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NOVEL MECHANISMS FOR THE VITAMIN D RECEPTOR (VDR) IN THE SKIN AND IN SKIN CANCER

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Abstract

The VDR acting with or without its principal ligand 1,25$(OH)_2$D regulates two central processes in the skin, interfollicular epidermal (IFE) differentiation and hair follicle cycling (HFC). Calcium is an important co-regulator with 1,25$(OH)_2$ D at least of epidermal differentiation. Knockout of the calcium sensing receptor (CaSR) in addition to VDR accelerates the development of skin cancer in mice on a low calcium diet. Coactivators such as Mediator 1 (aka DRIP205) and steroid receptor coactivator 3 (SRC3) regulate VDR function at different stages of the differentiation process, with Med1 essential for hair follicle differentiation and early stages of epidermal differentiation and proliferation and SRC3 essential for the latter stages of differentiation including formation of the permeability barrier and innate immunity. The corepressor of VDR, hairless (HR), is essential for hair follicle cycling, although its effect on epidermal differentiation in vivo is minimal. In its regulation of HFC and IFE VDR controls two pathways—wnt/β-catenin and sonic hedgehog (Shh). In the absence of VDR these pathways are overexpressed leading to tumor formation. Whereas VDR binding to β-catenin may block its activation of TCF/LEF1 sites, β-catenin binding to VDR may enhance its activation of VDREs. 1,25$(OH)_2$D promotes but may not be required for these interactions. Suppression of Shh expression by VDR, on the other hand, requires 1,25$(OH)_2$D. The major point of emphasis is that the role of VDR in the skin involves a number of novel mechanisms, both 1,25$(OH)_2$D dependent and independent, that when disrupted interfere with IFE differentiation and HFC, predisposing to cancer formation.

Keywords

Vitamin D receptor; calcium sensing receptor; hair follicle cycling; epidermal differentiation; coregulators; cancer

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1.0. Introduction

1.1

Under the influence of the UVB portion of sunlight 7-dehydrocholesterol in the epidermis is converted to vitamin D. This provides the major source of vitamin D for the body as food products unless supplemented have little vitamin D with the exception of some fatty fish such as salmon. The dominant cell of the epidermis, the keratinocyte, can further metabolize vitamin D to its active form 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) because it possesses both 25 hydroxylase (CYP27A1, CYP2R1) and 1α hydroxylase (CYP27B1) activity. Keratinocytes also express the vitamin D receptor (VDR) enabling them to respond to the $1,25(OH)_2D$ produced (1). The epidermis is stratified with the basal layer containing the stem cells. As cells proliferate and leave the basal layer they undergo a sequential process of differentiation until the formation of the permeability barrier in the stratum corneum. $1,25(OH)_2D$/VDR promotes this process by regulating proliferation in the basal layer (stratum basale) and stimulating the sequential differentiation of keratinocytes as they form the upper layers. Mutations or deletion of VDR and CYP27B1 results in hyperproliferation of the cells within the basal layer as well as defects in permeability barrier formation and the innate immune response. Loss of VDR also disrupts hair follicle cycling leading to alopecia, but this role of VDR appears to be independent of $1,25(OH)_2D$ in that mutations or deletion of CYP27B1 does not disrupt hair follicle cycling (2,3).

1.2

The effects of $1,25(OH)_2D$ and VDR are mediated at least in part by controlling the calcium levels of the keratinocytes. Calcium itself is a potent regulator of epidermal differentiation, although its role in hair follicle cycling is less clear. Within the epidermis there is a steep gradient of calcium from the stratum basale to the stratum granulosum, a gradient that corresponds to the differentiation status of the layers within the epidermis (4). The calcium sensing receptor (CaSR) is critical for controlling the response of the keratinocyte to calcium, and mice lacking the CaSR in their epidermis have a similar disruption of the permeability barrier and innate immune response as do mice lacking VDR or CYP27B1 (5). $1,25(OH)_2D$ induces the CaSR, and mice lacking CaSR have decreased levels of VDR. Like deletion of CYP27B1, deletion of CaSR does not result in alopecia.

1.3

The sequential role of VDR in regulating hair follicle cycling and basal cell proliferation on the one hand and more differentiated functions such as innate immunity and permeability barrier in the upper layers of the epidermis is attributed at least in part to the different coregulators that modulate its genomic actions. Hairless (Hr) plays a key role in hair follicle cycling in that in the absence of Hr, hair follicle cycling ceases after the first developmental cycle as it does in the absence of VDR (6). Hr also plays a cosuppressor role for VDR in the epidermis (7). The Mediator complex is required for hair follicle cycling and early stages of epidermal proliferation and differentiation, appearing to function at the stem cell level, whereas SRC2 and SRC3 exert their effects in the latter stages of differentiation, namely permeability barrier formation and innate immunity (8).

1.4

Given the importance of the VDR in regulating proliferation and differentiation, it is not surprising that the skin of mice lacking the VDR are susceptible to tumor formation. Both chemical carcinogens and UVR induce these tumors, which range from benign papillomas to invasive squamous cell carcinomas (9,10). Surprisingly, mice lacking $1,25(OH)_2D$ do not appear to be more susceptible to tumor formation by chemical means or UVB. This is reminiscent of hair follicle cycling in which the requirement for $1,25(OH)_2D$ appears limited, suggesting a common set of pathways under VDR but not $1,25(OH)_2D$ regulation, which we will explore subsequently. When mice lack both the VDR and CaSR, tumors form spontaneously without the requirement of UVR or chemical carcinogens (Jiang and Bikle, unpublished).

1.5

In this brief review we will examine these different actions of vitamin D and its receptor in regulating epidermal proliferation and differentiation, hair follicle cycling, and tumorigenesis, examining how these processes interact with those regulated by calcium.

2.0. Calcium and vitamin co-regulation of the differentiation process

In keratinocyte cultures, both calcium and $1,25(OH)_2D$ stimulate differentiation, and their combined effects are synergistic (11). In the promoter of at least one gene, involucrin, the AP-1 site critical for calcium induced involucrin expression lies adjacent to a vitamin D response element (VDRE). Mutation of the AP-1 site blocks both calcium and $1,25(OH)_2D$ induction of the gene, whereas mutation of the VDRE blocks only the induction by $1,25(OH)_2D$ (12). Formation of the E-cadherin/catenin complex is another example where vitamin D and calcium interact in a mutually dependent fashion (figure 1). This complex, that forms the adherens junction, provides not only cell-cell adhesion, but is a key signaling complex for the keratinocyte required for epidermal differentiation (13). Key members of this complex include E-cadherin itself, spanning the membrane with extracellular calcium providing the cell-cell junction, catenins including β-catenin and p120 essential for the stability of the complex, plakoglobin (γ-catenin) and α-catenin linking the complex to the actin infrastructure, and two enzymes phosphatidyl inositol phosphate 5 kinase 1α (PIP5K1α) that converts PIP to PIP2 and phosphatidyl inositol 3 kinase (PI3K) that converts PIP2 to PIP3. PIP3 is responsible for activating phospholipase C-γ1 (PLC-γ) that hydrolyzes PIP2 to produce two key second messengers that drive the differentiation process, diacyl glycerol (DAG) and inositol tris phosphate (IP3). DAG activates selected protein kinase Cs, whereas IP3 stimulates the release of calcium from intracellular stores. Calcium induces the formation of the E-cadherin complex, but this does not occur in cells lacking the VDR. Moreover, calcium and $1,25(OH)_2D$ each induce PLC-γ) (14,15). Formation of the calcium gradient is another mechanism jointly affected by calcium and vitamin D. Mice lacking CYP27B1, and so lacking $1,25(OH)_2D$, lack the normal calcium gradient in their epidermis, a feature also found in mice lacking the CaSR. Finally both calcium and vitamin D signaling are required for maintenance of the permeability barrier. Mice deficient in either VDR or CaSR have a reduced ability to form the permeability barrier (3,5,16) due to decreased levels of the enzymes critical for producing the long chain ceramides involved in formation

of the lipids that provide the waterproofing of the cornified envelope, the components of which are also induced by calcium and vitamin D.

3.0. The calcium sensing receptor

The CaSR in the keratinocyte is the same 7 transmembrane molecule as found in the parathyroid gland, kidney, bone and other tissues where it has been found (17). Activation of the CaSR in keratinocytes *in vitro* by increasing the extracellular calcium [Ca]o increases intracellular calcium [Ca]i in part through an immediate release of calcium from intracellular stores and in part from activation of PLC-γ1, which not only has a prolonged ability to stimulate the release of calcium from intracellular stores via IP3 formation, but also to increase the influx of calcium through TRPC channels in the membrane (18). The CaSR is required for calcium to induce the E-cadherin/catenin complex. Surprisingly, the formation of this complex does not require an initial increase in [Ca]i as it is not blocked by inhibitors of the IP3 receptor, calcium channels, or intracellular calcium chelators. Instead the CaSR appears to be acting through the Rho pathway (19). The CaSR is induced by $1,25(OH)_2D$ (20) and, surprisingly, keratinocytes lacking the CaSR also have low VDR levels suggesting a reciprocal relationship not yet fully explained (5).

4.0. VDR regulation of hair follicle cycling

The hair follicle cycle is divided into three main periods: anagen during which the follicle (and hair shaft) grow, catagen during which the cycling part of the hair follicle collapses, and telogen, the resting phase. The hair follicle cycle is initiated during embryonic development. This first cycle is the developmental cycle. Its initiation is not dependent on VDR. However, after day 13 postnatally the developmental cycle enters catagen. It is at this stage that lack of VDR is first manifest. Normal cycling requires activation of the stem cells in the bulge, a collection of cells approximately half way up the fully formed hair follicle, with a specialized collection of mesenchymal cells called the dermal papilla at the tip of the follicle. Normally during catagen the dermal papilla stays attached to the receding proximal (cycling) part of the hair follicle to end up adjacent to the bulge as the follicle enters telogen. In the skin of mice in which the keratinocytes lack the VDR the dermal papilla separates from the hair follicle during catagen, and anagen is not reinitiated (2). Both the wnt/β-catenin and sonic hedgehog pathway are important for hair follicle cycling, and as we will discuss in the section on skin cancer, part of the mechanism by which VDR controls hair follicle cycling may involve these pathways (21).

5.0. The role of coregulators

The major coactivator complexes regulating VDR in the keratinocyte are the Mediator complex and the SRC 2 and 3 complexes. They are differentially expressed during differentiation with the Mediator complex expressed in highest concentration in the undifferentiated keratinocyte and the SRC2 and 3 complex in the differentiated keratinocyte (figure 2) (8,22). This distribution also follows function in that deletion of Med 1 has its greatest impact on hair follicle cycling, keratinocyte proliferation, and early stages of keratinocyte differentiation including E-cadherin/catenin complex formation (23). Deletion of SRC3, on the other hand, has no impact on hair follicle cycling and little impact on E-

cadherin/catenin complex formation, but does result in deficient permeability barrier formation and innate immune response to wounding (16,22). Hairless (Hr) has a corepressor role for VDR at least for 1,25$(OH)_2$D regulation transcriptional activity (7). However, it is not clear what its role is with respect to VDR regulated hair follicle cycling. Both Hr and VDR are required for hair follicle cycling, and lack of one or the other produces a similar phenotype (2,21,24). Thus it is not obvious how Hr could serve as a corepressor for the VDR regulated hair follicle cycling.

6.0. VDR as a tumor suppressor

6.1

Deletion of VDR results in an increased susceptibility to tumor formation whether it is induced by chemicals such as DMBA (9) or by UV radiation (10,25). We have explored potential mechanisms. Both wnt/β-catenin and sonic hedgehog (SHH) pathways are associated with increased keratinocyte proliferation and decreased keratinocyte differentiation. As noted earlier, following calcium and vitamin D induced differentiation, β-catenin becomes incorporated into the E-cadherin complex in the plasma membrane where it presumably facilitates the differentiation process. Moreover, VDR appears to shift the β-catenin that would otherwise bind to its LEF/TCF sites in genes involved with differentiation to genes that are coregulated by VDR and β-catenin involving differentiation (26,27). Both VDR and 1,25$(OH)_2$D suppress β-catenin stimulated LEF1/TCF driven reporter activity (27). The SHH pathway is likewise regulated by VDR and 1,25$(OH)_2$D. The epidermis of the VDR null mouse shows an overexpression of SHH and most of the components of the SHH pathway (25). Moreover, 1,25$(OH)_2$D suppresses the expression of SHH and Gli1, the latter a key transcription factor of the SHH pathway. Activation of both the SHH and β-catenin pathways are known to be involved in tumor formation in the skin (basal cell carcinomas for overexpression of SHH pathway and folliculomas for overexpression of β-catenin pathway). Of interest is that in the absence of VDR, activation of the β-catenin pathway results in basal cell carcinomas as well (26).

6.2

Deletion of VDR also reduces the ability of the keratinocyte to clear UVB induced DNA mutations (28). This is due at least in part to the ability of 1,25$(OH)_2$D/VDR to induce one of the DNA repair enzymes, XPC. The net result is UVB induced DNA lesions such as cyclobutane pyrimidine dimers, the signature lesion of UVB DNA damage, and 6,4 photoproducts are slow to be repaired in keratinocytes lacking VDR (28).

6.3

More recently we have found that several long non coding RNAs (lncRNA) associated with tumor formation including squamous cell carcinomas are overexpressed in VDR null keratinocytes both *in vivo* and *in vitro* (29) (figure 3). H19 overexpression has also been linked to dysregulation of the wnt/β-catenin pathway (30) potentially tying together this pathway with the changes in expression of H19 in the VDR null keratinocyte. The significance of this observation is under investigation.

6.4

Calcium appears to play an important role in the ability of VDR to suppress skin cancer. When mice lacking both VDR and CaSR are placed on a low calcium diet, they spontaneously develop squamous cell carcinomas without induction by UVB or DBMA (figure 4). Whether the mechanisms for tumor suppression involve the same pathways as observed for $1,25(OH)_2D$/VDR remains to be seen. But the observation reinforces the concept that just as calcium and vitamin D collaborate in their roles to promote keratinocyte proliferation and differentiation, so they must in preventing the abnormal proliferation and differentiation resulting in malignant transformation.

Acknowledgments

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Highlights

- The VDR plays a key role in epidermal proliferation, differentiation, and hair follicle cycling.
- Calcium via the calcium sensing receptor (CaSR) is central to these actions of VDR.
- VDR utilizes coregulators such as Med 1, SRC3, and Hairless for its different actions.
- The ligand $1,25(OH)_2D$ is not required for all actions of the VDR. Mice lacking VDR and CaSR develop skin tumors spontaneously.

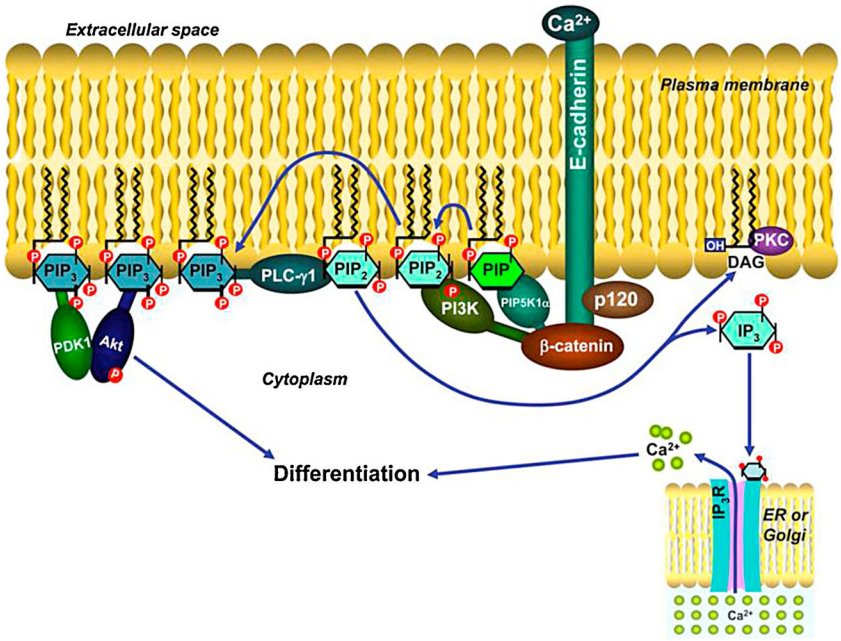

Figure 1. The E-cadherin/catenin complex

Both calcium and 1,25(OH)$_2$D induce formation of the E-cadherin complex. Major components include E-cadherin itself spanning the membrane and forming intercellular contacts through a calcium link, a number of catenins including β-catenin and p120, and enzymes involved with PIP phosphorylation to PIP3. PIP3 activates other enzymes involved in the differentiation process including PLC-γ1, Akt, and PDK1. PLC-γ hydrolyzes PIP2 to IP3 and DAG, which promote differentiation by increasing calcium release from intracellular stores and activating PKC, respectively. Figure modified from model originally published by Xie et al. Molec Biol Cell 20:1695–1704.

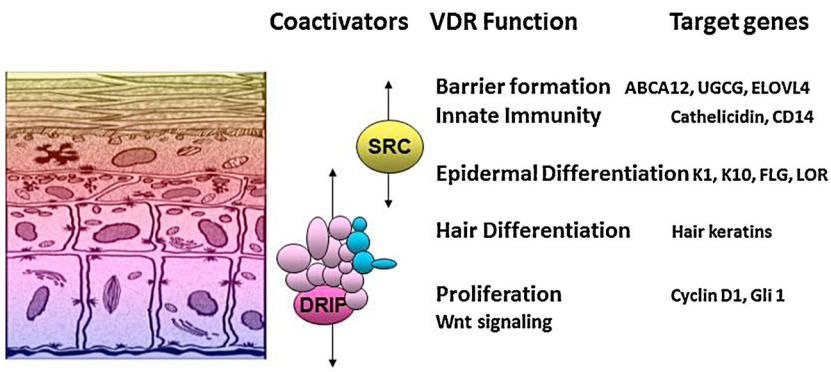

Figure 2. Coactivators of VDR function

The major coactivator complexes regulating VDR function in the keratinocyte are Mediator (aka DRIP) and SRC. The Mediator complex is most highly expressed in basal keratinocytes where it regulates proliferation and hair differentiation. The SRC complexes are most highly expressed in the more differentiated layers of the epidermis where they regulate barrier formation and innate immunity. Both complexes are involved in more intermediate stages of differentiation.

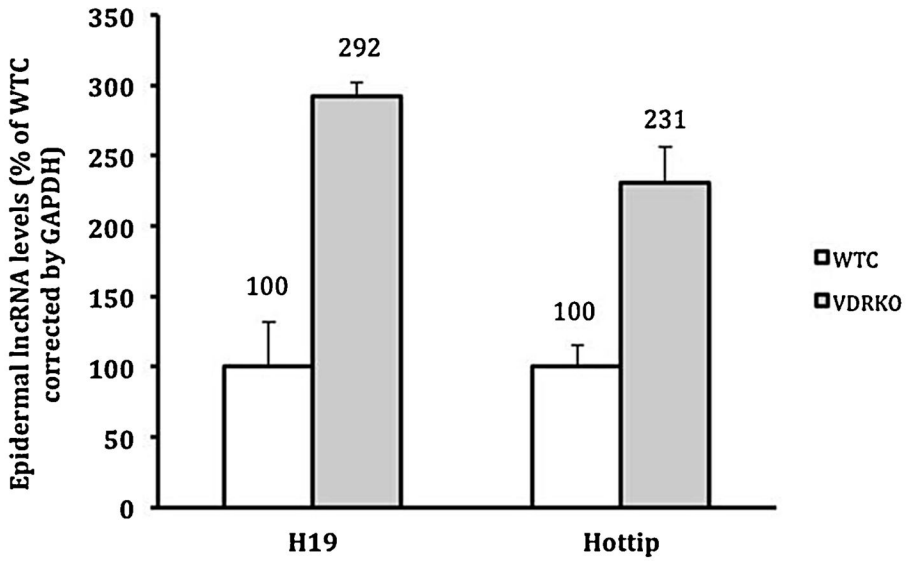

Figure 3. Oncogenic lncRNA expression in VDR null keratinocytes

H19 and Hottip, lncRNAs overexpressed in a number of tumors are over expressed in the epidermis and keratinocytes of VDR null mice. Data taken from Jiang and Bikle, Exper Dermatol 23:147–150

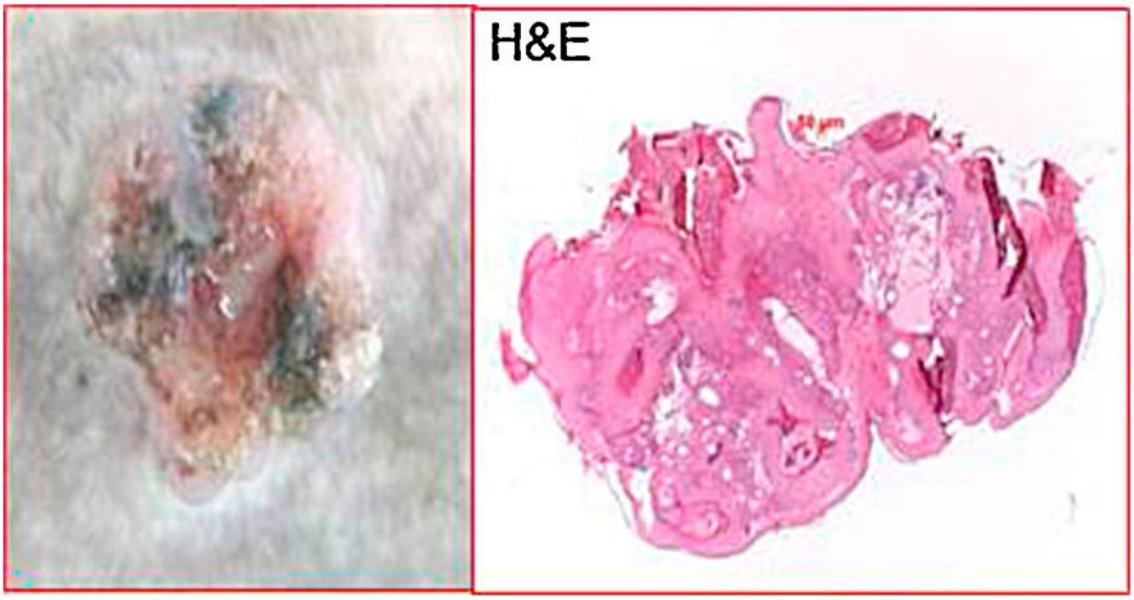

Figure 4. SCC formed spontaneously in a mouse lacking both VDR and CaSR

These tumors begin to appear about 6mo of age when the mice are placed on a low calcium diet.