Influence of Vitamin D on Corneal Epithelial Cell Desmosomes and Hemidesmosomes

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Purpose. We have observed noticeably weak epithelial attachment in vitamin D receptor knockout mice (VDR KO) undergoing epithelial debridement. We hypothesized that VDR KO negatively affects corneal epithelial cell desmosomes and/or hemidesmosomes.

Methods. Transcript levels of desmosome and hemidesmosome proteins in VDR KO corneas were assessed by qPCR. Western blotting and immunohistochemistry were used to detect proteins in cultured cells exposed to 1,25(OH)2D3 and 24R,25(OH)2D3.

Results. VDR KO resulted in decreased corneal desmosomal desmoglein 1 (DSG1) and desmocollins 2 (DSC2) mRNA, and hemidesmosomal plectin mRNA. DSG1 and plectin protein expression were reduced in VDR KO corneas. DSG1 protein expression increased in VDR wild types (VDR WT) and VDR KO mouse primary epithelial cells (MPCEC) treated with 1,25(OH)2D3 and 24R,25(OH)2D3. 24R,25(OH)2D3 treatment resulted in increased plectin and integrin β4 levels in VDR WT MPCEC, and decreased levels in VDR KO MPCEC. Treatment of human corneal epithelial cells (HCEC) with 1,25(OH)2D3 and 24R,25(OH)2D3 resulted in increased DSC2 and DSG1 protein expression. Plectin and integrin β4 were only increased in 24R,25(OH)2D3 treated HCEC.

Conclusions. VDR KO results in reduced desmosomal and hemidesmosomal mRNA and protein levels. 1,25(OH)2D3 and 24R,25(OH)2D3 increased DSG1 protein in all cells tested. For hemidesmosome proteins, 24R,25(OH)2D3 increased plectin and integrin β4 protein expression in VDR WT and HCEC, with decreased expression in VDR KO MPCEC. Thus, vitamin D3 is involved in desmosome and hemidesmosome junction formation/regulation, and their decreased expression likely contributes to the loosely adherent corneal epithelium in VDR KO mice. Our data indicate the presence of a VDR-independent pathway.

Keywords: cornea epithelium, vitamin D, hemidesmosome, desmosome
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**ECL** detection system were obtained from Bio-Rad. Membrane-associated protein disulfide isomerase family A member 5 (PdiA5) has been shown to be a secondary receptor for 1,25(OH)2D3, with each receptor separately activating its own downstream mediators. In addition to this traditional vitamin D metabolic pathway, vitamin D activation has also been demonstrated at the tissue and cell level. Our lab demonstrated the presence of the vitamin D metabolic enzymes in corneal epithelial cells as well as the ability of the corneal epithelium to activate vitamin D.

Vitamin D has been demonstrated to regulate a wide range of physiologic and pathologic processes, including cell growth, migration, immune response modulation, and differentiation. Our previous work demonstrated that 1,25(OH)2D3 influences the function of corneal epithelial cell gap junctions and tight junctions. In addition, we have observed weak epithelial attachment in vitamin D receptor knockout mice (VDR KO) undergoing epithelial debridement for wound healing studies (unpublished observation). Only a few studies have linked vitamin D with adherens junction regulation. Microarray data demonstrated that VDR deletion decreased epithelial adherens junction signaling in epithelium, and E-cadherin (CDH1) and DSG1 were decreased in the epidermis of VDR knockout mice and in a VDR/calcium-sensing receptor double knockout mouse. Conversely, vascular cell adhesion molecule-1 was found to be significantly abolished after incubation of human cardiac endothelial cells (ECs) with 1,25(OH)2D3.

We have observed in previous and ongoing corneal epithelial wound healing studies that the corneal epithelium is noticeably easier to remove from VDR KO mice than from VDR WT mice (unpublished observation). Based on this observation, we hypothesize that vitamin D regulates adherens junctions in the corneal epithelium and that VDR KO negatively affects corneal epithelial cell adherens junctions.

**METHODS**

**Materials**

1,25(OH)2D3 and 24R,25(OH)2D3 were purchased from Enzo Life Sciences (Farmingdale, NY, USA). Antibodies for DSG1, DSC2, and integrin β4 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Plectin and integrin β6 antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Premade protein markers were obtained from Bio-Rad (Hercules, CA, USA). Polyvinylidene difluoride (PVDF) membrane and the enhanced chemiluminescence from Bio-Rad (Hercules, CA, USA). Polyvinylidene difluoride (Danvers, MA, USA). Prestained protein markers were obtained from Biotechnology, Inc. (Santa Cruz, CA, USA). Plectin and integrin β6, integrin β4, and plectin respectively. The primers for mouse TATA box binding protein (TBP; NM_013684) and plectin mRNA levels. RT-PCR primers were generated from the Primer Bank database using National Center for Biotechnology Information (NCBI) sequence identification numbers (NM_013505, NM_181682, NM_001079818, NM_133663, NP_052427 for mouse DSC2, DSG1, integrin β6, integrin β4, and plectin respectively). The primers for mouse TATA box binding protein (TBP; NM_013684) were generated from the Universal Probe Library of Roche Life Sciences. Primers are listed in Table 1.

**Human Corneal Epithelial Cell Line**

The immortalized human corneal epithelial cell line (HCEC) has been previously described. While standard short-tandem repeat (STR)-based validation of this line is not possible due to lack of availability of the original source cells, we do routinely check the cells for the presence of keratin K12 and keratin K3 mRNA by PCR. All cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with fetal bovine serum (5%), 1% insulin-transferrin-selenium (ITS; BD Biosciences, Bedford, MA, USA), and 40 μg/mL gentamicin (Life Technologies, Grand Island, NY, USA). Cells were subpassaged using trypsin (Sigma, Ann Arbor, MI, USA) digestion, seeded in 35-mm dishes (Fisher Scientific, Breinigsville, PA, USA), and cultured in a humidified incubator at 37°C with 5% CO2. Culture medium was replaced every 2 days. HCEC and all cultured cells in this study were found to be mycoplasma negative (Mycoplasma Detection Kit; R&D Systems Inc, Minneapolis, MN, USA).

**Mouse Primary Corneal Epithelial Cells (MPCEC)**

VDR WT and VDR KO mice were obtained and bred from the Jackson Labs (Strain: B6.129S4-Vdr<sup>m11Bsd</sup>; Bar Harbor, ME, USA). All animal studies were approved by the University Institutional Animal Care and Use Committee, and animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Primary corneal epithelial cell cultures were established using a modification of the established explant culture method. Briefly, eyes were enucleated, and the cornea was washed with Ca<sup>2+</sup>-free PBS (pH 7.2). Each cornea was cut in half and placed in a 35-mm dish (Fisher Scientific) with the epithelial side up. One and half milliliters of DMEM with 10% serum containing 40 μg/mL gentamicin, 1% ITS, and 100 ng/mL chola toxin (LIST Biological Laboratories, Inc., Campbell, CA, USA) was added and the tissue was cultured in a humidified incubator at 37°C with 5% CO2. Culture medium was replaced every 2 days. Cells were passaged using 0.25% trypsin (Fisher Scientific), and subculturing in DMEM with 5% serum containing 40 μg/mL gentamicin, 1% ITS, and 100 ng/mL chola toxin.

**Real-Time PCR**

Total RNA was obtained from mouse epithelial cells. Real-time PCR was used to quantify DSC2, DSG1, integrin β6, integrin β4, and plectin mRNA levels. RT-PCR primers were generated from the Primer Bank database using National Center for Biotechnology Information (NCBI) sequence identification numbers (NM_013505, NM_181682, NM_001079818, NM_133663, NP_052427 for mouse DSC2, DSG1, integrin β6, integrin β4, and plectin respectively). The primers for mouse TATA box binding protein (TBP; NM_013684) were generated from the Universal Probe Library of Roche Life Sciences. Primers are listed in Table 1.

mRNA was isolated and cDNA was synthesized using the Bio-Rad RT-PCR system. First-strand synthesis was done at 42°C for 60 minutes, and inactivated at 85°C for 5 minutes. Equal amounts of cDNA were applied for PCR amplification in triplicate using the Bio-Rad system and SYBR probes. Amplification was performed at 95°C for 10 minutes, followed by 40 cycles at 95°C for 10 seconds and 62°C for 30 seconds. Quantitative values were obtained from the quantification cycle value (Cq), which is the point where a significant increase of fluorescence is first detected. TBP and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as internal RNA controls, and each sample was normalized on the basis of its gene content (ΔCq). The formula 2<sup>−(ΔΔCq)</sup> was used to analyze the results.

**Transmission Electron Microscopy**

Ten-week-old VDR WT and VDR KO mice were used for transmission electron microscopy (TEM; JOEL 2000; JOEL Ltd., Tokyo, Japan). Eyes were enucleated from euthanized mice and fixed using 4% paraformaldehyde, 2.5% glutaraldehyde, and 0.1 M cacodylate. Fixed eyes were embedded into resin blocks for sectioning. Images (~15,000) were taken of the epithelium and Bowman’s membrane to identify hemidesmosomes.
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Immunofluorescence Studies

Eyes were enucleated from euthanized mice and prepared for cryosectioning. Eyes were flash-frozen in liquid nitrogen and embedded in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA). Ten-micrometer thick cryosections were fixed for 10 minutes in 4% paraformaldehyde (4% PFA) and blocked with 10% goat serum in 0.1% Triton X-100/PBS for 1 hour at room temperature. Cryosections were incubated with primary antibodies (anti-DSC2, anti-DSG1, anti-integrin α6, anti-integrin β4, and anti-plectin) followed by incubation with secondary antibodies. Cryosections were examined using a Zeiss LSM 780 upright laser-scanning confocal microscope (Carl Zeiss Microscopy, White Plains, NY, USA).

Protein Extraction and Western Blot Analysis

Protein was isolated from confluent HCEC, VDR WT, and VDR KO MPCEC cells grown on 35-mm dishes, and from freshly isolated epithelial cells scraped from the corneas of three mice using a Gill corneal knife (Bausch + Lomb Storz Ophthalmic Instruments, Rochester, NY, USA). After washing cells with PBS, they were exposed to lysis buffer (50 mM Tris-HCl [pH 8], 150 mM NaCl, 0.02% N3Na, 100 μg/mL phenylmethylsulfonyl fluoride, 1% NP-40, 50 mM NaF, 2 mM EDTA, and protease inhibitor Sigma). Cell lysates were collected and Western blotting was performed as previously described. Blots were labeled with DSG1, DSC2, integrin β4, integrin α6, and plectin antibodies at a dilution of 1:1000. Membranes were washed and then incubated with anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3000). Detection was performed using the enhanced chemiluminescence method (Pierce Biotechnology, Rockford, IL, USA).

Statistical Analysis

All data are provided as the mean ± SE of at least three experiments. Data was analyzed using the unpaired Student’s t-test only comparing experimental groups against controls. \( P < 0.05 \) was considered statistically significant.

Results

Effects of VDR KO on Desmosome and Hemidesmosome mRNA Expression

Corneal epithelial cells from VDR WT and KO mice were collected, and transcript levels of desmosome and hemidesmosome proteins were assessed by qPCR. Figure 1 demonstrates that mRNA levels of the desmosomal proteins DSG1 and DSC2 were significantly reduced in VDR KO versus WT mice \( (P < 0.05) \). In addition, the mRNA level of the hemidesmosomal crosslinker protein plectin was significantly reduced in VDR KO mouse corneal epithelium \( (P < 0.05) \). There were no significant differences in the mRNA levels of the hemidesmosome proteins integrin α6 or integrin β4. Table 2 summarizes these results as well as all results from this study. TEM images showed decreased hemidesmosomes in VDR KO mouse corneal basal epithelium and Bowman’s membrane (Fig. 2). Desmosomes were not examined due to the difficulty in positively identifying them compared to other lateral membrane junction subtypes.

### Table 1. Summary of the Suggested Primers Pair Sets

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Primer Sequence</th>
<th>Amplicon Size</th>
<th>Temperature</th>
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</thead>
<tbody>
<tr>
<td>Mus musculus Desmocollin 2</td>
<td>Forward</td>
<td>5′-ATGGCGGCTGGGGATCTAT-3′</td>
<td>77</td>
<td>62°C</td>
</tr>
<tr>
<td>Mus musculus Desmoglein 1</td>
<td>Reverse</td>
<td>5′-GCAAGATCGGAGGTG-3′</td>
<td>142</td>
<td>62°C</td>
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<tr>
<td>Mus musculus Integrin α6</td>
<td>Forward</td>
<td>5′-TGGACGAGGCGGAAACATT-3′</td>
<td>175</td>
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<tr>
<td>Mus musculus Integrin β4</td>
<td>Reverse</td>
<td>5′-GACACCATCGACCCATGG-3′</td>
<td>124</td>
<td>62°C</td>
</tr>
<tr>
<td>Mus musculus Plectin</td>
<td>Forward</td>
<td>5′-GCGGAGAAACATTGGG-3′</td>
<td>139</td>
<td>62°C</td>
</tr>
<tr>
<td>Mus musculus TBP</td>
<td>Reverse</td>
<td>5′-GGGTTATCTTACACACCAGT-3′</td>
<td>83</td>
<td>62°C</td>
</tr>
</tbody>
</table>

**Figure 1.** DSC2, DSG1, and plectin mRNA levels were significantly decreased in VDR KO mouse corneal epithelium. Integrin subunit α6 and β4 mRNA levels were not changed \( (P < 0.05, n = 3) \).
immunostaining and Western blotting. Expression of DSG1 and DSC2 was clearly detectable throughout all epithelial layers (Figs. 3a, 3d). Figure 3a shows that DSG1 in VDR KO mice was decreased in corneal wing and basal epithelial cells. DSG1 protein labelling and expression was decreased in VDR KO mouse cornea epithelium compared with WT corneas (Figs. 3b, 3c). DSC2 protein expression was not affected by VDR KO (Figs. 3e, 3f).

Effects of VDR Knockout on Hemidesmosome Protein Plectin, Integrin \( \beta_4 \), and Integrin \( \alpha_6 \) in the Mouse Cornea

Plectin (Fig. 4a), integrin \( \beta_4 \) (Fig. 4d), and integrin \( \alpha_6 \) (Fig. 4e) expression were, as expected for hemidesmosome proteins, confined primarily to the basal cell/Bowman’s membrane interface. Plectin protein labelling and expression were decreased in VDR KO mouse cornea epithelium compared with WT corneas (Figs. 4a–c). There were no significant differences in integrin \( \alpha_6 \), \( \beta_4 \) protein expression in VDR KO versus WT mice (Western data shown in Supplementary data Fig. S1).

Effects of Vitamin D3 on DSG1 Protein Expression in VDR WT and VDR KO MPCEC

DSG1 protein expression was significantly increased in VDR WT MPCEC treated with 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\) (\( P < 0.05 \); Figs. 5a, 5b). DSG1 protein expression was also

### Table 2. Effects of 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\) Treatment on Desmosomal/Hemidesmosomal Components Compared With Untreated Cells

<table>
<thead>
<tr>
<th>Cell and VitD3 Junction Proteins</th>
<th>VDR WT MPCEC</th>
<th>VDR KO MPCEC</th>
<th>HCEC</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1,25(OH)(_2)D(_3)</td>
<td>24R,25(OH)(_2)D(_3)</td>
<td>1,25(OH)(_2)D(_3)</td>
</tr>
<tr>
<td>DSG1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Integrin ( \beta_4 )</td>
<td>NC</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Plectin</td>
<td>NC</td>
<td>+</td>
<td>NC</td>
</tr>
<tr>
<td>DSC2</td>
<td>NC</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Integrin ( \alpha_6 )</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

+, significantly increased; −, significantly decreased; NC, no change.
Increased desmosomal protein expression was observed in VDR KO MPCEC treated with 1,25(OH)2D3 and 24R,25(OH)2D3 (Figs. 5c, 5d).

**Effects of Vitamin D3 on Hemidesmosomal Protein Plectin and Integrin β4 in VDR WT and VDR KO MPCEC**

Plectin and integrin β4 protein expression levels were significantly increased in VDR WT MPCEC treated with 24R,25(OH)2D3 (P < 0.05; Figs. 6a, 6b, 6c, 6d). There were no significant changes in plectin or integrin β4 protein expression in VDR WT MPCEC treated with 1,25(OH)2D3. However, plectin expression was significantly reduced (P < 0.05) in VDR KO MPCEC treated with 24R,25(OH)2D3 (P < 0.05). Integrin α6 protein expression was unchanged in HCEC treated with 1,25(OH)2D3 or 24R,25(OH)2D3 (Fig. 9c).

**Discussion**

This study is the first in any tissue type to specifically examine the influence of vitamin D on desmosomes and in hemidesmosomes in a static epithelium. We initiated the study following our observation when performing corneal epithelial wound healing experiments, that the epithelium was much easier to remove from VDR KO mice than from WT mice. Proper expression of cell adhesion components is essential for corneal epithelial structural integrity. Specific desmosomal proteins expressed in corneas have been shown to be species specific. In bovine cornea, the desmosomal proteins DSC2 and DSG2 were detected in corneal epithelium, while DSC1, DSC3, DSG1, and DSG3 were absent. In rat epithelium, DSG1 and DSG2 were detected. In human corneal epithelial cells, DSG1 and DSG3 were found. Meanwhile, in mice, DSG1, DSG2, DSC1, DSC2, and DSC3 were all detected in the corneal epithelium. For hemidesmosomes, a variety of integrin heterodimers are expressed in the corneal epithelium, many of which have been linked to the adherence of epithelial cells to the stroma. Integrin subunits α6 and β4 were first detected in mouse and rabbit corneal epithelium. Integrin subunits α5, α6, β1, and β4 were detected in human corneal epithelium. In previous studies, plectin was found to be enriched in corneal (and skin) basal epithelial cells. Plectin was found to interact with integrin α6/β4 in migrating epithelial cells and with integrin α5/β6 to promote corneal wound healing. In the current study, we focused on the desmosomal proteins DSG1 and DSC2, and hemidesmosome proteins plectin and integrin subunits α6 and β4.

In previous studies, vitamin D deficiency was found to be associated with both poor wound healing and cell junction abnormalities. Our lab found that 10-week-old VDR KO mouse corneas showed decreased occludin and ZO-1 expression compared with WT, along with a decreased epithelial wound healing rate. Studies by Oda et al. have found that VDR ablation reduces DSG1 expression in the leading edge of healing cutaneous wounds. VDR KO mice have also recently been shown to have decreased lung expression of ZO-1, associated with both poor wound healing and cell junction abnormalities. ZO-1 has been shown to have decreased lung expression of ZO-1, associated with both poor wound healing and cell junction abnormalities. However, the current study found decreased desmosomal formation as observed using TEM in VDR KO mice. DSG1 mRNA and protein expression were significantly reduced in the VDR KO mouse corneal epithelium, and immunohistochemistry revealed reduced DSG1 in the basal epithelial cells of these mice. For hemidesmosomes, we found that plectin mRNA and protein expression were significantly decreased in VDR KO mouse corneal epithelium, while integrin levels were not affected. We conclude that VDR KO attenuates cell–cell adhesion and cell–matrix interactions in cornea not only through the reduction of the desmosomal protein DSG1, but also by inducing abnormal hemidesmosomal function through the reduction of plectin expression.

Oral vitamin D supplementation was found to improve wound healing of patients with diabetic foot ulcers compared with placebo. Vitamin D and calcium signaling in keratinocytes have been found to be required for the normal regenerative response of the skin to wounding. A previous study from our laboratory determined that 24R,25(OH)2D3...
increases corneal epithelial cell migration and proliferation. Moreover, it has been demonstrated that the remodeling of desmosomes is critical for cell proliferation, differentiation, migration. In the current study, we have determined that both 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\) increase DSG1 protein levels in mouse and human corneal epithelium. DSC2 protein levels were also increased in human corneal epithelium treated with 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\). We also determined that 24R,25(OH)\(_2\)D\(_3\) increased the expression of the hemidesmosomal proteins integrin \(\beta_4\) and plectin in mouse and human corneal epithelium. These results, along with our initial observations that VDR KO mice have easily removable epithelium, indicate that vitamin D plays a role in adhesion junction formation and maintenance in the cornea.

![Figure 5](image-url) Desmosomal molecular DSG1 protein expression in WT and VDR KO MPCEC treated with 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\). Representative Western blot (a) and blot densities (b) demonstrating increased DSG1 protein expression in VDR WT MPCEC treated with 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\) (t-test, \(\bar{x} \pm SE, ^*P < 0.05, n = 3\)). DSG1 protein expression (c, d) was also increased in VDR KO MPCEC treated with 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\) (t-test, \(\bar{x} \pm SE, ^*P < 0.05, n = 4\)). Uncropped blots and PVDF membrane images shown in Supplementary Figures S5 to S6.

![Figure 6](image-url) Hemidesmosomal plectin protein expression in WT and VDR KO MPCEC treated with 1,25(OH)\(_2\)D\(_3\) and 24R,25(OH)\(_2\)D\(_3\). Representative VDR WT MPCEC Western blot (a) and blot densities (b) demonstrating increased plectin protein expression in cells treated with 24R,25(OH)\(_2\)D\(_3\) (t-test, \(\bar{x} \pm SE, ^*P < 0.05, n = 5\)). Plectin protein expression in VDR KO MPCEC (c, d) was decreased following treatment with 24R,25(OH)\(_2\)D\(_3\) (t-test, \(\bar{x} \pm SE, ^*P < 0.05, n = 3\)). There was no change in plectin protein expression in WT or VDR KO MPCEC treated with 1,25(OH)\(_2\)D\(_3\). Uncropped blots and PVDF membrane images shown in Supplementary Figures S7 to S8.
It is generally believed that 24,25(OH)2D3 is an inactive form of vitamin D3. Previous work from our laboratory demonstrated that 24R,25(OH)2D3 stimulates both HCEC cell proliferation and migration, which are crucial for corneal epithelial wound healing.29 In the current study, 24R,25(OH)2D3 was found to increase DSG1, integrin $\beta_4$, and plectin protein levels in HCEC and VDR WT MPCEC. Thus, it is apparent that 24R,25(OH)2D3 is active in and beneficial to the anterior segment of the eye.

We found that the desmosomal protein DSG1 was significantly elevated in VDR KO MPCEC treated with 1,25(OH)2D3. Moreover, 24R,25(OH)2D3 was found to increase DSG1 and decrease integrin $\beta_4$ and plectin levels in VDR KO MPCEC. There was no change of integrin $\beta_4$ or plectin expression in mouse or human corneal epithelium, but the integrin $\beta_4$ protein level was decreased in VDR KO MPCEC treated with 1,25(OH)2D3. In our previous study, 1,25(OH)2D3 and 24R,25(OH)2D3 were both found to promote CYP24A1 and CYP27B1 protein expression in VDR KO MPCEC.29 Our data indicate that both VDR and VDR-independent pathways are involved in the regulation of 1,25(OH)2D3- and 24R,25(OH)2D3 on corneal epithelial wound healing.

**FIGURE 7.** Hemidesmosomal integrin $\beta_4$ protein expression in WT and VDR KO MPCEC treated with 1,25(OH)2D3 and 24R,25(OH)2D3. Representative VDR WT MPCEC Western blot (a) and blot densities (b) demonstrating increased integrin $\beta_4$ protein expression in cells treated with 24R,25(OH)2D3 ($t$-test, $x \pm SE$, *$P < 0.05$, $n = 5$). VDR KO MPCEC integrin $\beta_4$ protein expression (c, d) was decreased in cells treated with 1,25(OH)2D3 and 24R,25(OH)2D3 ($t$-test, $x \pm SE$, *$P < 0.05$, $n = 4$). There was no change of integrin $\beta_4$ protein expression in WT MPCEC treated with 1,25(OH)2D3. Uncropped blots and PVDF membrane images shown in Supplementary Figure S9.

**FIGURE 8.** Desmosomal DSG1 and DSC2 protein expression in HCEC treated with 1,25(OH)2D3 and 24R,25(OH)2D3. Representative Western blots and blot densities demonstrating increased DSG1 (a, b) and DSC2 (c, d) protein expression in HCEC treated with 1,25(OH)2D3 and 24R,25(OH)2D3 ($t$-test, $x \pm SE$, *$P < 0.05$, $n = 3$). Uncropped blots shown in Supplementary Figure S10.
24R,25(OH)2D3-induced corneal epithelial desmosomal and hemidesmosomal protein expression, along with the regulation of the expression of additional corneal proteins.

In conclusion, adherens junction proteins are affected by VDR KO. Poorly formed desmosomes and hemidesmosomes likely contribute to the easily removed corneal epithelium in VDR KO mice. Both 1,25(OH)2D3 and 24R,25(OH)2D3 appear to be involved in desmosome/hemidesmosome formation/regulation. Moreover, because both 1,25(OH)2D3 and 24R,25(OH)2D3 affect adherens protein expression levels in VDR KO mouse corneal epithelium, it is likely that vitamin D signaling related to adherens junctions is occurring through both VDR-dependent and -independent pathways. The results from this study indicate that severe vitamin D deficiency may lead to adherens junction alterations in the cornea. While patients with diseases linked to vitamin D deficiency (e.g., rickets, osteomalacia) do not typically present with primary ophthalmic defects linked to their disease state, these adherens alterations could lead to exacerbation of otherwise unassociated ophthalmic pathologies, such as recurrent erosions, diabetic keratopathy, or surgical complications.

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Disclosure: X. Lu, None; M.A. Watsky, None

References


**Figure 9.** Hemidesmosomal plectin, integrin β4, and integrin α6 protein expression in HCEC treated with 1,25(OH)2D3 and 24R,25(OH)2D3. Representative Western blots and blot densities demonstrating increased plectin (a) and integrin β4 (b) protein expression in HCEC treated with 24R,25(OH)2D3. There was no change in plectin or integrin β4 protein expression in HCEC treated with 1,25(OH)2D3 and no change in integrin α6 protein expression in HCEC treated with 1,25(OH)2D3 or 24R,25(OH)2D3 (t-test, α ≤ 0.05, n = 6). Uncropped blots shown in Supplementary Figure S11.
51. Paallysaho T, Tervo K, Tervo T, van Setten GB, Virtanen I. Distribution of integrins alpha 6 and beta 4 in the rabbit
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