

Vitamin D, vitamin D receptor and tissue barriers

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Tissue barriers are critical in the pathogenesis of human diseases, such as atopic dermatitis, inflammatory bowel diseases and various cancers. Preserving or restoring barrier functions of the epithelia cells is a therapeutic strategy to prevent and treat the illness. Mounting evidence indicates that vitamin D and the vitamin D receptor (VDR) play key roles in the pathogenesis of human diseases. In particular, we note an interesting link between vitamin D/VDR signaling and tissue barriers. In the current review, we summarize the recent progress on vitamin D and cell junction complexes. We focus on the functions of VDR and VDR-associated intracellular junction proteins, such as β -catenin and claudins. We also discuss the potential therapeutic functions of vitamin D in treating defective tissue barriers that involve skin, intestine, lung, kidney and other organs. However, the mechanisms for the vitamin D/VDR signaling in tissue barriers remain largely unknown. Further studies on vitamin D/VDR's multiple functions in physiological models will suggest new therapeutic targets for prevention and treatment diseases with defective barrier functions.

Introduction

Classically, vitamin D is known as a key player in calcium homeostasis, electrolyte and blood pressure regulation and immune response.¹ Vitamin D receptor (VDR) is a nuclear receptor² that mediates most known functions of 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}_3$], the active metabolite of vitamin D. Approximately 3% of the mouse and human genomes are regulated directly or indirectly by the vitamin D endocrine system, suggesting widespread functions of vitamin D and VDR in diseases.³⁻⁵ Sunlight exposure is the primary source of vitamin D. Provitamin D is formed in the skin through the action of UV irradiation. It is then metabolized into two different substances within the body: $25(\text{OH})\text{D}_3$ or calcidiol; and $1,25(\text{OH})_2\text{D}_3$ or calcitriol. Vitamin D can also be taken from the diet. Decreased sun exposure limits vitamin D synthesis. Current research has implicated that vitamin D deficiency is a critical factor in the pathology of varieties of cancer as well as inflammatory bowel diseases (IBD), bacterial infection, autoimmune diseases, diabetes, osteoarthritis, periodontal disease, skin disorders and more.⁶⁻¹⁴

Tissue barriers play an essential role in the pathogenesis of some human diseases. Preserving or restoring barrier functions of the epithelial cells is a therapeutic strategy to prevent and treat the illness. In particular, we note a link between vitamin D/VDR signaling and barrier functions. However, how the vitamin D/VDR signaling is involved in tissue barrier related to human diseases remains largely unknown. In the current review, we will summarize the recent progress in the roles of vitamin D/VDR signaling in diseases associated with vitamin D deficiency and tissue barrier defects. We will discuss the potential therapeutic functions of vitamin D/VDR in the related diseases.

Vitamin D and Adherens Junctions

Cell adhesion is the binding of a cell to a surface, extracellular matrix or another cell using cell adhesion proteins. Adherens junctions (AJs) represent a multiprotein complex located at the lateral plasma membrane of contacting epithelial cells. Epithelial AJs are composed by transmembrane proteins such as E-cadherin and nectin and cytosolic scaffolds, α -catenin, β -catenin and p120-catenin. Cellular adhesions are actively involved in signal transduction. β -catenin pathway controls a wide variety of normal and pathological processes, including embryogenesis, differentiation and carcinogenesis.¹⁵⁻¹⁸ Interestingly, the activity of β -catenin can be repressed by activation of VDR.¹⁸ The interaction exists between the activator function-2 domain of the VDR and C terminus of β -catenin. Acetylation of the β -catenin C terminus differentially regulates its ability to activate T-cell factor (TCF)- or VDR-regulated promoters *in vitro*.¹⁸ Vitamin D_3 and VDR affect the Wnt signaling through direct interaction with β -catenin, thus attenuating growth in colon cancer cells.¹⁹⁻²¹

Cancer cells are characterized by decreased expression of E-cadherin and nuclear relocalization of β -catenin. $1,25(\text{OH})_2\text{D}_3$ promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of β -catenin signaling.²² These were observed in human colon carcinoma SW480 cells expressing VDR (SW480-ADH) but not in that of a malignant subline (SW480-R) or metastatic derivative (SW620) cells lacking VDR. Taken together, the published data provide the rationale for using vitamin D in anticancer. The data also indicate the unique role of VDR in regulating structure and signaling functions of adhesion complexes.

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Vitamin D and Tight Junctions (TJs)

The TJs seal the space between adjacent epithelial cells. TJ structure plays a critical role in tissue barriers, host defense and inflammation. TJ protein complexes are composed of integral membrane proteins, cytoplasmic plaque proteins and cytoskeletal proteins. Among these, the claudin family membrane proteins are key components for the structure and function of TJs.²³ Molecular composition of TJs also involves occludin and members of “zonula occludens” (ZO) protein family, such as ZO-1, ZO-2 and ZO-3. Vitamin D₃ induces the expression of occludin, ZO-1, ZO-2 and vinculin. Vitamin D₃ treatment promotes the translocation of ZO-1 to the plasma membrane.²² Treatment with 1,25(OH)₂D₃ is able to abrogate podocytes injury, detected as desmin expression and loss of nephrin and ZO-1. 1,25(OH)₂D₃ administered with a therapeutic regimen may revert proteinuria, counteracting glomerular podocyte injury.²⁴

Corneal epithelial cells had increased transepithelial resistance (TER), decreased inulin permeability and increased occludin levels when cultured with 25(OH)D₃ and 1,25(OH)₂D₃.²⁵ Although 1,25(OH)₂D₃ treatment alone decreased TER in the cultured intestinal epithelial cells,²⁶ 1,25(OH)₂D₃ pretreatment protects cells from increasing permeability induced by Dextran sulfate sodium (DSS) in vitro.²⁷ TJ proteins, such as ZO-1, are upregulated in enterocytes by 1,25(OH)₂D₃. In the DSS-induced colitis mouse model, VDR^{-/-} mice are more susceptible to the DSS treatment compared with the VDR^{+/+} mice.²⁷ These data indicate that VDR may play a critical role in mucosal barrier homeostasis by preserving the integrity of junction complexes and the healing capacity of the colonic epithelium.²⁷

In vitro, VDR deletion in intestinal epithelial cells leads to decreased claudin 2 and 12 that contributed to vitamin D-dependent calcium homeostasis.²⁶ Claudin-2 is known as a “leaky” claudin that forms a paracellular water channel and thus mediates paracellular water transport in leaky epithelia.²⁸⁻³² Christakos et al. also reported that 1,25(OH)₂D₃ downregulates cadherin-17 and upregulates Claudin 2 and 12 in the intestine. Vitamin D mediated intestinal calcium absorption. These data indicate that 1,25(OH)₂D₃, by regulating these epithelial cell junction proteins, can redirect transepithelial transport of calcium toward the paracellular pathway.³³

We summarize the recent reports on vitamin D and cell structural proteins including E-cadherin, β-catenin, ZO-1, claudins and Occludin in Table 1. Further insights into the mechanisms responsible for VDR and barrier dysfunction are still needed in both in vitro and in vivo systems.

Human Diseases Associated with Vitamin D Deficiency and Defective Tissue Barrier

Vitamin D deficiency is caused by inadequate nutritional intake of vitamin D coupled with inadequate sunlight exposure. VDR is expressed in various tissues and mediates functions of 1,25(OH)₂D₃, the active metabolite of vitamin D. Therefore, it is not surprising that many human diseases associated with vitamin D deficiency also involve altered signaling and functions of

VDR. The traditional model of treating cells with vitamin D₃ that guided early vitamin studies is now giving way to a model with more complex mechanisms of action. Furthermore, recent studies provide new evidence for the key role of VDR in the pathogenesis of the diseases and defective tissue barriers. Atopic dermatitis (AD) is a multifactorial, heterogenous disease with defects in epidermal barrier integrity. A recent study has demonstrated that VDR signaling is important in keratinocytes that regulate skin barrier and homeostasis.³⁴

The intestinal epithelial cells play barrier, structural and host defense roles. Defective epithelial barriers have been implicated in IBD and can predict relapse during clinical remission.^{28,35-40} Changes of claudin 2 are associated with active IBD.^{28,38} Dysfunction of the junctional adhesion molecule, JAM-A, induced expressions of claudin 10 or 15.⁴¹ Genes implicated in mucosal barrier function (ECM1, CDH1, HNF4a and laminin B1) confer risk of ulcerative colitis (UC); furthermore, E-cadherin is the first genetic correlation between colorectal cancer and UC. Interestingly, these TJ and cell adhesion proteins are associated with VDR. Several recent studies suggest that VDR stabilizes TJ structure and modulates intestinal inflammation²⁷ and VDR status affects the development of *Salmonella*-colitis and DSS-colitis.^{42,43} In addition to VDR-dependent regulation of tight junctions increasing evidence demonstrates that VDR possesses anti-inflammatory activity.⁴⁴ Delineating the mechanisms that regulate intestinal VDR signaling will help us to understand how to modulate VDR signaling in order to restore normal function of this receptor and reduce chronic inflammation.

In lungs, deficiency of vitamin D is associated with accelerated decline in lung function. Chronic respiratory diseases associated with vitamin D deficiency include cystic fibrosis, interstitial lung disease and chronic obstructive pulmonary disease (COPD).⁴⁵⁻⁴⁸ Deficiency of vitamin D has also been associated with increased risk of respiratory infection from influenza A and *Mycobacterium tuberculosis*.⁴⁹ Absence of VDR in mouse lungs can lead to an early onset of emphysema/COPD because of chronic inflammation, immune dysregulation and lung destruction.⁵⁰ However, to our knowledge, there is no report on VDR regulation of cell junction proteins in lungs.

Therapeutic Potential of Vitamin D/VDR in Diseases Associated with Tissue Barrier

Significant progress has been recently achieved in applying vitamin D₃ analogs and VDR modulators to treat IBD, skin disorders, chronic kidney disease and other diseases. VDR activators, such as calcitriol, doxercalciferol, paricalcitol or alfacalcidol, are used to activate the vitamin D signaling pathways. Lavery et al. demonstrated that intrarectal administration of 1,25(OH)₂-16-ene-20-cyclopropylvitamin D had beneficial effects in the dextran sodium sulfate (DSS) mouse model, without causing hypercalcemia.⁵¹ To reduce the risk of hypercalcemia, Goff et al. used β-glucuronides of vitamin D to deliver 1,25(OH)₂D₃ to the colon to ameliorate colitis, while plasma calcium concentrations were lower in mice treated with β-gluc-1,25(OH)₂D than in mice treated with 1,25(OH)₂D₃.^{52,53} Recently, Miheller et al.

Table 1. Vitamin D and VDR influence the expression and function of cell junction proteins

VDR and proteins	In vitro	In vivo	Conclusion	References
ZO-1, E-cadherin and b-catenin		Rat	Rat treated with 1,25(OH) ₂ D ₃ is able to abrogate podocytes injury, detected as desmin expression and loss of nephrin and ZO-1.	24
	SW480		1,25(OH) ₂ D ₃ induced the expression of adhesion proteins and promoted the translocation of nuclear beta-catenin and ZO-1 to the plasma membrane.	22
Occludin	Corneal epithelium	Mouse, rabbit and human	Vitamin D enhances corneal epithelial barrier function. Cells showed increased TER, decreased IP and increased occludin levels when cultured with 25(OH)D ₃ and 1,25(OH) ₂ D ₃ .	25
	SW480-ADH		ROCK and MSK inhibition abrogates the induction of 1,25(OH) ₂ D ₃ 24-hydroxylase (CYP24), E-cadherin and vinculin and the repression of cyclin D1 by 1,25(OH) ₂ D ₃ .	68
	Caco-2	VDR ^{-/-} Mice, DSS-colitis model	1,25(OH) ₂ D ₃ enhanced TJs by increasing junction protein expression and TER and preserved the structural integrity of TJs in the presence of DSS. VDR knockdown reduced the junction proteins and TER. 1,25(OH) ₂ D ₃ can also stimulate epithelial cell migration in vitro.	27
Claudins			1,25(OH) ₂ D ₃ induces RANKL, SPP1 (osteopontin) and BGP (osteocalcin) to govern bone mineral remodeling; TRPV6, CaBP(9k) and claudin 2 to promote intestinal calcium absorption; and TRPV5, klotho and Npt2c to regulate renal calcium and phosphate reabsorption.	69
	SW480-ADH		1,25(OH) ₂ D ₃ activates the JMJD3 gene promoter and increases JMJD3 RNA in human cancer cells. JMJD3 knockdown or expression of an inactive mutant JMJD3 fragment decreased the induction by 1,25(OH) ₂ D ₃ of several target genes and of an epithelial adhesive phenotype. It downregulated the E-cadherin, Claudin-1 and Claudin-7.	70
		calbindin-D9k ^{-/-} mutant mice	1,25(OH) ₂ D ₃ downregulates cadherin-17 and upregulates claudin2 and claudin12 in the intestine, suggesting that 1,25(OH) ₂ D ₃ can route calcium through the paracellular path by regulating the epithelial cell junction proteins.	33
	Caco-2 cells	VDR ^{-/-} mice	Claudin2 and/or claudin12-based TJs form paracellular Ca ²⁺ channels in intestinal epithelia claudins-2 and -12 are up-regulated by 1,25(OH) ₂ D ₃ through the vitamin D receptor. This study highlights a vitamin D-dependent mechanism in calcium homeostasis.	26
	VDR and mucosal barrier	Caco-2 cells	DSS-colitis model	1,25(OH) ₂ D ₃ play a protective role in mucosal barrier homeostasis by maintaining the integrity of junction complexes and in healing capacity of the colon epithelium.

reported that two 0.25 µg doses of 1,25(OH)₂D₃ per day did not cause hypercalcemia and did improve the Crohn disease activity index in Crohn patients 6 weeks after treatment.⁵³

Oral vitamin D metabolites have been used to correct hypocalcemia in the chronic kidney disease patients with secondary hyperparathyroidism for many years.⁵⁴ VDR activators supplementation is used to enhance the VDR activation and is associated with better survival in the chronic kidney disease patients.^{55,56}

Vitamin D₃ analogs have been used in the topical treatment of psoriasis for a long time.⁵⁷

Cathelicidin antimicrobial peptide is directly regulated by the vitamin D/VDR signaling.⁵⁸ Vitamin D₃ also acts together with parathyroid hormone (PTH) or the shared N-terminal domain of PTH-related peptide (PTHrP), to synergistically

increase cathelicidin and immune defense.⁵⁹ PTH/PTHrP serves to compensate for inadequate vitamin D during activation of antimicrobial peptides.⁵⁹ Hata et al. showed increased cathelicidin in skin biopsies in atopic dermatitis patients after oral vitamin D supplementation.⁶⁰ Calcipotriol or calcipotriene is a synthetic derivative of calcitriol. Calcipotriol treatment could enhance cathelicidin during human skin wounding, which would improve wound healing by increasing re-epithelialization and granulation tissue formation.⁶¹ Calcipotriol also increased cathelicidin in lesional skin in psoriasis.⁶² The healing of wound involves different steps: hemostasis, inflammation, granulation tissue formation and re-epithelialization. Vitamin D-induced cathelicidin promotes the wound healing. However, we did not find any reports on the direct role of cathelicidin in enhancing tissue barriers.

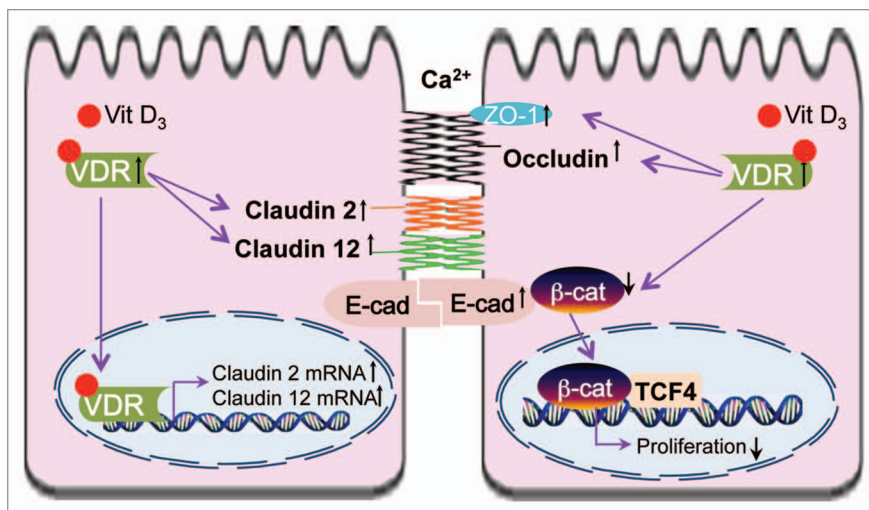


Figure 1. VDR and cell adhesion/TJs of epithelial cells. Activation of VDR suppresses the activity of β -catenin, thus decreasing nuclear β -catenin, suppressing transcription factor T cell factor (TCF) and inhibiting cell proliferation. Increased VDR level is also associated with increased claudin2 and 12, which may play roles in calcium homeostasis and barrier function. The other cell junction proteins involved in the vitamin D/VDR include E-cadherin, Occludin and ZO-1.

1,25(OH)₂D₃ pretreatment enhances the efficacy of allergen immunotherapy in mouse allergic asthma model induced by ovalbumin and aluminum hydroxide.⁶³ Administration of four doses of 2.5 mg vitamin D₃ did not affect time to sputum culture conversion in the whole study population of patients with pulmonary tuberculosis, but it did significantly hasten sputum culture conversion in patients with the tt genotype of the TaqI vitamin D receptor polymorphism.⁶⁴

There is an increasing interest in using vitamin D as an inexpensive and easy supplement for disease prevention. If we do not understand the vitamin D metabolism and VDR mechanisms, vitamin D taken by people may not be used effectively and efficiently. Recently, an alternative vitamin D signaling was reported.⁶⁵ A vitamin D hydroxyl analogs 20(OH)D₂ has antiproliferative and prodifferentiation activities through activation of VDR in a cell-type dependent manner without detectable toxic calcemic activity.⁶⁵⁻⁶⁷ This pathway of vitamin D₃ metabolism initiated by CYP11A1(25-OHase) and modified by CYP27B1 (1 α -OHase) activity.⁶⁵ With the increasing insights into the

vitamin D signaling pathways in defective tissue barriers, new therapeutic strategies may be developed in the near future.

Conclusion

Recent studies of vitamin D and its receptor revealed different cellular functions of VDR that are based on multiple intracellular signaling pathways and molecular targets of this protein. Specifically, VDR appears to regulate molecular composition and functions of different epithelial junctions. As summarized in **Figure 1**, VDR has physical interaction with β -catenin. Activation of VDR suppresses the activity of β -catenin, thus decreasing nuclear β -catenin and inhibiting cell proliferation. VDR status is also directly associated with the expression level and functions of TJ proteins, such as claudin2 and 12. Increased VDR level leads to increased claudin2 and 12, which may play roles in calcium homeostasis and barrier function (**Fig. 1**). The other cell junction

proteins involved in the vitamin D/VDR include E-cadherin, Occludin and ZO-1.

Taken together, vitamin D/VDR signaling regulates not only structural integrity but also transport functions of different epithelial barriers. Since preserving or restoring barrier functions of epithelial barrier has clear beneficial effects during tissue inflammation and other diseases, better understanding effects of VDR on epithelial junctions will lead to development of novel therapeutic strategies for human diseases accompanied by disruption of tissue barrier.

Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest.

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