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Review

Unraveling the complex link between vitamin D levels and cancer: A crucial understanding for designing future supplementation approaches

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Numerous studies have underscored the essential role of sunlight in vitamin D synthesis, while other studies have examined the association between dietary supplementation and vitamin D levels in different oncologic indications. In certain oncologic types, low levels of vitamin D correlate with a higher risk of cancer progression or poorer outcomes. On the contrary, the protective role of vitamin D remains ambiguous for some cancers. Given that the majority of cancer patients exhibit vitamin D deficiency or insufficiency, there have been suggestions to adopt supplementation strategies. However, vitamin D modulates and interacts with several molecular pathways. Therefore, it is crucial to contextualize the level and circumstances in which the action of vitamin D is observed. Distinct outcomes may emerge based on factors such as the method of assessing vitamin D levels, the size of the study populations, their genetic background, and the specific cancer type under investigation. In this article, we summarize some of the relevant studies examining the relationship between vitamin D levels and cancer. We further briefly outline the process of vitamin D synthesis and its effects on specific cellular pathways involved in cancer progression, highlighting essential considerations for future vitamin D assessments and supplementation approaches.

Keywords: Vitamin D. Cancer. Molecular pathway.

INTRODUCTION

A cell becomes cancerous when it accumulates mutations that lead to uncontrolled proliferation, and survival. These mutations can arise from DNA replication errors, inherited mutations, environmental factors, or a combination of these possibilities. While replication errors are the most contributing factor to the cancerous process, our lifestyle significantly influences the onset, progression, and even recovery from most cancers (Tomasetti, Li, Vogelstein, 2017). Examples are tobacco consumption and obesity, both of which have been associated with cancer (for reviews, see Arnold *et al*., 2015; Hecht, 1999). These examples indicate that cancer can be prevented, at least to some extent. Considering the dramatic global rise in cancer cases and the high costs of cancer care and treatment, it is essential to find strategies that help in both cancer prevention and treatment.

Several micronutrients have been studied to identify their potential protective effects against this disease (La Vecchia *et al*., 1994; Pelucchi *et al*., 2009). One of these micronutrients is Vitamin D, a fat-soluble secosteroid that is crucial for the intestinal absorption of other essential nutrients. Its deficiency has been associated with several pathologies, including cancer, diabetes, and cardiovascular diseases (Chiu, Chuang, Yoon, 2001; Garland *et al*., 1989; Skaaby *et al*., 2012; Skaaby *et al*., 2013).

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Different types of studies, such as geographical ecological, cross-sectional, case-control, nested case-control, retrospective, and prospective clinical trials, have yielded conflicting results regarding the association between vitamin D deficiency and cancer. This article briefly describes the complex relationship between vitamin D and cancer, highlighting both the epidemiological and molecular factors involved in vitamin D absorption.

Low levels of vitamin D and limited sunlight exposure increase the risk of colorectal cancer.

In 1980, Garland and Garland's pivotal study drew a connection between colon cancer mortality rates and vitamin D deficiency. Their research showed that the population in the northeast of the United States, with less solar exposure, exhibited higher colon cancer mortality rates compared to those in the south, southeast, and west of the country, where solar exposure was high. The study further analyzed the yearly amount of solar radiation that penetrated the atmosphere at each location, suggesting an association between sunlight exposure and cancer mortality rates (Garland, Garland, 1980). These results led to several studies aiming to establish a link between low vitamin D levels and elevated risk of cancer. However, this relationship remained somewhat elusive.

Shortly after, Garland *et al.,* (1989) undertook another study evaluating the relationship between 25-hydroxyvitamin D (25(OH)D) levels in the blood serum and the risk of colorectal cancer among 25,620 participants. Their findings indicated that concentrations of 25(OH)D at 20 ng/ml or higher were associated with a three-fold reduction in the risk of colon cancer (Garland *et al.*, 1989). Likewise, a meta-analysis showed a dose-response relationship between vitamin D and the prevention of colorectal cancer, indicating that vitamin D intake of ≥ 1000 IU/day or circulating 25(OH)D levels ≥ 33 ng/mL could potentially reduce the risk of incidence rates of colorectal cancer by 50% (Gorham *et al.*, 2007). Subsequent to these findings, a strong body of evidence has suggested a protective effect of vitamin D against colon and rectal cancer, with notable implications on patient survival (reviewed by Dou *et al.*, 2016). Several studies using different approaches have since been conducted to understand the relationship between vitamin D and other types of cancers (Figure 1).

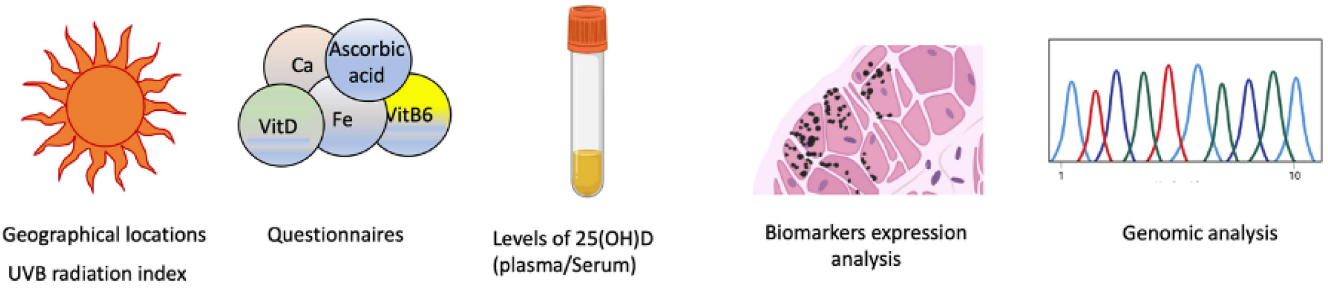

FIGURE 1 - Different approaches used to study the relationship between cancer and vitamin D.

Impact of ethnic backgrounds and lifestyle on vitamin D action.

Although the evidence suggests that solar UVB exposure can offer protection against cancer, the results varied when considering different ethnic backgrounds (Grant, Garland, 2006), suggesting that activation of vitamin D might depend on multiple factors. Thus, elements like obesity, alcohol and tobacco consumption, sedentary lifestyles, and occupation leading to reduced sun exposure could collectively compromise the protective impact of vitamin D against cancer. Moreover, smoking diminishes the availability of the active form of vitamin D and decreases the expression of its receptor. (Okrit *et al.*,

2021; Yang *et al*., 2021). Similarly, alcohol consumption affects the relationship between 25(OH)D and upper gastrointestinal cancers (Abnet *et al*., 2010). These results demonstrate that the interaction of different risk factors influences the action of 25(OH)D at different levels.

Nutrient consumption and vitamin D protective role against cancer.

To further clarify the association between vitamin D and cancer, subsequent studies evaluated the association between different dietary compositions and vitamin D levels and its potential protective role against cancer. In 1994, La Vecchia *et al.* reported that only beta-carotene, ascorbic acid, folate, and nitrate intake produced a protective effect against gastric cancer, whereas vitamin D did not show such an effect. However, consecutive studies demonstrated that nitrates might increase cancer risk, especially in relation to prostate, breast, and colorectal cancer (Chazelas *et al*., 2022; Schullehner *et al*., 2018). Upon analyzing the effects of various micronutrients only beta-carotene and ascorbic acid maintained a slightly protective effect (La Vecchia *et al*., 1994). Likewise, an Italian case-control study found no significant association between the risk of gastric cancer and several micronutrients, including calcium, vitamin B6, and vitamin D. In this study, micronutrient intake was estimated using validated and reproducible food frequency questionnaires, which were based on an Italian food composition database. The study was conducted between 1997-2007 and included 230 patients with histologically confirmed gastric cancer, as well as 547 matched controls (Pelucchi *et al*., 2009).

Interestingly, a prospective study across two cohorts – the Health Professional Follow-up Study, including 46,771 men aged between 40 and 75, and the Nurse's Health Study, including 75,427 women aged between 38 and 65 – revealed an intriguing result. This study found that a high intake of vitamin D was associated with a lower risk of pancreatic cancer, even after adjusting for total calcium intake. In both cohorts, the baseline dietary patterns were assessed through a semiquantitative food-frequency questionnaire, which was filled out in 1984 for the Health Professional Follow-up Study and in 1986 for the Nurse's Health Study. The values for nutrient contents in foods were adjusted to calculate total energy intake and were sourced from the Harvard University Food Composition Database (Skinner *et al*., 2006). However, in 2011, another study failed to confirm these results (Zablotska *et al*., 2011). Consequently, the analysis of micronutrient intake provided little support for a vitamin D protective role against cancer.

Levels of vitamin D and its association with cancer risk.

A retrospective study conducted by Vyas *et al*., (2016) revealed an association between lower vitamin D levels in serum and gastric adenocarcinoma. This study encompassed patients who were diagnosed with gastric adenocarcinoma and whose vitamin D levels between 2005 and 2015 were documented. Despite the limited sample size, with only 49 patients (and their corresponding matched-controls) included in the study, a higher prevalence of gastric adenocarcinoma was observed in patients with 25(OH)D deficiency (<20 ng/mL) or 25(OH)D insufficiency (between 20-29 ng/mL) in comparison to those with normal levels (≥ 30 ng/mL) (Vyas *et al*., 2016). A similar trend was obtained in another study that analyzed 197 patients with gastric carcinoma (Ren *et al*., 2012). Conversely, another prospective study, including 545 esophageal squamous cell carcinomas (ESCC) and 353 patients with gastric cardia adenocarcinomas, found no discernible link between low vitamin D status and these particular cancer types (Chen *et al*., 2007). Correspondingly, no definitive correlation has been established between low 25(OH)D levels in plasma and cancers, such as pancreatic, ovarian, endometrial, kidney, upper gastrointestinal, and non-Hodgkin lymphoma, (as reviewed in Helzlsouer, Gallicchio, 2013). The relationship between circulating 25(OH)D concentrations and cancer risk necessitates meticulous examination. For example, the pooled nested case-control study performed by Stolzenberg-Solomon *et al*., (2010), which consolidated data from eight cohorts under the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (VDPP), using data from 1974 to 2006 with a median follow-up of 6.5 years, found a significant result. Elevated concentrations of 25(OH)D exceeding 100 nmol/L (40 ng/mL), were associated with a 2-fold increase in the overall risk of pancreatic cancer (odds ratio = 2.12, 95% confidence interval: 1.23-3.64)

(Stolzenberg-Solomon *et al.*, 2010). Furthermore, low levels of 25(OH)D were not associated with a heightened risk of pancreatic cancer. Hence, the available evidence suggests that low levels of vitamin D in plasma don't universally correlate with cancer risk. Given that vitamin D impacts several biological functions and affects multiple organ systems, it is conceivable that its action may differ depending on the specific context of each cancer type. Prospective and nested case-control studies show that an elevated concentration of 25(OH)D in the blood serum was associated with a decreased incidence of colorectal cancer, as well as other cancers such as bladder and lung. Nevertheless, this association becomes weak or even inconclusive in the context of breast and pancreas cancers (Mondul *et al.*, 2017). Yin *et al.*, (2010) also failed to find a significant association between vitamin D levels in plasma and breast cancer. The meta-analysis incorporated ten articles with both case-control and prospective studies where 25(OH)D measurements were taken years before diagnosis. While the case-control studies implied a protective effect of high 25(OH)D levels (measured after diagnosis), this inverse relationship was not corroborated in the cohort studies (Yin *et al.*, 2010). Different types of studies used to analyze the association between vitamin D and cancer are summarized in Table I.

Different approaches to measure Vitamin D and the discrepancy in study findings

In 2006, Giovannucci *et al.,* (2006) employed two distinct approaches to determine vitamin D status. Firstly, vitamin D exposure was assessed based on various factors, including vitamin D intake, use of vitamin D supplements, skin pigmentation, adiposity, leisure-time physical activity, and geographic location. Secondly, plasma 25(OH)D concentration was measured (Figure 1). The study was conducted within a cohort of 1,095 men from The Health Professionals Follow-Up study. Multiple linear regression models, along with a multivariable Cox proportional hazard model, were used to ascertain the relationship between the 25(OH)D levels and cancer risk within a larger cohort (comprising 47,800 men) from the same Health Professionals Follow-Up Study. The results suggested that low levels of 25(OH)D in the plasma might be related to an increase in cancer incidence and mortality, particularly for digestive cancers (Giovannucci *et al.*, 2006). Although measuring vitamin D levels in plasma seems to be a more direct way to assess the association between vitamin D and cancer, subsequent studies employing this approach gave mixed results. In this direction, the nested case-control study by Abnet *et al.*, (2010), which utilized information from the VDPP to investigate 1,065 cases failed to identify a significant association between the risk of gastric and esophageal cancer and low levels of 25(OH)D in plasma. Interestingly, the study revealed that among Asian populations and those who never smoked, lower levels of 25(OH)D in plasma (<25 nmol/L) were actually associated with a decreased risk of upper gastrointestinal cancers (Abnet *et al.*, 2010). Likewise, in a prospective study by Skaaby *et al.*, (2014) no distinct association was established between low levels of 25(OH)D and total or specific types of cancers. However, the results did indicate a higher risk of non-melanoma skin cancer in cases where vitamin D status was higher. This study included 12,204 individuals aged 18 to 71 years. Particularly, it analyzed three cohorts from the general Danish population: the Monica10 study from 1993 until 1994 including 2,656 individuals; the Inter99 study spanning from 1999 to 2001, comprising 6,784 individuals; and the Health2006 study conducted from 2006 to 2008, involving 7,931individuals. Nonetheless, the methodology for measuring 25(OH)D levels in plasma varied across these three cohorts. Specifically, the Monica10 study measured 25(OH)D levels using the IDS-SYS 25-Hydroxy Vitamin D method, while the Inter99 study employed high-performance liquid chromatography to determine 25(OH)D levels, and the Health2006 study utilized an immunoassay called Cobas e411 from Roche Diagnostics (Skaaby *et al.*, 2014). Despite the extensive longitudinal population analysis in Skaaby *et al.*'s (2014) study, coupled with long-term follow-ups and standardized registry-based diagnoses, the utilization of different devices to estimate 25(OH)D levels might reduce the predictive accuracy. This could potentially mask the true association between vitamin D and the cancers under investigation.

The different methodologies used to measure vitamin D levels could account for the contradictory results obtained in these different studies, eclipsing a clear interpretation regarding the true association

between vitamin D and cancer. These results indicate that beyond the advantages of large longitudinal populations, long-term follow-ups, and standardized registry-based diagnoses, it is crucial to have a comprehensive understanding of the study's methodologies and its inherent limitations.

TABLE I - Type of studies to analyze the association between vitamin D and cancer

Sample	Number of participants or number of studies	Year published	Type of study	findings	Relevant cancer or risk factor	References
Serum (25(OH)D)	25,620	1989	Geographical	High leves is associated with with a three-fold decrease in colon cancer	colon	Garland *et al*., 1989
Serum (25(OH)D)	5	2007	Meta-analysis	Circulating 25(OH)D levels ≥ 33 ng/mL reduce the risk of incidenceof colorectal cancer	colorectal	Gorham et al., 2007
UVB index	Data for Caucasians age-adjusted of total U.S population (period: 1950-1994)	2006	Ecological study	Solar UVB, through photosynthesis of vitamin D, is inversely associated with cancer mortality rates	several cancers	Grant and Garland, 2006
Serum (25(OH)D)	24 (11340 participants included)	2021	Meta-analysis	Smokers present lower levels of circulating vitamin D	smoking	Yang et al, 2021
Ciculating 25(OH)D (plasma or serum)	1,065 cases and 1,066 controls	2010	Case-control studies	Administration of vitamin D fail to diminish risk of upper gastrointestinal cancers	esophageal and Gastric Cancer	Abnet et al, 2010
questionnaire	723 patients of gastric cancer and 2024 controls	1994	Case-control studies	Vitamin D levels is not associated with a protective role against gastric cancer	gastric cancer	La Vecchia et al 1994
questionnaire	230 gastric cancer patients and 547 matched controls	2009	Case-control studies	No significant relationship between gastric cancer risk and vitamin D among other micronutrients	gastric cancer	Pelucchi et al., 2009
semiquantitative food-frequency questionnaire	46,771 men (the Health Professionals Follow-up Study), and 75,427 women (the Nurses' Health Study)	2006	Prospective studies	Potential role for vitamin D in prevention of pancreatic cancer.	pancreatic cancer	Skinner et al, 2006
semi-quantitative food-frequency questionnaire.	532 pancreatic cancer patients	2011	Population-based case–control study	Increased risk of pancreatic cancer associated with dietary intake of vitamin D and of calcium	pancreatic cancer	Zablotska et al, 2011
Serum (25(OH)D)	49 patients and 49 control	2016	Retrospective case control study	Lower vitamin D levels in serum are associated with gastric adenocarcinoma	gastric adenocarcinoma	Vyas et al, 2016
Serum (25(OH)D)	197 gastric carcinoma patients	2012	Retrospective study	Patients with high vitamin D levels group had a higher overall survival compared with the low vitamin D levels group	gastric adenocarcinoma	Ren et al, 2012
Serum (25(OH)D)	545 esophageal squamous cell carcinoma and 353 gastric cardia adenocarcinoma patients	2007	Prospective study	No associations for gastric cardia or noncardia adenocarcinoma were found. However, in subjects with low vitamin D status, higher serum 25(OH)D concentrations were associated with significantly increased risk of ESCC in men, but not in women	esophageal squamous cell carcinoma and gastric cardia and non cardia adenocarcinoma	Chen et al, 2007
Serum (25(OH)D)	952 cases and 1,333 controls	2010	Pooled nested case-control study (including ATBC Study, CLUE, the Cancer Prevention Study II Nutrition Cohort (CPS-II), the New York University Women's Health Study (NYU-WHS), the Multiethnic Cohort Study (MEC), the PLCO, and the Shanghai Women's and Men's Health Studies (SWHS and SMHS).	No significant associations were observed for participants with lower 25(OH)D status. Higher levels of 25(OH)D was associated with a 2-fold increase in pancreatic cancer risk overall	primary pancreatic adenocarcinomas	Stolzenberg-Solomon et al, 2010
plasma 25(OH)D	69 studies included (17 cohort and 21 nested case-control, 30 case-control studies)	2010	Meta-analysis	A protective effect of vitamin D is observed in case-control studies when stratifying in premenopausal and postmenopausal women, but not significant association is found in cohort studies	breast cancer	Yin et al, 2010
plasma 25(OH)D	12,204	2014	Prospective population-based study	Not significant association between low levels of 25(OH)D and total or specific cancers	several cancers	Skaaby T et al, 2014
Serum (25(OH)D)	1546 African American women and 1426 white women	2002	National Health and Nutrition Examination Survey (1988-1994)	Dark-skin populations present lower levels of 25(OH)D in plasma	risk-factor	Nesby-O'dell *et al.*, 2002

Limitations in assessing vitamin D levels and its influence on cancer.

Analyzing factors such as UVB radiation exposure, micronutrient intake, or directly measuring 25(OH) D levels in the plasma might provide only a partial answer on the relationship between vitamin D levels and cancer. This is especially true considering that these parameters change over time in response to a variation in an individual's lifestyle.

The genetic and nutritional heterogeneity of the individuals included in the studies can further affect vitamin D assessment. Moreover, the consumption of certain animal products, notably meat and eggs, can raise vitamin D levels in the blood. Consequently, it is crucial for studies to consider the dietary habits of their subjects, determining whether they mainly adhere to vegetarian diets or consume meat-based diets. Skin pigmentation plays a pivotal role in vitamin D synthesis (Mosekilde, 2008), and should be carefully taken into account when examining heterogeneous cohorts. Furthermore, individuals with darker skin tend to have lower levels of 25(OH)D in plasma compared to those with a white Caucasian background (Nesby-O'dell *et al.*, 2002). Likewise, gender distinctions can present similar challenges, especially for ESCC (Chen *et al.*, 2007).

Additionally, there are other factors to consider. For instance, Plasma levels of 25(OH)D fluctuate with the changing seasons; as a consequence, taking a single blood sample at a specific time of the year (like spring) poses a statistical risk in capturing accurate vitamin D levels. The follow-up time can also affect the accurate assessment of the individual's true vitamin D levels. In prospective studies with extended follow-up times durations (exceeding 3 to 4 years), there is usually a failure to identify an association between vitamin D levels and the incidence of cancer, which is not surprising considering the long gap between the time the blood sample was collected and the eventual point of study evaluation. Collecting multiple blood samples at the onset and during the course of the study could mitigate the aforementioned problem (Giovannucci *et al.*, 2006). In addition, other limitations may arise from the stage of the disease at the time of diagnosis, which can influence 25(OH)D concentrations. Thus, to truly understand the impact of vitamin D levels on cancer, it is necessary to complement the previously mentioned studies with insights from genetic backgrounds, lifestyle factors (like physical activity, alcohol, and tobacco consumption), and investigations into molecules that are downstream in the vitamin D signaling pathway.

From vitamin D to calcitriol and its effect on gene expression.

Indeed, evidence indicates that the main source of vitamin D is solar radiation (290-315nm), which facilitates the production of vitamin D in the skin by transforming 7-dehydrocholesterol into vitamin D3 (Grant, 2018). Animal foods such as salmon, liver, meat, and eggs; along with fortified foods like milk and orange juice, are secondary contributors of vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Vegetable-based foods are even less efficient sources of vitamin D (Feldman *et al.*, 2014; Stolzenberg-Solomon *et al.*, 2010).

The process by which vitamin D is converted to its activated form has been extensively reviewed elsewhere. Here, we provide a brief overview of this process and highlight the most important molecular targets. Upon circulation, both vitamin D3 and D2 can bind to vitamin D-binding proteins (DBP) and transport to the liver, where a hydroxylation reaction occurs at the carbon-25 position. Most of the studies focus on measuring this circulating form of vitamin D, known as 25(OH)D. Several cytochrome P450 enzymes (CYP) have been described as responsible for the hydroxylation process; however, the prevailing evidence points to CYP2R1 as the major player (for review see, Feldman *et al.*, 2014). Then, 25(OH)D3 is transported to the kidneys, where it is further modified by cytochrome CYP such as CYP24A1 or CYP2R1 (Cheng *et al.*, 2003) and CYP27B1 (Takeyama *et al.*, 1997) (Figure 2). Importantly, CYP24A1 (24-hydroxylase) targets both 1,25(OH)2D3 and 25(OH) D3 for excretion, whereas CYP27B1 (1α-hydroxylase) is responsible for converting 25(OH)D into 1,25(OH)2D3 (calcitriol) (for review see, Prosser, Jones, 2004). This calcitriol is considered the predominant active form of

vitamin D, although it is a less quantifiable form than the traditional 25(OH)D.

Changes in the expression pattern of CYP27B1 have been observed during cancer progression, with a reduction observed in less differentiated tumors (Bises *et al.*, 2004). Administration of 1,25(OH)2D3 can reduce tumor progression, and mutations in CYP27B1 within mammary tissue have been shown to accelerate tumor growth in mouse models of breast cancer (Li *et al.*, 2016). Moreover, 1,25(OH)2D3 plays an important physiological role, where it regulates the expression of calcium transporter 1 (calcium channel) in the duodenal tissue. This, in turn, influences calcium absorption or excretion and acts as an endocrine hormone in different tissues (Song *et al.*, 2003).

The active form 1,25(OH)2D3 binds to the vitamin D receptor (VDR), which is a ligand-activated transcription factor belonging to the steroid hormone receptor superfamily (Yoshizawa *et al.*, 1997). While VDR is mainly located in the nucleus, it is also present in the cell cytoplasm (Clemens *et al.*, 1988; Fleet, *et al.*, 2012). In the nucleus, the calcitriol-VDR complex forms a heterodimer with the retinoid X receptor (RXR), which binds to response elements (VDREs) located in regulatory regions across different genome locations. It attracts a range of co-activators, including histone acetyltransferases and the mediator complex subunit 1 (MED1), thereby regulating gene expression (reviewed in Deeb, Trump, Johnson, 2007; Feldman *et al.*, 2014; Pike, Meyer, Bishop, 2012) (Figure 2). Some of the target genes activated by this mechanism are integral parts of the osteoblast program, including r*BGP*, m*SPP1*, m*LRP5*, and m*RANKL*. Meanwhile, other target genes play roles in crucial processes such as mineral regulation, detoxification, metabolism, and cell cycle control (reviewed in Haussler *et al.*, 2013). Vukić *et al.*, (2015) demonstrated a strong correlation between serum 25(OH) D3 levels and the mRNA expression of twelve VDR target genes in peripheral blood mononuclear cells. Among these genes, eight of them – *STS*, *BCL6*, *ITGAM*, *LRRC25*, *LPGAT1*, *TREM1*, *DUSP10*, and *CD14* – together with parathyroid hormone (PTH) serum levels were proposed as a tool for monitoring the in vivo response to vitamin D supplementation, both for long-term and short-term periods (Vukic *et al.*, 2015). Moreover, ChIP-chip, ChIP-sequencing, and transcriptome-wide analysis of several genes responsive to vitamin D supplementation have unveiled protein-protein and DNA-protein interaction, helping understand the extended and complex action of 1,25(OH)2D3 (Heikkinen *et al.*, 2011, Ramagopalan *et al.*, 2010).

The calcitriol-VDR-RXR complex has the ability to modify the chromatin landscape by recruiting nuclear receptor corepressor(s), histone deacetylases, and demethylases in the vicinity of target genes (Haussler *et al.*, 2013), leading to the repression of genes such as the gene that encodes cytochrome p450 27B1 (CYP27B1) (Kim *et al.*, 2009). Importantly, VDR, in some cases, binds to regulatory regions without the presence of calcitriol. These binding regions can be found either within intronic sections or intergenic areas, regardless of their proximity to the regulated genes.

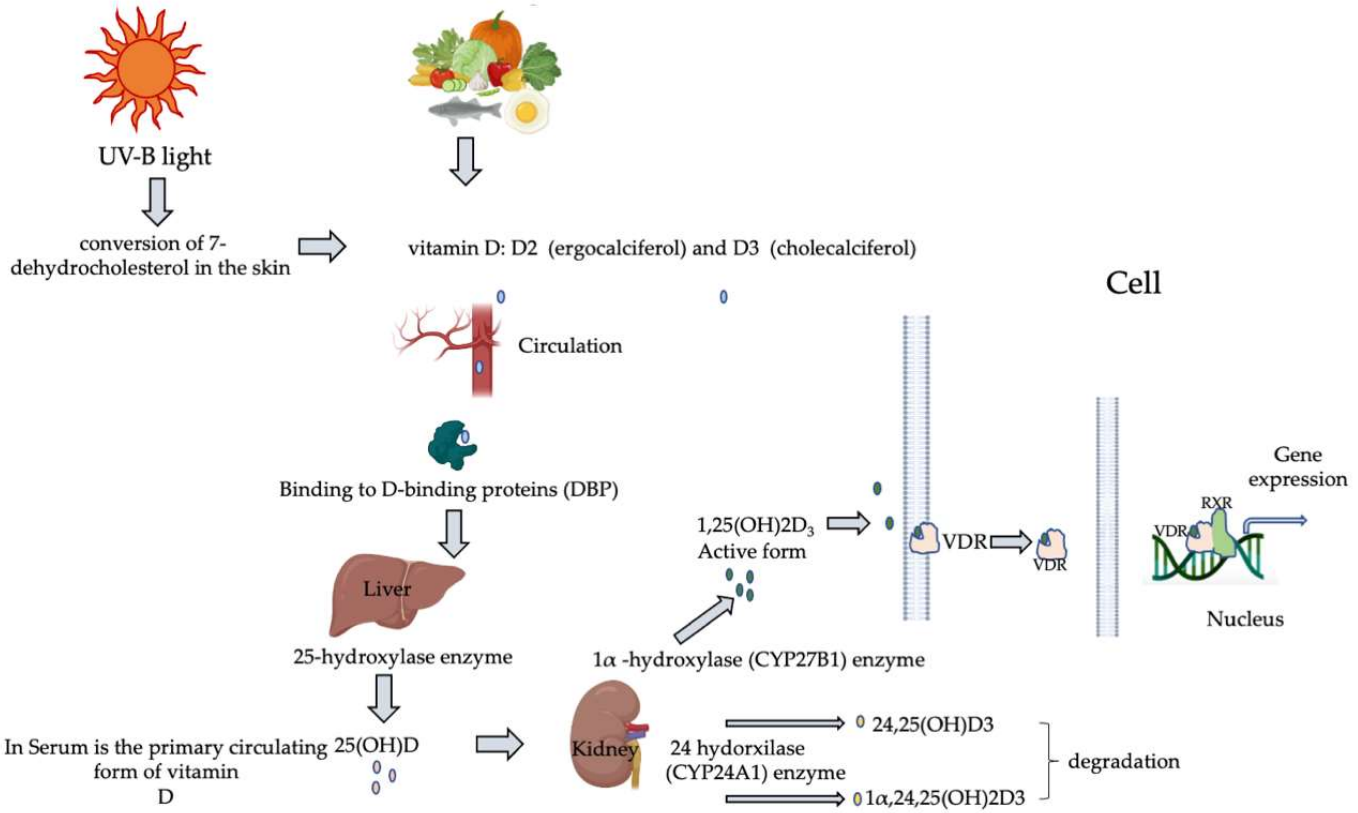

FIGURE 2 - Vitamin D synthesis pathway. The sunlight transforms the precursor 7-dehydrocholesterol into vitamin D3. The diet is another source of vitamin D3. Once in the body, vitamin D3 binds to D-binding proteins and travels to the liver, where it is converted to 25-hydroxyvitamin D (25(OH)D) by 25-hydroxylase enzymes. As 25(OH)D travels to the kidney, where it is converted into its active form, 1,25-dihydroxyvitamin D (1,25(OH)2D3), also known as calcitriol, by 1α-hydroxylase enzyme. On the other hand, the 24-hydroxylase enzyme promotes excretion. Calcitriol binds to vitamin D receptor (VDR), forming a heterodimer with the retinoid X receptor (RXR), and response elements (VDREs) on the genome, promoting the recruitment of several co-activators, histone acetyltransferases, and the mediator complexes.

Action of calcitriol on cell signaling pathways: a brief overview of the molecular mechanism and its potential effect in cancer.

The calcitriol levels can modulate various oncogenes and tumor repressor genes. However, this modulation seems to occur by affecting signaling pathways rather than through the direct interaction of VDR with VDREs. For example, 1,25(OH)2D3 induces the expression of *CDH1*, which encodes E-cadherin, promoting the translocation of β-catenin from the nucleus to the plasma membrane. Consequently, this shift favors cell differentiation and restrains cell growth by inhibiting β-catenin target gene expression (Palmer *et al.*, 2001). Furthermore, calcitriol can repress the glucose transporter 1 (GLUT1), several glycolytic proteins, glycolysis, and Wnt/ β-catenin target genes, including *CCND1*, encoding cyclin D1, and *c-MYC*, reducing cell growth and proliferation in human colorectal cancer cells (Huang *et al.*, 2021). Likewise, functional analysis conducted on a non-transformed prostate epithelial cell line, RWPE1, suggests that 1,25(OH)2D3 has an anti-prostate cancer effect by suppressing important pathways such as Wnt, Notch, NF-κB, IGF1, and inflammation (Kovalenko *et al.*, 2010). Calcitriol also has the capability to inhibit the Vascular Endothelial Growth Factor (VEGF) in cell lines, as well as in nude mice models implanted with MDA-435S breast carcinoma cells and MCF-7 breast cancer

cells that have an overexpression of VEGF (Mantell *et al.*, 2000). Additionally, 1,25(OH)2D3 plays a crucial role in the differentiation, maturation, and functioning of tolerogenic dendritic cells (Piemonti *et al.*, 2000), Treg development (Hafkamp *et al.*, 2022) and in T cell helper 2 activation (Boonstra *et al.*, 2001). On the other hand, there are reports indicating that the expression of the SNAIL transcription factor in human colon tumors can repress the human VDR gene promoter, thus diminishing the anticancer efficacy of calcitriol (Palmer *et al.*, 2004). During osteogenesis and in situations of high glucose-induced oxidative stress, 1,25(OH)2D3 activates Wnt/β-catenin signaling, promoting osteoblast proliferation and inhibiting apoptosis (Xiong *et al.*, 2017). Such findings suggest that the response to calcitriol is context-dependent.

Besides, calcitriol can upregulate the mitogen-activated protein kinase 5 (DUSP10), and by doing so can suppress the expression of p38 stress kinase signaling and the pro-inflammatory program (Feldman *et al.*, 2014). In addition, kinase pathways such as phosphatidylinositide 3-kinase (PI3K), phospholipase Cγ (PLCγ), Ras, and ERK1 signaling are also modulated by 1,25(OH)2D3. Along these lines, Yang *et al.*, (2015) reported that the pathways MEK/ERK and PTEN/PI3K/AKT/mTOR are essential for vitamin D-induced autophagy. Autophagy is a cellular process that helps in maintaining cellular homeostasis in nutrient-rich environments or providing energy during conditions of cellular starvation (Yang *et al.*, 2015). Likewise, in osteoblast, the activation of the VDR/PI3K/AKT pathway exhibits an antiapoptotic effect (Zhang, Zanello, 2008).

Another example is the biological effect of calcitriol on MEK1/2, ERK1/2, JNKs signaling pathways, and p38 kinases during acute myeloid leukemia (AML), which ends with differentiation of AML cells (reviewed in Gocek, Studzinski, 2015). In colon cancer cells, calcitriol also induces differentiation through the activation of PKC- and JNK-dependent JUN signaling. Alternatively, calcitriol exerts a pro-apoptotic effect mainly by suppressing BCL2 and inducing the expression of BAX, BAK, and BAD (Blutt *et al.*, 2000; Deeb, Trump, Johnson, 2007). In epithelial ovarian cancer cells, studies have shown that calcitriol can destabilize telomerase reverse transcriptase (*TERT*) mRNA. This leads to the induction of apoptosis due to the down-regulation of telomerase activity (Jiang *et al.*, 2004). Moreover, calcitriol can stimulate transforming growth factor-β (TGFβ) signaling, inhibiting cell growth and promoting differentiation (Welsh, 2012). It also contributes to the reduction of angiogenesis in human tumoral cells by overexpression of thrombospondin 1 (THBS1) (Fernandez-Garcia *et al.*, 2005), inhibition of hypoxia-inducible factor (HIF)-1 pathway and suppression of VEGF. Yet, in certain scenarios, calcitriol might actually foster angiogenesis by inducing multipotent mesenchymal stromal cells via the activation of the PI3K/AKT pathway (Ye *et al.*, 2020) (Figure 3). Thus, understanding how calcitriol can affect the interplay among the signaling pathways involved in cancer opens new opportunities for clinical approaches.

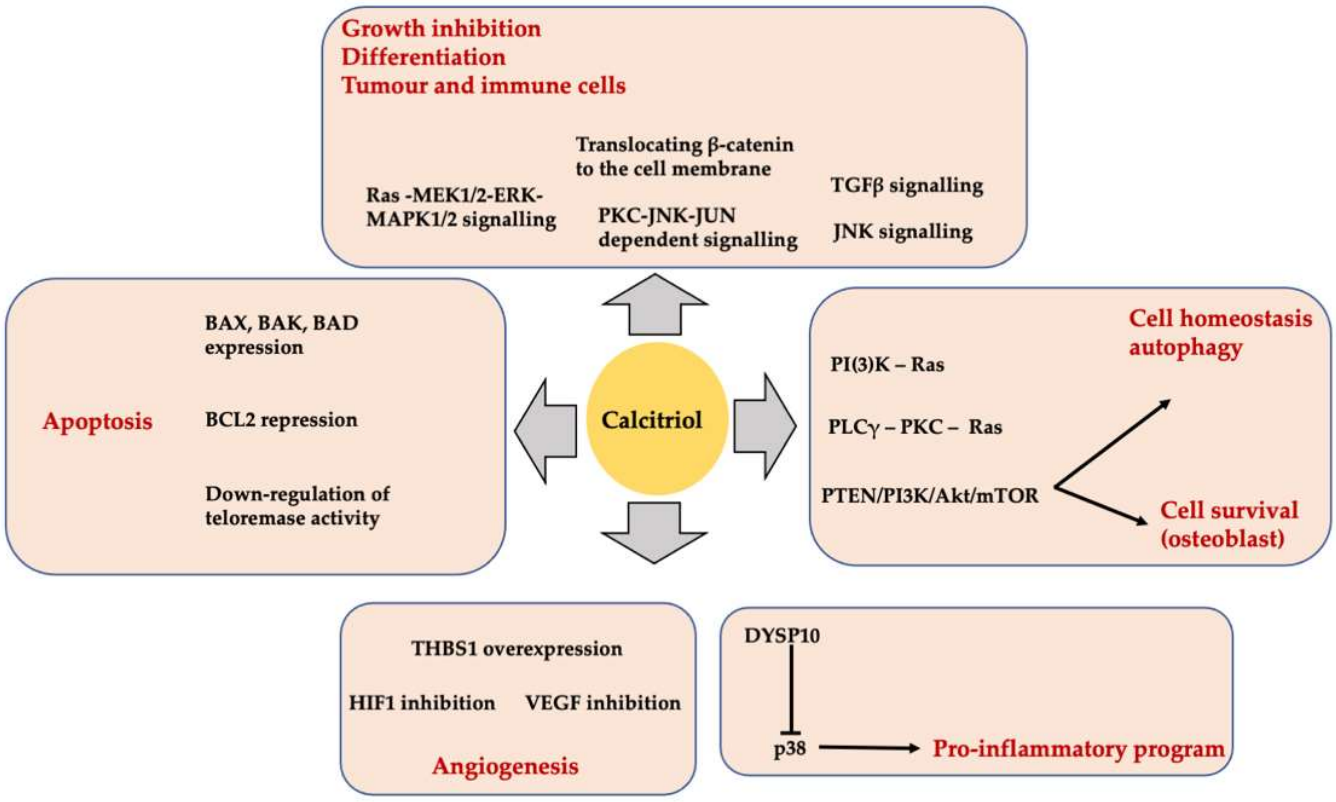

FIGURE 3 - Calcitriol influence on different biological processes. Briefly, calcitriol modulates the pro-inflammatory response, the angiogenesis process, cell survival and autophagy, cell death, and several pathways involved in cell differentiation and growth.

Vitamin D supplementation as an anticancer strategy.

The impact of calcitriol on different signaling pathways has been validated in cell lines and xenograft mice models. Nonetheless, caution must be exercised when directly extrapolating these findings to cancer patients. Each cancer situation is unique, and patients possess distinct genetic backgrounds and comorbidities. For instance, the analysis of the VITAL randomized clinical trial shows that being overweight hampers the protective effect of vitamin D supplementation against the incidence of advanced (metastatic or fatal) cancer (Chandler *et al.*, 2020).

It has been reported that patients with diseases such as cancer and rheumatoid arthritis present vitamin D deficiencies. We recently analyzed 312 patients (comprising 90 males and 222 females) with different oncologic indications (averaging 60 ± 12 years old). We observed that these patients displayed low 25OH vitamin D levels, with an average value of 21± 8 ng/mL (results not published yet). The challenge lies in identifying whether this deficiency is associated with a modifiable lifestyle behavior that can be addressed or if it arises from disruption in enzymes involved in the transformation of vitamin D to calcitriol or from the dysregulation of specific cell signaling pathways. The understanding of the molecular action of calcitriol has led to new treatment opportunities, such as by combining vitamin D supplementation with inhibitors, including RAD001 (Everolimus) (Yang *et al.*, 2010), as well as COX1 and COX2 inhibitors (Jamshidi *et al.*, 2008) (Figure 4).

The expression of VDR is vital for the anticancer effect of vitamin D supplementation (Matthews *et al.*, 2010; Palmer *et al.*, 2004). Moreover, the decreased VDR expression is associated with tumor progression

(Kallay *et al.*, 2002). In the context of colon cancer, VDR is expressed during the early stages but experiences downregulation as the disease progresses, attributed to SNAIL upregulation (Palmer *et al.*, 2004). This phenomenon has been confirmed in patients diagnosed with malignant gastric tumors. The VDR expression is downregulated, especially in poorly differentiated gastric tissues (57.61% compared to 73.64% in premalignant tissues and 82.61% in normal tissues) (Wen *et al.*, 2015). These results suggest that these patients may exhibit a low activation of 1,25(OH)2D3-induced biological responses. Patients showing a reduction in DBP or an aberrant expression of CYP24A1 might also lack the protective effect of vitamin D (Horvath *et al.*, 2010). Thus, measuring the levels of these molecules in cancer patients could aid in the design of vitamin D supplementation strategies and in structuring clinical trials involving vitamin D metabolites.

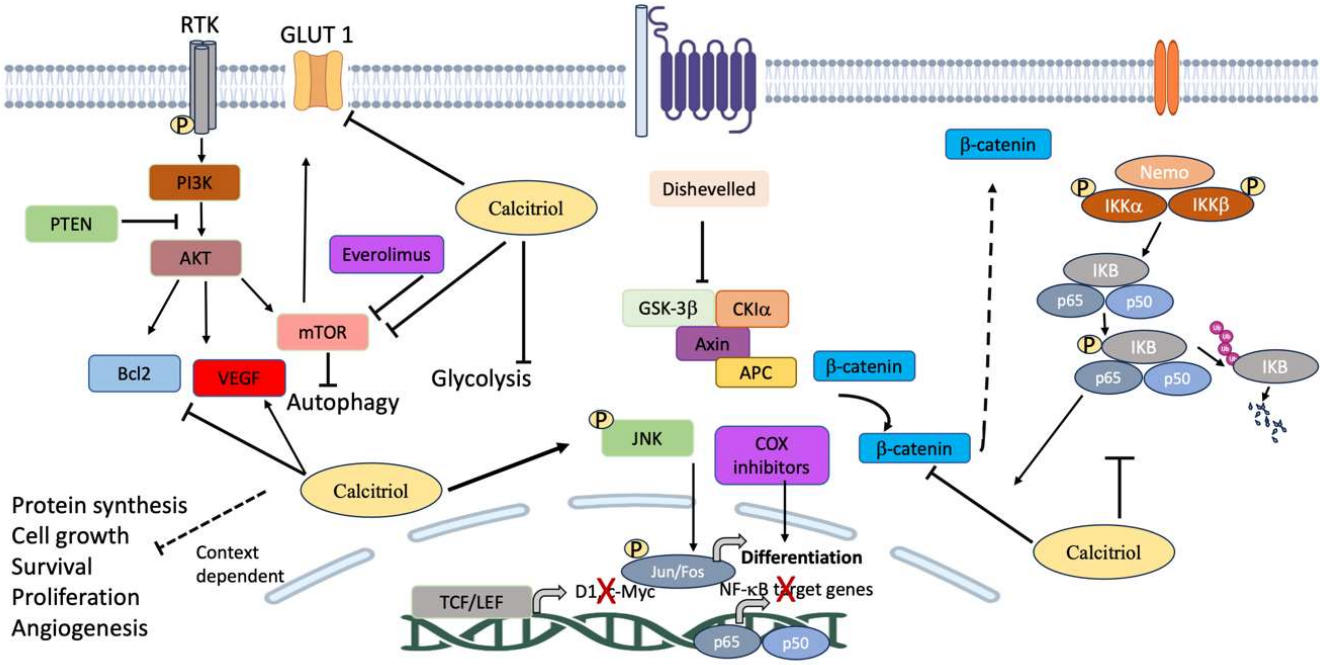

FIGURE 4 - Calcitriol effect on different signaling pathways. Calcitriol can module crucial signaling pathways such as a Wnt, NF-κB, JNK, and RTK/PI3K/AKT, activating or repressing different target genes and processes. Briefly, calcitriol inhibits β-catenin nuclear translocation, repressing cyclin D1 and c-Myc. Likewise, calcitriol can inhibit the glucose transporter GLUT1, glycolysis, mTOR, and BCL2, promoting the repression of cell proliferation, survival, protein synthesis, and angiogenesis. Calcitriol is also able to regulate inflammation by repressing NF-κB target genes. The action of calcitriol potentiates the effect of cancer drugs such as Everolimus and COX inhibitors.

DNA polymorphism affecting the action of calcitriol.

The existing evidence strongly suggests that DNA polymorphisms can also influence the physiological and molecular activity of calcitriol. In rheumatoid arthritis, patients with a high prevalence of osteoporosis and hip fractures are characterized by low levels of 25(OH) D in serum, which is associated with a genetic (GC) polymorphism in the gene encoding the DBP (Yoshida *et al.*, 2014). Moreover, in humans, two alternative transcripts of the VDR gene are expressed, encompassing a "normal" version and an alternative version that arises from post-transcriptional splicing and the use of an upstream in-frame start codon (Sunn *et al.*, 2001). Both these alternative versions coexist, and the differences in their

functionalities have been described elsewhere (Esteban *et al.*, 2005). Thus, variations in the VDR gene can also shape the body's response to vitamin D administration (Usategui-Martin *et al.*, 2022). The VDR gene is located on chromosome 12q13. While there are over 470 identified single nucleotide polymorphisms, only a few, including Fok1 (rs2228570), Bsm1 (rs1544410), Taq1 (rs731236), Apa1 (rs7975232), and Poly A (rs17878969) have been fully studied. The FokI polymorphism comprises a T-to-C transition in exon 2 of the VDR, resulting in a conversion from ATG to ACG, which leads to the formation of a short version of the VDR (Arai *et al.*, 1997, van Etten *et al.*, 2007). This modification can affect the VDR mRNA stability and protein function, ultimately impacting the body's response to vitamin D intake and the process of bone turnover (Wang *et al.*, 2012). The presence of the VDR Fokl polymorphism has been associated with increased susceptibility to several types of cancer, including gastric (Cong *et al.*, 2015), prostate (Li *et al.*, 2007), breast, colon cancer (reviewed in Mccullough, Bostick, Mayo, 2009), and even susceptibility to COVID-19 (Zeidan *et al.*, 2022). These polymorphisms add variability to the individual's response to vitamin D supplementation and its potential anticancer effects.

CONCLUSION

Vitamin D triggers a complex cascade of events, modulating several molecular and physiological pathways. Its effect depends not just on its concentration but also on the level at which it can effectively fulfill its biological role. To date, several epidemiological studies have shown that sunlight exposure and vitamin D supplementation are crucial for reducing cancer risk.

Our in-house data, along with multiple other studies have demonstrated that vitamin D deficiency is prevalent among populations in Chile, North America, and Europe (for review, see Deeb, Trump, Johnson *et al.*, 2007), especially in oncological patients. The available evidence underscores the significance of monitoring vitamin D levels. This can be achieved either by measuring its concentration at the plasma level or by assessing different key molecules involved in the vitamin D metabolic pathway. While measuring plasma levels of vitamin D is a routine and feasible procedure, it is advisable to complement this analysis by evaluating proteins, such as DBP or VDR, or proteins involved in the catabolism of vitamin D, at least in some cases. Low levels of VDR are associated with poor cancer prognosis, whereas high levels of CYP24A1 reduce the availability of calcitriol, thus increasing the risk of an unfavorable prognosis. Polymorphisms associated with low metabolism of vitamin D may also diminish the efficacy of treatments that involve supplementation with vitamin D or its analogs. Although vitamin D demonstrates antitumor properties in several cancers, uncovering the molecular mechanisms behind selective resistance to vitamin D is critical for designing and implementing novel vitamin D analogs for clinical use and optimizing future vitamin D supplementation strategies.

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