apoptosis controlled in the epidermis? One model for a possible explanation is protooncogen bcl-2 protein is expressed only in the proliferating basal layer of normal human keratinocytes.[66,67] Cells destined either to die by apoptosis or to undergo terminal differentiation move up to suprabasal layers. Thereby the loss of bcl-2 is involved in the signals to move up. Suprabasal keratinocytes stop proliferating, possibly in response to downregulation of c-myc or synthesis of TGF-β, which is synthesized in suprabasal cells and may be a prerequisite for the apoptotic pathway.[27,68] In HL-60 cells, pretreatment with 1α,25$(OH)_2D_3$ has been shown to downregulate also the expression of the bcl-2 gene.[69] Cytosolic free Ca^{++} concentrations have revealed that overproduction of bcl-2 does not prevent rises in intracellular Ca^{++}, suggesting that bcl-2 blocks an apoptotic signal downstream of this event.[70] Furthermore, p53 may also play an important role in normal epidermal cell growth. p53 mRNA, which is rapidly induced, is a necessary mitogen for the growth of keratinocytes under serum-free conditions. 1α,25-$(OH)_2D_3$ as a signal molecule is closely connected with inducing terminal differentiation. The elucidating of such mechanisms of cell-cycle regulation and the identification of individual control points of the apoptotic pathway will provide new insights to the pathogenesis of psoriasis and other skin diseases.

Vitamin D_3 and its synthetic analogues have already undergone clinical evaluation. Calcipotriol has been studied most extensively. Compared to 1α,25-$(OH)_2D_3$, calcipotriol was about 200 times less potent in its effect on calcium metabolism, though similar in its receptor affinity. This has set the stage for the development of a new class of compounds with potential usefulness for hyperproliferative, differentiative and immune-mediated diseases as well as for various tumors. Approximately 30 new recently developed vitamin D_3 analogues possess higher antiproliferative, differentiative, and/or immunosuppressive potential compared

with vitamin D_3 and show simultaneously reduced hypercalcemic side effects. It has been demonstrated that topical calcipotriol (MC903) is both efficacious and safe for the short- and long-term treatment of psoriasis.[71,72] The pathogenesis of psoriasis partly remains unclear. But in psoriasis patients, the number of proliferating versus post-mitotic quiescent cells is largely increased because of an abnormal shortened cell cycle.[66] Psoriatic skin lesions evidence abnormal bcl-2 and transglutaminase expression[67] as well as IL-6 overproduction.[73] It is known that vitamin D_3 receptors are expressed not only in normal, but also in psoriatic skin.[1,4,74] Furthermore, calcipotriol can increase VDR expression in psoriatic lesions. Therefore, vitamin D_3 analogues act as multifunctional agents in the treatment of psoriasis.

Besides MC903, other new vitamin D_3 analogues have been developed that also play a pharmacological role. In vivo, 1α,25-dihydroxy-16-ene-23-yne-vitamin D_3 has an antineoplastic effect that increases the life span of mice with leukemia.[75] 1α,24-$(OH)_2D_3$ is comparable to 1α,25-$(OH)_2D_3$ in its effects on keratinocyte differentiation, but exerts a weaker influence on calcium-metabolism than MC903.[76] The 20-epi-vitamin D_3 analogue is a significantly more potent modulator of cell proliferation and cell differentiation than vitamin D_3 in vitro, and the vitamin D analogue KH1060 has a 14,000 times greater antiproliferative effect than vitamin D_3 and is a stronger immunosuppressive than cyclosporine.[77]

The hair follicle is suggested to be a highly hormone-sensitive organ. It was reported that 1α,25$(OH)_2D_3$ stimulated human hair follicle growth and hair fiber production in whole-organ cultures in vitro.[78] The recognized VDR expression was also demonstrated in outer root sheath keratinocytes and dermal papilla cells and suggested to hair cycle-associated changes.[79] Vitamin D-dependent rickets Type II is an autosomal recessive disease caused by target organ resistance to the action of active form 1α,25$(OH)_2D_3$. Mutations in the VDR gene are known to be associated with alopecia.[80] Although this rare syndrome of a defect in the target tissue of 1α,25$(OH)_2D_3$ has been well known, it is not still interpreted why patients with vitamin D-dependent rickets Type II do not have any significant abnormality in their skin other than alopecia. One possible explanation for the alopecia is an ectodermal defect due to absent 1α,25$(OH)_2D_3$ action during a critical stage of hair follicle development that precludes subsequent hair growth from the follicle[81] or 1α,25$(OH)_2D_3$ caused a subtle biological effect which is not absolutely essential for the development, differentiation, and growth of the skin and the hair follicle.[82]

Recently, vitamin D_3 and its analogues were shown to interact positively with retinoids in inducing cell differentiation.[83] The fact that VDR may form transcriptionally active heterodimers with RXRα and possibly RAR, and that these complexes may recognize different binding sites at the DNA level is of particular interest.[84] Therefore, an attractive hypothesis is that synergistic effects between vitamin D_3 and various retinoids occur directly at this level. The combination of vitamin D_3 analogues with retinoids open interesting therapeutic perspectives. But vitamin D_3 and retinoids also act on distinct, but complementary, sets of genes via independent pathways indicating the possibility of non-synergistic effects. Therefore, further investigations are necessary to clarify interactions between both substance classes.

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