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Mitochondrial and lipogenic effects of vitamin D in differentiating and proliferating human keratinocytes

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Abstract

Even in cells which are resistant to the differentiating effects of vitamin D, the activated vitamin D receptor (VDR) can down regulate the mitochondrial respiratory chain and sustain cell growth through enhancing the activity of biosynthetic pathways. The aim of this study was to investigate whether vitamin D is effective also in modulating mitochondria and biosynthetic metabolism of differentiating cells. We compared the effect of vitamin D on two cellular models: the primary human keratinocytes, differentiating and sensitive to the genomic action of VDR, and the human keratinocyte cell line HaCaT, characterized by a rapid growth and resistance to vitamin D. We analysed the nuclear translocation and features of VDR, the effects of vitamin D on mitochondrial transcription and the consequences on lipid biosynthetic fate. We found that the negative modulation of respiratory chain is a general mechanism of action of vitamin D, but at high doses the HaCaT cells became resistant to mitochondrial effects by upregulating the catabolic enzyme CYP24 hydroxylase. In differentiating keratinocytes vitamin D treatment promoted intracellular lipid deposition, likewise the inhibitor of respiratory chain stigmatellin, whereas in proliferating HaCaT this biosynthetic pathway was not inducible by the hormone. By linking the results on respiratory chain and lipid accumulation we conclude that vitamin D, by suppressing respiratory chain transcription in all keratinocytes, is able to support both proliferation and the specialized metabolism of differentiating cells. Through mitochondrial control vitamin D can have an essential role in all the metabolic phenotypes occurring in healthy and diseased skin.

Key words: vitamin D receptor, keratinocyte, mitochondria, respiratory chain, lipid deposition.

Introduction

Vitamin D ($1,25(OH)_2D_3$) exerts its pleiotropic effects through a genomic and non-genomic signaling mediated by its receptor VDR. In genomic modality VDR operates as a transcription factor, migrating into the nuclear compartment upon ligand binding and together with its binding partner RXR it recruits coactivators or corepressors to enhance or repress transcription. The non-genomic activity of VDR, on the other hand, occurs at plasmamembrane sites, where VDR modulates calcium fluxes (1,2). Recently our work has demonstrated a third novel mechanism of action of VDR that takes place in mitochondria (3). In fact we have shown that the receptor acts as modulator of mitochondrial activity and consequently as facilitator of the metabolic switch essential for cancer cell proliferation.

Healthy differentiated cells are responsive to the differentiating properties of vitamin D. The skin is one of the tissues most dependent on vitamin D for regular differentiation, integrity and its physiological properties as a barrier. The skin is capable of vitamin D synthesis and activation, express the VDR and responds to VDR activation by induction or repression of a multitude of genes, demonstrating therefore an autocrine/paracrine regulation by the hormone. Epidermal keratinocytes undergo a complex program of differentiation giving rise to the different cell layers and the barrier function of the skin (4,5). When the balance between the proliferating reservoir and the fully differentiated epidermal layers is perturbed, a skin disease can arise, and vitamin D has demonstrated its efficacy in restoring epidermal homeostasis (6). Investigation of permeability and activity of vitamin D analogues in human skin has been facilitated by the recent generation of a new animal model with an engineered bioluminescent readout (7).

The nuclear effects of VDR in keratinocytes have been extensively investigated, as well as the important modulation of calcium homeostasis exerted by VDR in these cells. The sequential role of VDR in regulating firstly basal cell proliferation and then the differentiated function in the upper layers of the epidermis is attributed at least in part to the different coregulators that modulate its genomic actions. The major coactivator complexes regulating VDR in the keratinocyte are

distinctly expressed during differentiation (8-10). On the contrary, several cancer cells are insensitive to the differentiating and antiproliferative action of vitamin D (11-15). We have demonstrated that in HaCaT cells resistant to vitamin D the mitochondrial effects of VDR facilitate cell growth (3). To date, nothing is known about the effects of VDR on mitochondrial activity of differentiated cells that are normally responsive to the genomic signaling of the receptor.

The aim of this work was to study the effects of vitamin D on mitochondrial transcription of differentiating cells, and to investigate the impact on cellular metabolism. In order to understand the different results of VDR activity on differentiating and proliferating skin cells, we compared the effect of vitamin D on two cellular models: the primary human keratinocytes, which are differentiating and sensitive to the genomic action of the hormone, and the human keratinocyte cell line HaCaT, which is characterized by a rapid growth and resistance to the antiproliferative properties of vitamin D. We analysed the nuclear translocation and features of VDR, the effects of vitamin D on mitochondrial and nuclear transcription of COXII, COXIV and CYP24A1 genes, and also on lipid deposition in differentiating primary keratinocytes and immortalised HaCaT cells. We describe a common mechanism of action through which vitamin D modulates mitochondrial activity, but we demonstrate a different impact on cellular metabolism, which depends on the proliferative or differentiated status of the cell.

Methods

Cell culture and treatment

The immortalized human epidermal keratinocyte cell line (HaCaT) was purchased from American Type Culture Collection (ATCC), USA, and were cultured in Dulbecco's modified Eagle's medium (DMEM) that had been supplemented with 10% fetal bovine serum and 1% antibiotics [penicillin-streptomycin (Sigma-Aldrich)] at 37°C in a humidified atmosphere containing 5% CO_2. Differentiating and quiescent primary keratinocytes were obtained from Banca della Cute, AOU Città della Salute e della Scienza, Torino, Italy, and were prepared as previously reported (16). Primary cultures were used at confluency in early passages. When treated, the cells were maintained in DMEM that had been supplemented with 1% fetal bovine serum and were incubated for up to 48 hours with 10 nM or 100 nM $1,25(OH)_2D_3$ or 10 nM stigmatellin (Sigma-Aldrich).

Extract preparation and western blotting analyses

Subcellular fractionation and western blotting analyses were carried out as previously described (17). Lysates were subjected to differential centrifugation to isolate the nuclear and mitochondrial fraction. Proteins were extracted from nuclei and mitochondria by incubation in boiling sample buffer followed by sonication. In HaCaT cell lysates, nuclear fractionation was achieved by incubation of nuclei in buffer C (420 mM NaCl, 1 mM EDTA, 20 mM HEPES pH 7.9, 25% glycerol, 1 mM protease inhibitors, 1 mM PMSF) for 20 min at 4 °C with agitation. After centrifugation for 15 min at 16,000 × g at 4 °C, supernatants (corresponding to nucleosol) were separated from nuclear pellets, which were resuspended in boiling sample buffer followed by sonication. Thirty µg of each fraction were separated using 10% SDS-PAGE and analysed by western blotting. Mouse anti-VDR (sc-13133) and rabbit antibody anti-PARP (sc-7150) were from Santa Cruz, CA, USA.

DNase treatment

Pelleted nuclei were washed in isotonic buffer (10 mm Tris HCl, pH 7.4, 150 mm NaCl) and then incubated for 20 min at 37 °C in 0.5 ml of digestion buffer containing 10 mm Tris HCl, pH 7.4, 10 mm NaCl, 5 mm $MgCl_2$, 0.2 mm PMSF, and 200 units of DNase I (Sigma) (18). Nuclei were pelleted at 14,000 rpm, and the supernatant, containing the DNA-released material, was taken. Nuclear pellet was resuspended in boiling sample buffer followed by sonication. The protein content of the nuclear pellet fraction was quantified and 50 µg were separated using 10% SDS-PAGE and analysed by western blotting.

RNA extraction and real-time PCR

RNA was extracted using TRIzol (Invitrogen) and 1 µg of total RNA that had been treated with DNase (Roche) was reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's recommended protocol. Real-time PCR was performed using iQ SYBR Green (Bio-Rad) with the following primers:

COXII, fwd 5′-CGACTACGGCGGACTAATCT-3′, rev 5′-TCGATTGTCAACGTCAAGGA-3′;

COXIV, fwd 5′-CGAGCAATTTCCACCTCTGT-3′, rev 5′-GGTCAGCCGATCCATATAA-3′;

CYP24, fwd 5'- CGTTTGGACGATGATGGTCAC, rev 5'-TTTCTTGAAGCCGATTCTGGTG;

β-actin, fwd 5′-CATGTACGTTGCTATCCAGGC-3′, rev 5′-CTCCTTAATGTCACGCACGAT-3′

Beta-actin was used as an internal control. The real-time PCR parameters were as follows: Cycle 1, 50°C for 2 minutes; cycle 2, 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds and then 60°C for 1 minute. The 2-ΔΔCT method was used to analyse the data.

Oil Red O staining

To assay lipid deposition after 48 hours of treatment with 10 nM stigmatellin (Sigma-Aldrich) or 10 nM vitamin D or just regular medium, cells were stained with Oil Red O (ORO, Sigma-Aldrich) according to Kim et al. (19). Briefly, cells were fixed with 4% paraformaldehyde overnight and washed with 60% isopropanol. After drying cells, 0.21% ORO in 60% isopropanol was applied to

the cells for 10 minutes followed by 4 times washing with distilled water. Stained ORO was extracted with 100% isopropanol and absorbance was measured at 520 nm. Pictures were taken before extraction.

Bands quantification and statistical analysis

Bands from protein electrophoresis were quantified by scanning digital densitometry using an ImageJ software analysis (ImageJ version 1.29, Sun Microsystems Inc., Palo Alto, CA). Statistical analysis of data was performed using ANOVA test with Tukey's post-hoc correction. p values <0.05 were considered significant. All data were expressed as mean ± S.D

Results

Differentiating keratinocytes show ligand-dependent nuclear translocation of VDR, whereas HaCaT cells are insensitive to vitamin D treatment

In order to verify that our *in vitro* model of primary human keratinocytes corresponded to a differentiating tissue responsive to the nuclear effects of vitamin D, we assessed VDR translocation into nuclear compartment, driven by ligand binding and necessary for the transcriptional activity of the receptor. At both the concentrations of vitamin D tested (10 and 100 nM), we were able to detect a strong nuclear translocation of VDR (Fig. 1A), with small differences in amplitude depending on the culture analysed. Although different primary cell preparations responded slightly differently to stimulation, the nuclear levels of VDR were consistently higher in treated cells compared with control (Fig. 1B). On the contrary, in our previous work we have reported that HaCaT cells display high levels of nuclear VDR but have lost the modulation of the nuclear shuttling of the receptor, and the absence of ligand-dependent nuclear translocation is associated with the loss of the antiproliferative effects of vitamin D (3,17). In this study HaCaT cells represented therefore a model of proliferating human keratinocytes resistant to the differentiating properties of vitamin D. In order to better characterize the abundant nuclear VDR of HaCaT cells, the nuclear extracts were further processed and we obtained the soluble fraction (nucleosol) and the particulate fraction (nuclear pellet, comprehensive of chromatin, associated proteins and the components of nuclear envelope). The analysis of nucleosol and nuclear pellet of treated and control cells confirmed that VDR levels were not modulated by ligand binding, and most interestingly we detected a nuclear VDR present only in the particulate fraction (Fig. 1C). This partition is not always typical of nuclear factors, for example we found that the levels of the nuclear enzyme poly (ADP-ribose) polymerase (PARP) were distributed quite in the opposite way between the two fractions. To verify whether the receptor was retained in the nuclear particulate fraction as a result of strong binding to chromatin, we treated the nuclear pellets with DNase, thereby digesting the chromatin and releasing any bound protein. After this treatment VDR was lost from pellet, as

well as PARP, which is notoriously an enzyme working on DNA (Fig 1D) (20). The results of these experiments demonstrated that HaCaT cells possess a nuclear VDR, tightly docked to DNA, which upon ligand stimulation does not increase its already robust nuclear presence.

Vitamin D treatment inhibits the transcription of the respiratory chain

In our previous work, we demonstrated that in HaCaT cells VDR exerted profound effects on mitochondrial activity by downregulating the transcription of respiratory chain subunits (3). Also in primary keratinocytes VDR is localized in the mitochondrial compartment, as shown by western blotting analysis of mitochondrial extracts (Fig. S1). In this study we tested the effects of two concentrations of vitamin D on the transcription rate of the two considered cellular models and we found interesting differences. We treated the cells with 10 and 100 nM of vitamin D up to 48 hours and by real time PCR analysis we confirmed that VDR was able to downregulate the transcription of both subunit II and IV of complex IV (cytochrome c oxidase COX II and IV), whose transcripts are of mitochondrial (the former) and nuclear (the latter) origin. Both nuclear and mitochondrial-encoded proteins are required for the formation of active respiratory complexes; in our assay we evaluated COX II as a marker of mitochondrial transcription activity and COX IV as a marker of the nuclear contribution to respiratory chain modulation. Vitamin D at the concentration of 10 nM was effective in restraining transcription of COX II and IV both in the differentiating keratinocytes and in proliferating HaCaT (Fig. 2A and 2B upper panels). However, the higher dose of 100 nM, which was still effective in differentiating keratinocyte (Fig. 2A middle panel), in HaCaT was able to downregulate transcription only for the first 24 hours, and the effect was reverted in the following 24 hours (Fig. 2B middle panel). HaCaT cells demonstrated therefore a delayed resistance to the mitochondrial effects of vitamin D. It has been reported that vitamin D controls its own activity by transcriptional induction of the catabolic enzyme CYP24A1 (21). The increased activity of this enzyme has been deemed responsible also for the resistance to vitamin D detected in several cancer cells (22,23). We measured the levels of CYP24 transcript in both cellular systems,

and we found that in HaCaT cells CYP24 was indeed strongly potentiated in the same experimental conditions that reversed the effect of vitamin D (100 nM at 48 hours, Fig. 2B lower panel). In differentiating keratinocytes a strong induction was not evident, since CYP24 did not reached the levels seen in HaCaT at 48 hours (Fig. 2A lower panel). The analysis of the effects exerted by vitamin D on mitochondria demonstrated a common mechanism of action in both differentiating and proliferating keratinocytes, but proliferating HaCaT cells were resistant to $1,25(OH)_2D_3$ activity, at least in part due to the high induction of CYP24A1 catabolic activity

Vitamin D promotes lipid accumulation in differentiating keratinocytes but not in proliferating HaCaT cells

The finding that in keratinocytes, whether they be differentiating or proliferating, vitamin D hampers mitochondrial transcription prompted us to investigate the impact of a reduced activity of respiratory chain on metabolism, which must be different in order to sustain a differentiation cellular program or a proliferative state. In our previous work (3) we have demonstrated that in HaCaT cells VDR acts as a promoter of acetyl-CoA utilization outside of the mitochondria in the mevalonate biosynthetic pathway. In proliferating cells the most important final product of the biosynthetic fate of acetyl-CoA is cholesterol, essential for duplication processes. In this study we considered which biosynthetic pathway would mostly benefit from an increased availability of acetyl-CoA in differentiated cells. The most superficial terminally differentiated layers of epidermis produce lipids that are secreted into the extracellular space where they contribute to the formation of the hydrophobic lipid envelope responsible for the barrier properties of the skin. Therefore we sought to investigate whether differentiating keratinocytes could respond to vitamin D by enhancing intracellular lipid deposition. The same analysis was carried out in HaCaT cells, as it had never been performed before. We treated both cellular models for 48 hours at low vitamin D concentration (10 nM) to avoid the resistant phenotype observed in HaCaT cells, and we detected the intracellular lipid deposition by Oil-red O staining. We also treated the cells with an inhibitor of

the respiratory chain, stigmatellin, which with a different mechanism of action should affect the respiratory chain even more than VDR, therefore it should have a similar impact on metabolism. Actually, the two treatments produced equivalent results on differentiating keratinocytes, promoting a strikingly evident accumulation of lipids (Fig. 3A). On the contrary, HaCaT cells treated with vitamin D did not show any difference from control, while stigmatellin was still able to cause a fair lipid deposition (Fig. 3A). The results of different experiments were quantified and averaged (Fig. 3B), and we were able to demonstrate that vitamin D treatment leads to intracellular lipid accumulation only in differentiating keratinocytes, whereas in proliferating HaCaT this biosynthetic pathway is not inducible by the hormone.

Discussion

To date, the effects of vitamin D activity on mitochondria of differentiated cells were not known, therefore in this study we decided to compare the response to vitamin D in two different cellular phenotypes: proliferating and differentiating human keratinocytes. As validation of our cellular models, we found that the differentiating keratinocytes responded to vitamin D by activating the classical pathway of nuclear translocation of VDR, whereas HaCaT, previously characterized as vitamin D-resistant cells (3), displayed a vitamin D-independent nuclear expression of the receptor. Interestingly, our analysis revealed that VDR was tightly bound to chromatin, again independently from ligand stimulation. The binding of VDR to VDRE regions and its ability to enhance or silence gene transcription is the result of complex interactions with dimerization partners, coactivators and corepressors, as extensively described by the work of Bikle and coll. (8,9) and Campbell and coll. (24,25). Transformed keratinocytes are less responsive to the antiproliferative actions of $1,25(OH)_2D_3$ than are normal keratinocytes despite having normal levels of VDR (11,12,26). This altered response has been ascribed to the perturbation of nuclear signaling of the hormone (8,24). Deregulation of corepressor binding could be the main mechanism of resistance to vitamin D. In transformed cells the normal replacement operated by vitamin D, that dissociates corepressor hairless (Hr) and recruits coactivators on VDR (27), could be defective and the corepressor could bind a silent VDR to chromatin. Actually, Hr has been found to repress VDR transactivation in HaCaT cells (28,29). Other studies have given evidences of an epigenetic resistance in cancer that often involves elevated expression of the corepressor NCoR2/SMRT (24,25). Thus our novel finding of a VDR permanently bound to DNA in HaCaT cells is not surprising, and it is in agreement with the resistance of these cells to the antiproliferative properties of vitamin D.

The negative modulation of the respiratory chain transcription and activity exerted by VDR in HaCaT cells had been described in our previous work (3), and this mechanism of action found a confirmation in the present study on differentiating keratinocytes. VDR is present in the mitochondrial compartment of primary keratinocytes, it is therefore reasonable to hypothesize that

also in these cells vitamin D exerts a combined mitochondrial and nuclear transcriptional control on respiratory chain, as suggested by the investigation of VDR in HaCaT (3). Moreover, by testing different concentrations of vitamin D, in this study we discovered that in HaCaT high doses of the ligand induce insensitivity to the mitochondrial effects of the hormone. This mitochondrial resistance is acquired upon potent induction of CYP24A1, the catabolic enzyme target of VDR that turns off a massive signaling of vitamin D. Elevated levels of CYP24 are common in cancer (30-32), thus CYP24 activity has been identified as one of the causes of resistance to vitamin D, although to date the role of CYP24 has been described in genomic resistance only. Vitamin D can induce 24-hydroxylase expression in transformed lines as well as in normal keratinocytes (11,33), even when it cannot induce enzymes required for differentiation. The difference is due to the failure of transformed keratinocytes to switch coactivator complexes; this unbalance disturbs the readout of the genes required for differentiation whereas it facilitates CYP24 transcription. Our observations on CYP24 induction are therefore reasonable and in agreement with other reports, however we describe for the first time the impact of this mechanism of resistance on mitochondrial activity.

The other major finding of this study was the analysis of lipid production. In our previous work (3) we demonstrated that the negative modulation of respiratory chain activity promoted the diversion of acetyl-CoA from the energetic catabolism toward the biosynthetic pathways. In proliferating HaCaT cholesterol was one of the main products of the metabolic shift promoted by VDR. In this study, we found that the same mitochondrial modulation occurred in differentiating keratinocytes, but the metabolic switch triggered by vitamin D in these cells promoted lipid deposition. Lipid biosynthesis and secretion is a metabolic specialization of differentiated keratinocytes. Lipids are synthesized in the keratinocytes of the stratum granulosum, assembled within organelles named lamellar bodies (34) and then secreted and processed in the extracellular space, eventually forming the lamellar bilayers that mediate permeability barrier function (35). Stratum corneum contains large quantities of lipids, which are primarily comprised of mixtures of ceramides, cholesterol, and free fatty acid (36). The biosynthetic pathway of the most abundant lipids ceramides is tightly

controlled by VDR. In fact it has been demonstrated that VDR and SRC coactivators are required for the production of epidermal-specific glucosylceramide and subsequent barrier formation (37). Mice deficient in 25-hydroxylase, which is required for the formation of active $1,25(OH)_2D_3$, have impaired barrier repair after acute disruption due to a decrease in lamellar body secretion (38). In our study we have confirmed in differentiating keratinocytes the important role of vitamin D in lipid accumulation and we have related the lipogenic effect with the downregulation of electron transport chain. The link is supported by the enhanced lipid biosynthesis observed after treatment of differentiating keratinocytes with stigmatellin, which inhibits the respiratory chain and increases lipid deposition. Proliferating HaCaT cells, on the other hand, do not respond to vitamin D-dependent mitochondrial modulation by variation in lipid droplets content, in agreement with the previously demonstrated utilization of acetyl-CoA in cholesterol biosynthesis to sustain proliferation (3).

From our data we concluded that the negative modulation of mitochondrial respiratory chain is a common mechanism of action of VDR, however the outcome of the consequent rerouting of intermediates is different depending on metabolic demands, as shown in Fig. 4. In differentiating keratinocytes VDR is able to trigger the nuclear effects and the downregulation of electron transport chain. Spared acetyl-CoA is thus rewired toward the proper biosynthetic fate, represented mainly by lipid droplet deposition and secretion (upper model). In proliferating keratinocytes (and cancer cells) the negative modulation of respiratory activity is not associated with a differentiating action of vitamin D. Therefore the diversion of acetyl-CoA results in enhanced cholesterol biosynthesis, necessary to the proliferative state of the cell (lower model).

The differential effects of vitamin D on differentiating and proliferating human keratinocytes described in this work lay the molecular basis that can help to understand the physiological role of vitamin D as regulator of both proliferation and differentiation processes present in healthy skin. In addition to nuclear dynamics controlled by coregulators (reviewed in (39)), our work highlights

how VDR can modulate mitochondrial and cellular metabolism to sustain the different cellular phenotypes composing the skin.

Moreover, the data presented here shed light on the dual role given to VDR as tumor suppressor/facilitator, which could be particularly relevant in skin cancers. A recent epidemiologic study has reported a positive association between baseline vitamin D levels and basal cell carcinoma and melanoma incidence and a negative association with squamous cell carcinoma incidence (40). The results of our study, that highlight how vitamin D can support both differentiating and proliferating metabolism, would explain why vitamin D has been considered both a procarcinogenic and anticarcinogenic agent (41-46).

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M.C., M.V., S.C., C.C. and F.S. performed the research, F.S. and G.P. designed the study, all authors analysed the data, F.S. and G.P. wrote the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Characterization of ligand-dependent nuclear localization of VDR in primary keratinocytes and HaCaT cells. (A) Primary keratinocytes responded to the 24 h treatment with vitamin D 10 nM (D10) or 100 nM (D100) by translocating VDR into nuclear compartment. Thirty μg of nuclear proteins from untreated (control, C) or treated cells were analysed by western blotting for VDR. The blot is representative of three independent experiments, in which different primary cultures were used. (B) Bands from experiments based on three different cultures (named KER 119, 120, 121) were quantified, normalized for loading as a ratio to PARP expression and data plotted on graph as percentage of control. (C) HaCaT cells showed basal levels of nuclear VDR which were not modulated by vitamin D treatment. Nuclear extracts from HaCaT cells were separated in nucleosol (N) and nuclear pellet (NP) fractions and 30 μg of proteins from untreated or treated cells were analysed by western blotting for VDR. PARP expression was used as control of equal loading. (D) After DNase treatment of HaCaT nuclear extracts, proteins still present in nuclear pellet were analysed by western blotting for VDR and PARP. The blots are representative of three independent experiments.

Figure 2. Vitamin D treatment inhibits the transcription of respiratory chain complexes. Primary keratinocytes (A) and HaCaT cells (B) were treated for 48 hours with vitamin D 10 nM (D10) and 100 nM (D100). After 24 and 48 hours of treatment mRNAs were purified and assayed by real time PCR for COX II, COX IV and CYP24 transcript expression. The values plotted on the graphs represent the fold change in transcript expression in treated versus untreated cells and are displayed as the means ± SD of three independent experiments. * $P<0.05$ compared to the untreated cells. § $P<0.05$ compared to D10 treated cells.

Figure 3. Treatment with Vitamin D and with an inhibitor of respiratory chain activity induces intracellular lipid accumulation in primary keratinocytes but not in HaCaT cells. Primary

keratinocytes and HaCaT cells were treated for 48 hours with 10 nM stigmatellin or 10 nM vitamin D. Cells were stained with Oil red O and pictures were taken (A). The image is representative of a set of three independent experiments. Afterwards, staining was solubilized and quantified by spectrophotometer (B). Data represent the means ± SD of three independent experiments. * $P<0.05$ compared to the untreated cells.

Figure 4. A working model of the molecular mechanisms underlying VDR effects on metabolism of differentiating primary keratinocytes and proliferating HaCaT cells. Vitamin D through its receptor VDR exerts a general role as inhibitor of mitochondrial electron transport chain (ETC) and as facilitator of the diversion of mitochondrial acetyl-CoA toward biosynthetic pathways; the impact on metabolism depends on the differentiated or proliferative status of the cell. Upper panel: in differentiating keratinocytes vitamin D is effective in nuclear compartment as inducer of differentiation; this condition forces the utilization of acetyl-CoA into lipid biosynthesis and secretion. Lower panel: in proliferating keratinocytes nuclear resistance hampers differentiating effects of vitamin D; the unaltered proliferative status requires an enhanced biosynthesis of cholesterol, which is achieved by channeling acetyl-CoA into the cholesterol biosynthetic pathway that sustains proliferation. TCA cycle: tricarboxylic acid cycle; AS: ATP synthase.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Legend for supplemental illustration

Figure S1. Mitochondrial localization of VDR in primary keratinocytes. Equal amounts of proteins from the mitochondrial extracts of HaCaT (H) and primary keratinocytes (K) were analysed by western blotting and VDR expression was detected in both cells.

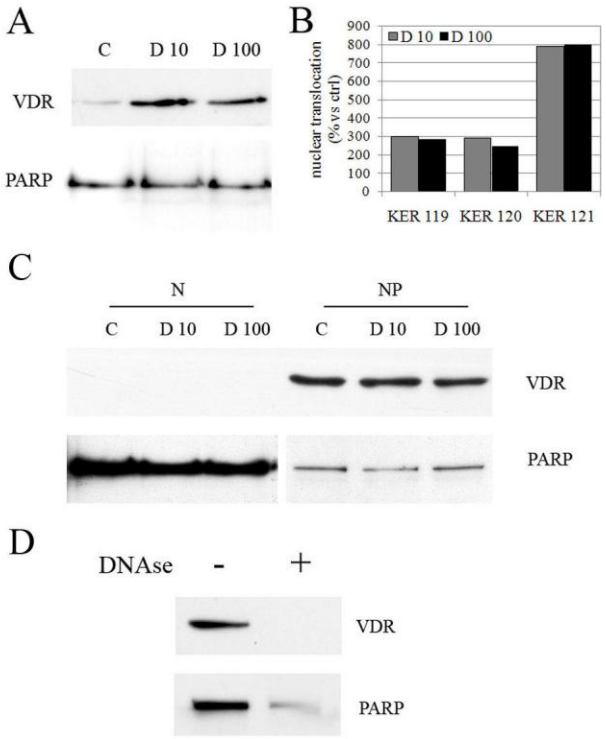

Figure 1

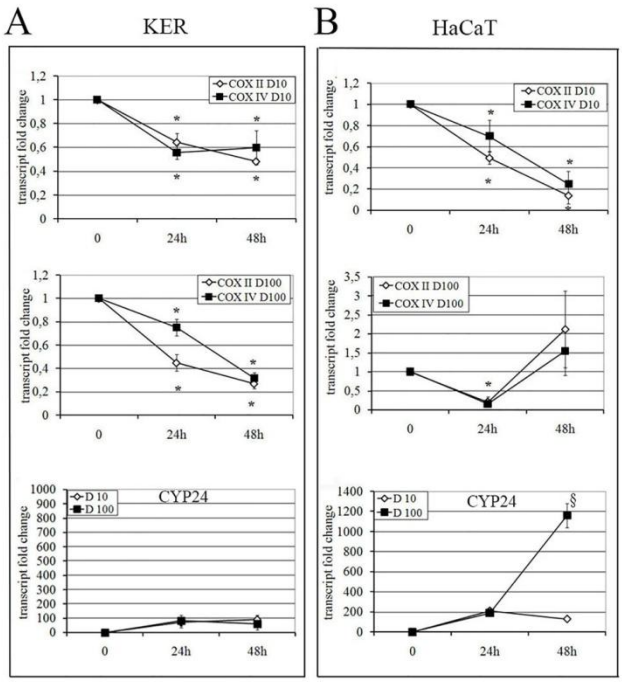

Figure 2

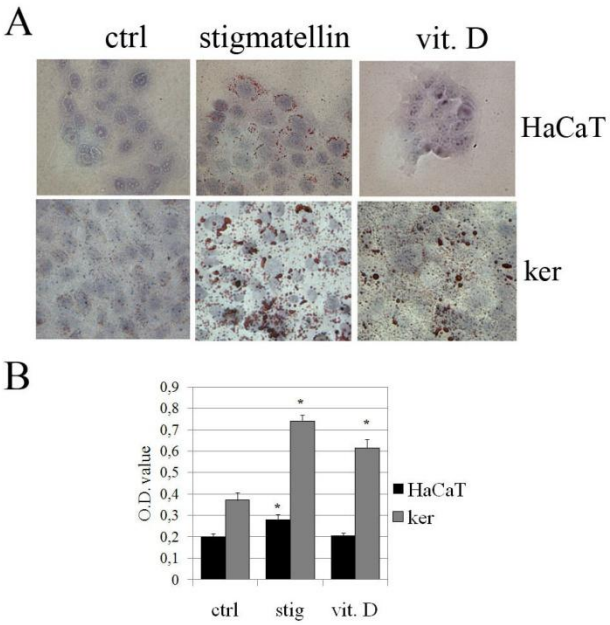
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Figure 3

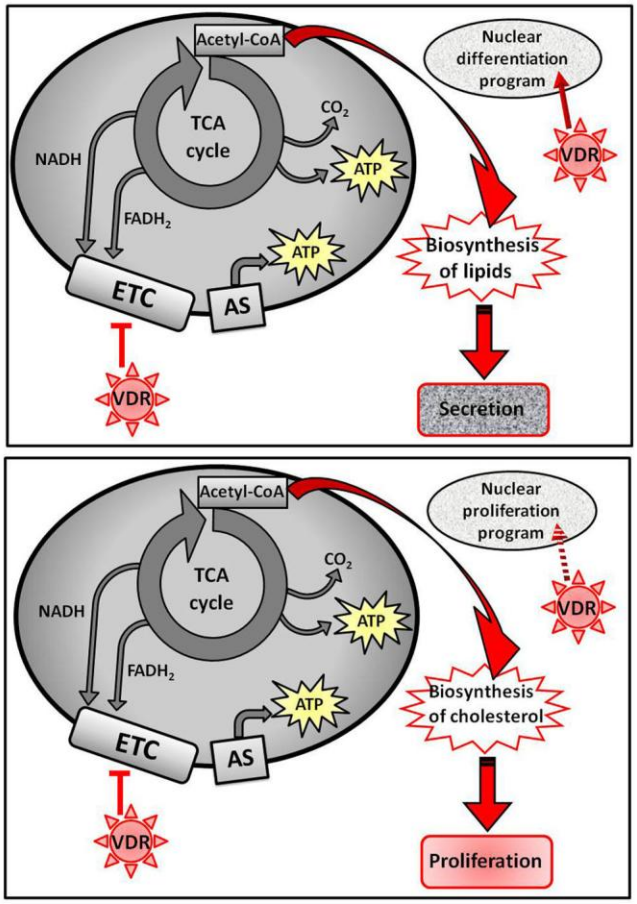

Figure 4

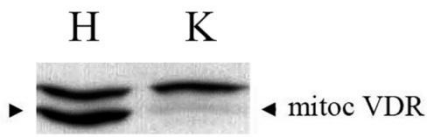

Figure S1