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RESEARCH HIGHLIGHT

# Vitamin D-dependent microbiota-enhancing tumor immunotherapy

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The antitumor immune response plays a crucial role in successful cancer treatment. In a recent article in Science, Giampazolias et al. [1] reported a vitamin D (VitD)-mediated antitumor mechanism in murine intestinal epithelial cells (IECs) associated with a specific microbiota signature (*Bacteroides fragilis*) that enhances the anti-tumor immune response. The strong positive association between VitD-dependent microbiota and the antitumor immune response provides a proof-of-concept for supplementing immunotherapy regimens with VitD to strengthen antitumor immunity and improve immunotherapy success.

Unlike traditional treatments, immunotherapy does not target the cancer itself but instead trains the immune system to fight cancer. Several types of immunotherapies, including T-cell transfer therapy, immune system modulators, vaccines, monoclonal antibodies, and immune checkpoint inhibitors (ICIs), are now approved for the treatment of many cancer types. Unfortunately, despite clinical success, only a subset of patients respond to immunotherapy (https://www.cancer.gov/about-cancer/treatment/types/immunotherapy) (Fig. 1).

The microbiota (or microbiome) refers to the populations of microorganisms that reside throughout the human body in different niches, such as the oral cavity, esophagus, lungs, stomach, skin, and gut [2]. Recent advances have demonstrated the key role of the microbiota in the diagnosis, development, and treatment of many diseases, including malignancies [2, 3]. In fact, nearly 20% of malignant cancers are associated with microbial infections [4]. Modulation of the microbiota is a promising tool for improving immunotherapy efficacy. The mechanisms underlying these effects are unknown but are likely to involve immune responses and microbiota-modified/derived metabolites [2].

Humans and other mammals can obtain vitamins from their diet or other external sources, or they can be derived/produced by the gut microbiota, and their production is regulated by the bidirectional crosstalk between the immune system and the gut microbiome. One vitamin that influences gut microbiota diversity and immune system regulation is VitD (Fig. 1A) [5]. VitD (calciferol) is produced by the skin *via* an ultraviolet light-dependent photolytic process as $VitD_2$ (ergocalciferol from ergosterol) and $VitD_3$ (cholecalciferol from 7-dehydrocholesterol) and from the diet (20% of total requirements). $VitD_2$ and $VitD_3$ are converted by at least five enzymes in the liver to the inactive prohormone 25-hydroxycholecalciferol (25-$(OH)D_3$), which is hydroxylated by CYP27B1 in the kidney to its active, circulating form 1,25-dihydroxyvitamin D (1,25-$(OH)_2D_3$ or calcitriol) (Fig. 1A). Although VitD is not produced in large quantities by the gut microbiota, its metabolites may have direct or indirect effects on the immune system.

VitD availability is regulated by the blood carrier protein group-specific component (Gc) globulin. In IECS, 1,25-$(OH)_2D_3$ interacts with the VitD receptor (VDR), a ubiquitously expressed nuclear receptor that functions as a ligand-activated transcription factor, to regulate the expression of VitD-responsive genes [5]. Notably, a role for VitD in immune system actions in health and disease has been proposed [6]. In this context, a recent publication by Giampazolias et al. [1] identified a novel regulatory link between VitD, microbiota-specific responses and the enhancement of antitumor immune response, suggesting that VitD is a potential key player in improving the success of immunotherapy (Fig. 1).

Giampazolias et al. [1] used a Gc-deficient mouse model that has low blood levels of VitD [7] to test whether Gc suppresses anticancer $CD8^+$ T-cell responses in transplantable tumors and showed that loss of Gc increased $CD8^+$ T-cell-dependent tumor control and enhanced the response to immunotherapy. These observations demonstrated immune-dependent transmissible tumor resistance, consistent with previous results observed in $sGsn^{-/-}$ mice (mice lacking secreted gelsolin, an extracellular protein circulating in the plasma and secreted by tumor cells) (Fig. 1B).

The authors investigated whether $Gc^{-/-}$ mice and wild-type (WT) controls had differences in microbiota by performing fecal transplantation and coprophagy studies. The results indicated that $Gc^{-/-}$ mice have a genotype-driven divergence in microbiota composition and that $Gc^{-/-}$related components of the microbiota can be transferred in a dominant fashion by coprophagy. Remarkably, fecal transplantation from $Gc^{-/-}$ donors to WT mice suppressed the growth of tumors derived from transplanted melanoma cells, and the administration of antibiotics (vancomycin, metronidazole or neomycin) to $Gc^{-/-}$ mice attenuated this effect. However, the antitumor effect of the fecal transplant from $Gc^{-/-}$ mice was not accompanied by signs of gut inflammation or histological changes in the intestinal barrier. Subsequent analysis to investigate the immune dependency associated with transferable resistance to transplanted tumors indicated a key role for innate and adaptive immunity in the tumor growth suppression effects conferred by the microbiota of $Gc^{-/-}$ mice.

Because $Gc^{-/-}$ mice have lower plasma levels of $VitD_2$ and $VitD_3$ [7], the authors investigated the role of the major VitD ($VitD_3$) in mice.

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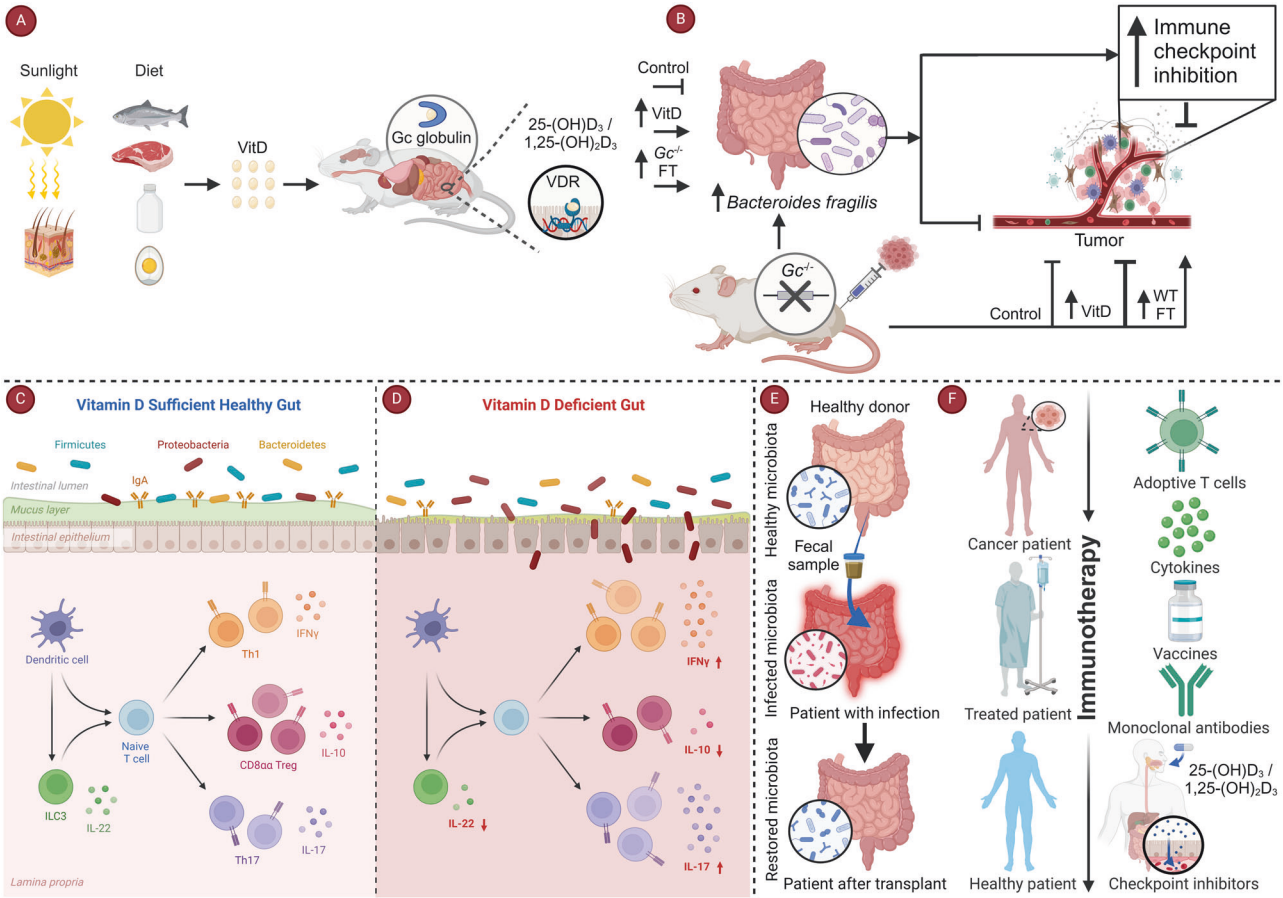


**Fig. 1 Vitamin D availability powers the gut microbiome to improve cancer immunotherapy efficacy. A** Origin, transport, metabolism and mechanism of action of vitamin D (VitD). Overview of the source of VitD through solar ultraviolet radiation on the skin and a VitD-enriched diet (i.e., fatty fish, meats, milk and dairy products, and eggs). **B** Specific microbiota associated with VitD availability. Representation of the transit of the precursor form of VitD (calciferol) in mice through intestinal uptake, its binding to the VitD-binding protein (Gc globulin) and subsequent hepatic and renal processing to generate the metabolic precursors and active VitD compounds 25-hydroxycholecalciferol (25-$(OH)D_3$) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2D_3$), respectively. The binding of active (1,25-$(OH)_2D_3$) to the VitD receptor (VDR) in intestinal epithelial cells (IECs) promotes changes in transcriptomic gene expression that indirectly promote the development of a microbiota rich in *Bacteroides fragilis* that is capable of acting as a repressor/inhibitor of tumor development and increasing the efficacy of anticancer immunotherapy in mice. **C** Schematic of the cellular integrity and immune response in IECs under conditions of optimal and healthy levels of VitD in the gut. **D** Scheme of the effects of severe deficiency of VitD in the gut on the cellular integrity and immune response of IECs. **E** Schematic of the transfer of a microbiome from a healthy donor to a sick patient with a pathological microbiome using fecal transplantation (FT) to strengthen the immune response. **F** Summary of current immunotherapeutic agents used in patients with cancer. The potential role of VitD as a supplement/adjuvant to boost these approaches is related to its impact on the selection or promotion of a microbiota capable of intensifying the activity of the immune system to eliminate tumors. This figure was created with Biorender.com

WT and $Gc^{-/-}$ mice were fed a $VitD_3$-deficient diet for 4 weeks to eliminate VitD reservoirs. This starvation protocol abolished the enhanced ability of $Gc^{-/-}$ mice to suppress tumor growth. In contrast, increased dietary $VitD_3$ supplementation resulted in increased total VitD serum levels and reduced tumor growth in WT mice to the same levels as those in $Gc^{-/-}$ mice fed standard chow. These findings suggest that restoring VitD levels through dietary supplementation increases resistance to transplantable tumors. Further analysis revealed that high VitD levels favor a microbiome that strengthens the antitumor immune response and that the effect can be reversed by fecal transplantation in a VitD-dependent manner.

The authors found that other models of immunologically deficient mice (*Rag1*$^{-/-}$, *Batf3*$^{-/-}$, or *Myd88*$^{-/-}$) did not show a VitD-driven increased antitumor immune response because these mice did not have impaired high $VitD_3$-associated microbiota. However, the fecal material of the above immunodeficient mice fed a high-$VitD_3$ diet induced greater tumor growth suppression when transplanted into WT mice. These results showed that the ability of high VitD availability to alter the microbiome is independent of the immune system. Gene expression analysis of WT mice fed a high-$VitD_3$ diet failed to reveal strong compositional differences in specific immune cell populations but did reveal significant differences in cellular signaling, cell junctions, organization, and defense. This profile was consistent with the ability of VitD to directly regulate the expression of multiple genes *via* VDR. To directly assess the relevance of VDR in IECs, the authors generated a $Vdr^{\Delta IEC}$ strain of mice lacking VDR expression in IECs. However, a diet high in VitD3 did not increase the tumor suppression effect in this strain. Furthermore, under these conditions, fecal material was no longer able to suppress tumor growth compared with that in control mice. These observations suggest that VitD acts through IECs to promote a gut microbiome that enhances immune-mediated cancer control. In addition, metagenomic analysis of fecal samples from mice fed diets with different genotype combinations identified gene products and two taxa that were consistently regulated by VitD availability across conditions. Higher VitD availability increased the abundance of *Bacteroides fragilis*

at the expense of *Prevotella brevis*. This result was confirmed by three rounds of oral gavage of *B. fragilis*, which increased resistance to subsequent tumor transplantation. In contrast, the tumor growth suppression induced by *B. fragilis* was prevented when recipient mice were fed a diet deficient in $VitD_3$. Furthermore, the availability of VitD was necessary to maintain the niche of competent *B. fragilis* to induce tumor growth suppression. Therefore, VitD levels in mice favor a microbiome that potentiates antitumor immune response (Fig. 1B).

Previous findings in humans have indicated the correlation of polymorphisms in genes associated with VitD with biological functions, cancer risk, changes in the microbiota, and/or changes in immune parameters (https://www.ebi.ac.uk/gwas/). The authors created a gene signature (VitD-VDR) consisting of 237 VDR target genes from different human cell types identified using chromatin immunoprecipitation and sequencing datasets. They then examined the VitD-VDR signature in several cancers (skin cancer, sarcoma, hepatocellular carcinoma, breast cancer, and prostate adenocarcinoma) using data from The Cancer Genome Atlas and showed that lower expression of the VitD-VDR signature correlated with poorer survival or more advanced disease. Finally, they analyzed more than 1000 patients treated with ICIs (the CPI1000 cohort) across seven cancer types using bioinformatics tools. They found that low expression of the VitD-VDR signature (and to a lesser extent, low expression of VDR) was associated with resistance to ICIs and faster disease progression. Collectively, these data suggest that in humans and mice, low VitD tissue availability is associated with poorer overall immune-mediated control of tumor growth and cancer outcomes (Fig. 1B).

Several human epidemiological studies have associated high serum levels of total and free VitD with reduced cancer incidence and prolonged patient survival; however, these studies were inconclusive and limited by small sample sizes. Nevertheless, combined analyses from Danish Cancer Registry institutions are consistent with data from preclinical mouse models, suggesting that low serum VitD levels may be a prospective risk factor for cancer development in humans [1].

Giampazolias et al. [1] suggested that VitD homeostasis through a microbiota-mediated VitD-VDR signature plays a major role in cancer incidence, survival, and/or response to immunotherapy. However, significant gaps remain in our understanding of the precise mechanisms that mediate VitD availability and microbiota interactions and how this affects human health and disease outcomes. Future studies should focus on the following questions: (i) Why are there no exacerbated inflammatory/anti-inflammatory responses of the intestine observed in $Gc^{-/-}$ mice to high VitD levels or to the absence of VitD? (Fig. 1C and D). (ii) Could the novel findings of Giampazolias et al. [1] be extrapolated to human settings involving selective microbiota (*B. fragilis*), the immune system, and inflammatory response-associated diseases? (iii) How does VitD-VDR signaling through IECs promote *B. fragilis* expansion? (iv) Can the endogenous endocrine system be reprogrammed in other tissues/organs to modulate VitD-associated immune system responses? (v) What is the immune threshold in each tissue/organ and what is their degree of dependence/independence on the VitD-VDR axis? (vi) What is the impact of VitD/VDR-induced changes in the overall microbiota and the control of VitD levels by the microbiota, and what are the contributions of these changes to differences in inflammatory disease intensity and tropism? (vii) Given the complex etiology of inflammatory disorders during aging, how is VitD-mediated control of tissue immunity maintained in the chronic state? (viii) Through what mechanisms does VitD increase the abundance of *B. fragilis* at the expense of *P. brevis* to favor tumor growth suppression by enhancing local/universal immunity? (ix) Is the regulation of the VitD-VDR axis selective in favoring gain-of-function from a particular microbiota population, and how does sexual dimorphism and the tissue-dependent action of sex hormones during life and aging affect this event? (x) Do VitD-enriched diets and/or fecal supplementation therapies have adjuvant effects on improving the efficacy of immunotherapy strategies? (xi) What are the host factors that allow gut-resident microbes to modulate anticancer immune responses? (xii) How does *B. fragilis* act, and is it sufficient to boost immunity against cancer? (xiii) Should the analysis of serum 25-$(OH)D_3$ levels be included in routine blood tests, and should VitD supplementation be encouraged to maintain optimal circulating levels ($> 20–30$ ng/ml) [5] to avoid the effects of solar ultraviolet radiation and the associated risk of tumor/melanoma development? (xiv) How does local VitD influence tissue pathology in an environmental context dependent on age/aging, development and other environmental conditions, and could microbiota transplantation and healthy dietary habits be used as targets to influence tissue immune systems? (xv) Could massive multidimensional and multifaceted research on VitD regulatory metabolism (and its metabolites) and VitD supplementation trials change public health policies aimed at addressing immune diseases, malignancies, infectious diseases and inflammation/aging-related disorders?

Interestingly, the gut-brain axis is emerging as a major driver of organ function, metabolism, nutritional status, and immune system responses [8]. For example, an immune insult has been shown to strongly activate the body-brain axis to regulate immune responses [8]. More intriguingly, there is evidence to suggest that immune system aging may drive the aging of other organs/tissues [9] and that inhibiting the immune system may slow organ/tissue aging and even rejuvenate organs/tissues [10].

Overall, the findings of Giampazolias et al. [1] establish the functional interplay between microbiota enhancement and the immune response in relation to the VitD-VDR axis and underscore the impact of dietary patterns on these interactions. This exceptional study improves our understanding of the physiological roles of VitD availability and highlights potential strategies for improving the response to cancer immunotherapy. For example, this study highlights the role of the microbiota in tumor immunology and potential approaches to harness the antitumor microbiome for cancer therapy (Fig. 1E and F).

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## COMPETING INTERESTS

The author declares no competing interests.

## ADDITIONAL INFORMATION

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