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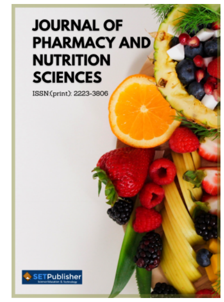

The Role of Vitamin D in Preventing Colorectal Carcinogenesis: A Review of Molecular Mechanisms

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Abstract:

Introduction: Colorectal carcinoma is one of the cancers with a high disease burden globally. Previous observational studies have found a connection between colorectal cancer incidence with sunlight exposure and vitamin D levels. Subsequent studies investigated this relationship further and found various anti-tumoral pathways regulated by vitamin D in colorectal tissue. This paper aims to elucidate the actions of those pathways in preventing the malignant transformation of the colorectal cell by reviewing relevant literature.

Methods: A search was conducted on several medical literature electronic databases for original research studying the effects of vitamin D treatment on colorectal adenoma and colorectal cancer and its underlying anti-tumoral mechanism. A total of 122 studies were included for evaluation.

Results: Twenty-seven studies passed for analysis. These *in vitro* and *in vivo* study reveals that vitamin D treatment can suppress cell proliferation, induce apoptosis, maintain cellular differentiation, reduce the pro-inflammatory response, inhibit angiogenesis, and hinder metastatic progression in colorectal cancer and colorectal adenoma cells by regulating associated gene transcription or directly prevents activation of selected signalling pathways. Five studies have also shown that adding calcium to vitamin D treatment increases the anti-tumoral activity of vitamin D through cross-talk between both of their pathways.

Conclusion: Vitamin D could potentially impede colorectal cancer transformation and growth through interaction with various signalling pathways and regulating gene transcription. Further clinical studies are needed to confirm whether vitamin D can be used as the basis of targeted colorectal cancer therapy using its inherent anti-tumoral properties.

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INTRODUCTION

Colorectal carcinoma (CRC) is one of the leading causes of death due to cancer in the world. Most recent epidemiological studies by GLOBOCAN ranked colorectal cancer second in mortality with an estimate of almost 900.000 deaths worldwide [1]. Although newer screening and treatment options have decreased these mortality rates compared to decades ago, there is an increasing trend of colorectal cancer incidence, especially in younger people [2]. Since colorectal cancer risk has been linked with lifestyle and diet, various studies are currently looking for nutrients that could help prevent or increase the survival rates of its patients. One of the most investigated ones was vitamin D.

Vitamin D has been traditionally known as the regulator of calcium homeostasis and bone metabolism [3], but it could also modulate various anti-tumoral activities. A review of vitamin D activity describes that vitamin D can regulate the expression of various genes that control tumor growth. This genomic modulation by vitamin D was mediated by Vitamin D Receptor (VDR). VDR would bind to calcitriol ($1,25(OH)_2D_3$), creating a VDR-calcitriol complex. The complex then moves into the nucleus and binds with Vitamin D Response Elements (VDRE) in the promoter region of target genes. This binding would initiate the recruitment of various coactivators or corepressors to control the gene transcription and regulate their function [4].

Observational studies in the eighties and nineties found evidence of the correlation between vitamin D and colorectal cancer. An epidemiological study found that the colon cancer mortality rate in the USA was tied to the daily mean solar radiation received [5]. The follow-up study also found that the risk of getting colon cancer was significantly reduced in people with serum vitamin D levels above 20 ng/ml [6]. These findings create the notion that vitamin D has a protective effect against colorectal cancer. To date, many *in vitro* and *in vivo* studies have been conducted to prove that notion by discovering the anti-tumor pathways mediated by vitamin D in cancers. This literature review will collect, interpret, and evaluate those studies to create a picture regarding the molecular mechanism of vitamin D in preventing colorectal carcinogenesis.

METHODS

We conducted a search on PUBMED and COCHRANE electronic databases for literature focusing on the molecular mechanism of vitamin D action against colorectal carcinogenesis published between 1980 to 2020. We used the following terms for the search keywords: (Colorectal cancer[MeSH Terms]) AND (Vitamin D [MeSH Terms]) AND ((carcinogenesis) OR (tumorigenesis) OR (oncogenesis)). The literature search was limited to articles published in English with available full text. Figure **1** shows the flowchart of our searches.

We identified an initial hit of 122 studies. Excluding non-English studies (n=1) and without available full-text (n=10) gave us the remaining articles of 111 studies. Screening for duplicates further removes 8 studies. These papers are then assessed by screening the title and abstracts. All original research concerning vitamin D activity on colorectal cancer were included for assessment. After screening, 62 articles were included for full-text review. Forty articles were excluded for reasons such as unexplained molecular mechanisms or inaccessible full text. Their references were also manually screened for additional citations. A total of 27 studies were included for analysis, consisting of *in vitro* studies on colorectal cancer cell lines and *in vivo* studies on mice and humans. Table **1** summarize our findings.

RESULTS

Proliferation

Twelve studies have pointed out that the mechanisms in the anti-proliferation activity of vitamin D are tied to the Wnt-β-catenin signalling pathway. $1,25(OH)_2D_3$ is able to regulate colon cancer growth by arresting the cell cycle at G1/G0 through inhibition of Cyclin D1 expression [7–10] and upregulation of $p21^{waf1}$ Cyclin-Dependent Kinase (CDK) inhibitor [11]. $1,25(OH)_2D_3$ is also able to modulate the expression of various oncogenes, such as c-myc or H19, that is overexpressed in colon cancer through transcriptional regulation mediated by VDR [12–14]. β-catenin, a vital component in activation of the pro-proliferative gene in the Wnt signalling pathway, was regulated by $1,25(OH)_2D_3$ by binding it to E-cadherin, thus preventing its transcriptional activity [15–17]. Calcium was also found to work synergistically with $1,25(OH)_2D_3$ in its anti-proliferative activity on colorectal cancer. Vanadium, a micronutrient that can influence calcium mobilization, has been reported in a study by Samanta *et al*. [18] to have a synergistic effect with $1,25(OH)_2D_3$ in preventing DNA damage by inhibiting the formation of methylated DNA adducts and suppressing

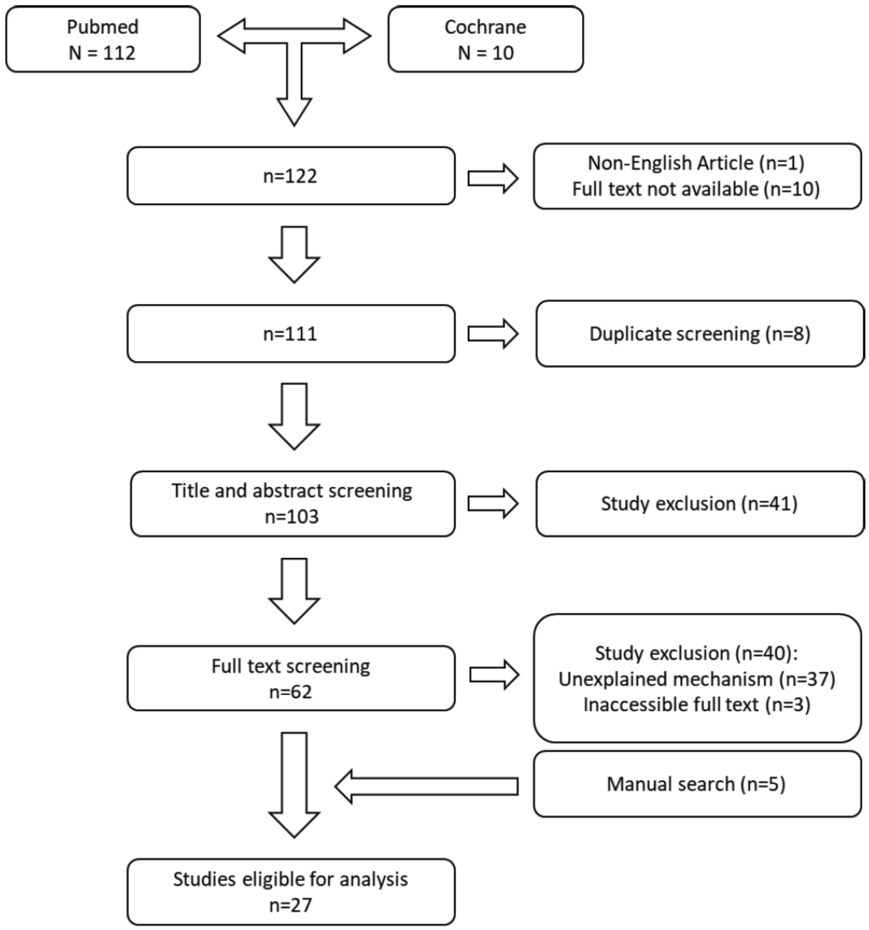

Figure 1: Flowchart of search strategies.

Table 1: Study Results and Characteristics

No	Author/Year	Study type	Subject	Intervention	Results	Effects
1	Franceschi *et al.* / 1987	*In vitro*	SW480 human colon adenocarcinoma	1a,25-dihydroxyvitamin D3	Increased FN synthesis in cancer cell	Induces cell differentiation
2	Evans *et al.* / 1999	*In vitro*	HT29 human colon cancer cell	1a,25-dihydroxyvitamin D3 and Vitamin D analog (Ro 25-6760)	Upregulation of p21waf1 CDK inhibitors and initiation of PARP-mediated apoptosis	Induces G1/G0 cell cycle arrest and apoptosis
3	Iseki *et al.* / 1999	*In vivo*	Wistar rats injected with AOM	Vehicle vs 1a-hydroxyvitamin D3 Vs 1a,25-dihydroxyvitamin D3	Reduction in vessel count and VEGF expression	Inhibits angiogenesis
4	Diaz *et al.* / 2000	*In vitro*	AA/C1, RG/C2 (adenoma cell); HT29, SW620, PC/JW (adenocarcinoma cell)	1a,25-dihydroxyvitamin D3 and vitamin D analog (EB1089)	Reduction of Bcl-2 and Bcl-XL expression and increased Bax and Bak expression	Induces apoptosis
5	Palmer *et al.* / 2001	*In vitro*	SW480 human colon adenocarcinoma	1a,25-dihydroxyvitamin D3	Upregulation of E-cadherin transcription and inhibition of the β-catenin signal pathway	Induces cell differentiation

(Table 1). Continued.

No	Author/Year	*Study type*	Subject	Intervention	Results	Effects
6	Makishima *et al.* / 2002	*In vivo*	Mice	VDR ligands (1a(OH)D3 and EB1089) vs PXR ligand (PCN) vs lithocholic acid (LCA) vs vehicle	Activation of VDR-specific gene by LCA and upregulation of CYP3A mRNA by VDR ligands and LCA	Detects and prevents LCA-induced neoplastic transformation
7	Wilson *et al.* / 2002	*In vitro*	SW837 colon cancer cell	Butyrate vs 1a,25-dihydroxyvitamin D3 vs sulindac (NSAID) vs Trichostatin A	Reduction of c-Myc expression	Suppresses proliferation
8	Wali *et al.* / 2002	*In vivo*	Male Fisher-344 rats injected with AOM or saline	AIN-76A vs AIN-76A + UDCA (ursodeoxycholic acid) vs AIN-76A+F6-D3 (vitamin D analog)	Inhibition of Cyclin D1 expression, Upregulation of E-cadherin expression, inhibition of COX2 and iNOS	Induces G1/G0 cell cycle arrest and cell differentiation and inhibits pro-inflammatory signaling pathway
9	Murillo *et al.* / 2005	*In vivo*	Mice injected with AOM	1a(OH)D5 vs control	Inhibition of nuclear β-catenin and PPAR-B expression	Suppresses proliferation
10	Fichera *et al.* / 2007	*In vivo* & *In vitro*	A/J mice injected with Azoxymethane (*In vivo*) HCA7 mucinous colon carcinoma cell (*In vitro*)	Vitamin D analog (Ro26-2198)	Inhibition of COX2 activation and reduction in c-myc and ERK expression	Prevents activation of pro-inflammatory signaling pathway and suppresses proliferation
11	Aguilera *et al.* / 2007	*In vitro* & *In vivo*	SW480 human colon adenocarcinoma (*In vitro*) colorectal cancer sample obtained from surgery (*In vivo*)	1a,25-dihydroxyvitamin D3 (*In vitro*)	Increased DKK-1 RNA and protein through VDR-mediated transcription	Induces cell differentiation
12	Ben-Shoshan *et al.* / 2007	*In vitro*	PC3 & LNCaP prostate cancer cell, CL1 lung cancer cell, MCF7 breast cancer cell, SW480, HCT116, & HCT116 HIF1a -/- colon cancer cell	1a,25-Dihydroxyvitamin D3	Inhibition HIF1a expression and transcription in colon cancer cell	Inhibits angiogenesis
13	Samanta *et al.* / 2008	*In vivo*	Sprague–Dawley rats injected with carcinogen DMH or Saline	Vanadium vs Vitamin D vs Vanadium + Vitamin D vs control	Inhibition of DNA adduct formation, upregulation of p53 expression, and downregulation of Bcl-2 expression in vanadium + vitamin D group	Suppresses proliferation and induces apoptosis (synergistically with vanadium)
14	Yang *et al.* / 2008	*In vivo*	Apc 1638/N+ mice	AIN-76A vs AIN-76A + western diet vs AIN-76A + western diet + calcium + vitamin D	Inhibition of cyclin D1 expression and downregulation of Bcl-2 expressionin vitamin D + calcium group	Induces G1/G0 cell cycle arrest and apoptosis
15	Maier *et al.* / 2009	*In vitro*	SW387 and DLD-1 colon adenocarcinoma cell	Butyrate and 1a,25-dihydroxyvitamin D3	Inhibition of cyclin D1 expression	G1/G0 cell cycle arrest

(Table 1). Continued.

No	Author/Year	*Study type*	Subject	Intervention	Results	Effects
16	Xu *et al.* / 2010	*In vivo*	Apc min/+ heterozygous C57BL/6J mice and wild-type (wt) C57BL/6J-Apc+/+ mice	1a,25-dihydroxyvitamin D3 vs vitamin D analog QW vs vitamin D analog BTW vs Vehicle	Inhibition of CD44 expression and upregulation of osteopontin and E-cadherin expression	Suppresses proliferation and induces cell differentiation
17	Murillo *et al.* / 2010	*In vitro* & *In vivo*	Caco2, HCT116 and HT29 colon cancer cell (*in vitro*) CF1 mice exposed to AOM/DSS carcinogen	1,25(OH)2D vs 25(OH)D vs 1α(OH)D5 vs control	Reduction in NFKB expression through regulation of TLR4 pathway	Prevents activation of pro-inflammatory signaling pathway
18	Hopkins *et al.* / 2011	*In vivo*	Colorectal adenoma patients	1a,25-dihydroxyvitamin D3 vs calcium vs 1a,25-dihydroxyvitamin D3 + calcium	Reduction of inflammatory markers (CRP, TNFα, IL-6, IL-8, IL-1β) through VDR-mediated transcriptional repression	Inhibits pro-inflammatory cytokine production
19	Ahearn *et al.* / 2012	*In vivo*	Colorectal adenoma patients	1a,25-dihydroxyvitamin D3 vs calcium vs 1a,25-dihydroxyvitamin D3 + calcium	Increased APC and E-cadherin expression and decreased β-catenin expression	Induces cell differentiation and suppresses proliferation
20	Bessler *et al.* / 2012	*In vitro*	HT29 & RKO colon cancer cell incubated with peripheral blood mononuclear cell	1a,25-Dihydroxyvitamin D3	Reduced generation of pro-inflammatory cytokines TNFa and IL-6 by suppressing activation of NFKB pathway	Prevents activation of pro-inflammatory signaling pathway
21	Aggarwal *et al.* / 2014	*In vivo* & *In vitro*	SCID mice implanted with HT29 colon cancer cell (*In vivo*) HT29 & Caco2 colon cancer cell (*In vitro*)	Low/High vitamin D diet (*In vivo*) Calcium & Vitamin D (*In vitro*)	Decreased cyclin D1 expression and increased Bax/Bcl-2 ration in the group with wild-type CaSR	Suppresses proliferation and induces apoptosis (synergistically with calcium)
22	Meeker *et al.* / 2014	*In vivo*	Smad3-/- Mice fed high vitamin D diet vs maintenance	Helicobacter broth vs control	Suppression of MAPK and NFKB pathway activation	Prevent activation of pro-inflammatory signaling pathway
23	Chen *et al.* / 2015	*In vitro*	SW480 & HT29 colon cancer cell	1a,25-dihydroxyvitamin D3 and/or TGFB1/TGFB2	Inhibition of TGFB1/B2-induced cell invasion & migration, suppression of TGFB1/B2-induced E-cadherin transition, and inhibition of MMP-2 & MMP-9 expression	Reduces metastasis potential
24	Deevi *et al.* / 2016	*In vitro*	Caco-2 vs Caco-2 ShPTEN colon cancer cells	1a,25-Dihydroxyvitamin D3	Upregulation of PTEN/CDC42/ PRKCZ signaling to prevents cribriform morphology cell transformation	Activates pro-differentiation signaling pathway
25	Chen *et al.* / 2017	*In vitro*	HT29 and DLD1 human colon adenocarcinoma	1a,25-Dihydroxyvitamin D3	Inhibits H19 oncogene expression by modulating c-Myc/Mad-1 network	Suppresses cancer proliferation and migration

(Table 1). Continued.

No	Author/Year	*Study type*	Subject	Intervention	Results	Effects
26	Liu *et al.* / 2017	*In vivo*	Colorectal adenoma patients	1a,25-dihydroxyvitamin D3 vs calcium vs 1a,25-dihydroxyvitamin D3 + calcium	Increased APC and E-cadherin expression and decreased β-catenin expression	Induces cell differentiation and suppresses proliferation
7	Xin *et al.* / 2017	*In vivo* & *In vitro*	C57BL/6 mice injected with AOM and DSS (*In vivo*) SW480 colon cancer cell (*in vitro*)	Cholecalciferol at a different dose (*In vivo*) 1a,25-dihydroxyvitamin D3 or DMSO (*In vitro*)	Reduction of the β-catenin transcriptional activity and increased expression and binding affinity of E-cadherin	Induces cell differentiation

AOM: Azoxymethane, APC: Adenomatous Polyposis Coli, CaSR: Calcium-Sensing Receptor, CDC42: Cell division cycle 42, CDK: Cyclin-Dependent Kinase, CRC: Colorectal Cancer, CRP: C-reactive Protein, DNA: Deoxyribonucleic acid, DMH: 1,2-Dimethylhydrazine, DMSO: Dimethyl Sulfoxide, DSS: Dextran Sodium Sulfate, FN: Fibronectin, IL-6/8/1β: Interleukin-6/8/1 Beta, iNOS: inducible nitric oxide synthase, LCA: Lithocholic Acid, MAPK: Mitogen-Activated Protein Kinase, MMP: Matrix MetalloPeptidase, NFKB: Nuclear Factor Kappa-light-chain-enhancer of activated B cells, PARP: Poly (ADP-ribose) polymerase, PRKCZ: Protein Kinase C Zeta, PTEN: Phosphatase and Tensin homolog, TLR4: Toll-Like Receptor 4, TNFα: Tumor Necrosis Factor Alpha, TGF-β1/β2: Transforming Growth Factor-Beta 1/Beta 2, VDR: Vitamin D Receptor. VEGF: Vascular Endothelial Growth Factor.

proliferation through upregulation of p53 expression. Aggarwal *et al*. [10] showed that Calcium-Sensing Receptor (CaSR), a protein that interacts with Ca^{2+} in maintaining calcium homeostasis, was observed to be regulated by VDR-mediated transcription and enhanced the anti-proliferative activity of $1,25(OH)_2D_3$ on cancer cell with functional CaSR. They also describe their previous research that found CaSR activity in inhibiting the Wnt/β-catenin pathway and suggest CaSR as the link between the cooperative action of calcium and vitamin D in suppressing tumor proliferation. Vitamin D is shown to modulate colorectal cancer cell proliferation by regulating the expression of genes involved in the cell cycle checkpoint and Wnt/β-catenin signaling pathway and has a synergistic action with calcium in suppressing proliferation.

Differentiation

Ten studies describe the effects of $1,25(OH)_2D_3$ activity on colon cancer cell differentiation, prominently through the inhibition of the Wnt/β-catenin pathway. Abnormal Wnt pathway activation was commonly observed in colorectal cancer and caused by mutations of its component such as APC, E-cadherin, or β-catenin. Treatment using $1,25(OH)_2D_3$ or its analog was reported able to increase APC and E-cadherin expression [7,16,17,19–21]. *In vivo* study by Ahearn *et al.* [16] shows that $1,25(OH)_2D_3$ induces E-cadherin production through transcriptional upregulation by VDR complex. However, its author hasn't found the mechanism by which $1,25(OH)_2D_3$ upregulates APC expression. APC and E-cadherin will prevent β-catenin nuclear translocation by binding it in the plasma membrane. A study by Palmer *et al*. [19] also showed that VDR directly competes with the TCF (T-cell transcription factor) receptor in the nucleus for β-catenin binding to prevent the activation of Wnt transcription through the β-catenin-TCF complex. Another mechanism for $1,25(OH)_2D_3$ to inhibit Wnt/β-catenin pathway is through the induction of Dickkopf-1 (DKK-1) gene. This gene encodes DKK-1 protein that acts as an extracellular Wnt antagonist by interacting with the LRP6 co-receptor on the cell surface and preventing it from activating the Wnt signal pathway. Aguilera *et al.* [22] in their study observed that $1,25(OH)_2D_3$ was able to upregulate DKK-1 gene transcription through indirect stimulation by induction of epithelial adhesive phenotype.

Several studies have reported other mechanisms than inhibition of the Wnt/β-catenin pathway in preventing the neoplastic transformation of colon tissue. An *in vitro* study by Deevi *et al*. [23] mentioned that $1,25(OH)_2D_3$ can regulate the PTEN tumor suppressor gene through VDR-mediated transcription. This gene modulates the CDC42/PRKCZ/PARD apical polarity complex that maintains spindle alignment and orientation, the arrangement of epithelial structure, and tissue homeostasis in colon cells. $1,25(OH)_2D_3$ treatment upregulates PTEN, activates CDC42 and PRKCZ signaling, and suppresses the development of cribriform morphology in the Caco-2 colon cancer cell line. The second mechanism is by inducing the synthesis of fibronectin through the stimulation of its gene transcription by VDR. Franceschi *et al*. [24] reported that $1,25(OH)_2D_3$ treatment stimulates fibronectin synthesis in various cancer cell lines, including the SW-480 colon cancer cell line. Upregulation of fibronectin will help maintain cell adhesion and prevent loss of cytoskeletal architecture induced by malignant transformation of colon cancer cells. The third mechanism is through detecting and detoxifying secondary bile acid LCA (lithocholic acid) in the colon. Makishima *et al*. [25] in their study explained that LCA overexcretion is associated with high-fat diets and could cause DNA strand breaks, create DNA adducts, and suppress DNA repair in enteric tissue. Their study shows that LCA is able to bind to VDR as ligands, and in subsequent study using mice, shows this binding increases the expression of CYP3A that detoxifies LCA. Vitamin D is observed to maintain cellular differentiation in the colorectal cell by regulating Wnt/β-catenin pathway and promoting expression of the gene that helps maintain cellular structure.

Apoptosis

Five studies reported the mechanism of $1,25(OH)_2D_3$ in activating the apoptotic pathway. *In vitro* studies of colonic adenoma and CRC cell line by Aggarwal *et al*. [10] and Diaz *et al*. [26] reported that $1,25(OH)_2D_3$ treatment triggers the apoptotic pathway through the upregulation of pro-apoptotic proteins Bax and Bak and downregulation of anti-apoptotic proteins Bcl-2 and Bcl-Xl. Poly(ADPribose)polymerase (PARP) proteolytic cleavage induced by treatment with $1,25(OH)_2D_3$ and its analog and the subsequent activation of caspase that leads to cell death has been described by Evans *et al*. [11] as another possible mechanism in vitamin D-induced apoptosis. Studies by Yang *et al*. [8] using mice show a synergistic effect when combining $1,25(OH)_2D_3$ with calcium in increasing tumor apoptotic rate. The author, based on similar research, suggested that calcium action in apoptosis is mediated through CaSR. Identical to its anti-proliferative effects, Aggarwal *et al*. [10] observed that CaSR expression also regulates the apoptotic activity of $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ treatment in cells with overexpressed CaSR increases the activity of caspases 3 & 7 to increase cell death rate. Vanadium treatment with vitamin D also increases the tumor apoptosis rate in mice. The study by Samanta *et al*. [18] showed that the upregulation of p53 expression by vanadium also initiates the p53-mediated apoptotic pathway, differed from $1,25(OH)_2D_{3,}$ whose apoptotic pathway was p53-independent. Vanadium is also able to inhibit Bcl-2 expression similar to $1,25(OH)_2D_3$, creating a synergistic effect in promoting apoptosis. These studies showed that vitamin D promotes apoptosis by upregulating pro-apoptotic proteins and downregulating anti-apoptotic ones and has a synergistic effect with calcium in increasing their tumor apoptotic rate.

Inflammation

Six studies evaluated vitamin-D mediated effects on suppression of the inflammatory pathway. *In vitro* study by Bessler *et al*. [27] on peripheral blood mononuclear cells found that $1,25(OH)_2D_3$ capable of halting the NFKβ (Nuclear Factor Kappa Beta) inflammatory signaling pathway. Experiments by Murillo *et al*. [28] on colon cancer cell lines and mice fed with carcinogen supported this evidence, showing that $1,25(OH)_2D_3$ is able to suppress NFKβ activation by regulating the expression of TLR4 (Toll-like Receptor 4) pathway genes. TLR4 activation is associated with the initiation of the NFKβ signaling pathway in its downstream and treatment with $1,25(OH)_2D_{3,}$ or its analog, reduces TLR4 mRNA expression, NFKβ activation, and NFKβ nuclear expression. They described that, based on previous studies, $1,25(OH)_2D_3$ inhibits NFKβ activation through suppressing transcription of Re1B, an NFKβ transcription coregulator, and by directly binding to NFKβ to prevent its function. The blockade of the NFKβ signaling pathway will also result in the inhibition of inflammatory mediator production. Two *in vivo* studies by Wali *et al*. [7] and Fichera *et al*. [13] on mice injected with carcinogen azoxymethane (AOM) describes that the downregulation of COX-2 synthesis was an indirect result of NFKB pathway suppression by $1,25(OH)_2D_3$, which prevents NFKB from binding with COX-2 gene. Another example of an inflammatory pathway inhibited by vitamin D is the p38 MAPK pathway. Studies by Meeker *et al*. [29] on mice fed with helicobacter show that $1,25(OH)_2D_3$ supplementation reduces p38 MAPK pathway protein expression through upregulation of MAPK phosphatase-1. Besides blocking the inflammatory pathway, vitamin D is also capable of mediating the production of pro-inflammatory cytokines (IL-6, TNFα, IL-1β, etc.) through transcriptional repression of their gene by VDR [30]. Vitamin D, as reported in these studies, can regulate inflammatory processes in colorectal cells by inhibiting pro-inflammatory signaling pathways and mediating the expression of pro-inflammatory cytokines

Angiogenesis

Two studies discussed vitamin D effects in inhibiting angiogenesis in colon cancer. *In vivo* study by Iseki *et al*. [31] found that administration of $1,25(OH)_2D_3$ or its analog reduced vessel counts on colorectal cancer through downregulation of VEGF (Vascular Endothelial Growth Factor) expression. A study by Ben-Shoshan *et al*. [32] on several cancer cells, including colon cancer cell lines, explain this mechanism further. It is mentioned in their study that the anti-angiogenesis effect of 1,25$(OH)_2D_3$ was mediated through the HIF-1 (Hypoxia-inducible factor 1) pathway. HIF-1 was expressed during hypoxia and is responsible for the transcription of various pro-survival genes, including VEGF and its receptor. 1,25$(OH)_2D_3$ was found to inhibit HIF-1 during its translational process, thus preventing its transcription of VEGF. Vitamin D is shown to suppress angiogenesis by downregulating VEGF expression through inhibition of the HIF-1 pathway.

Metastasis

One *in vitro* study by Chen *et al*. [33] explained the mechanism of vitamin D in impairing tumor invasion and migration. The potential for cancer to metastasize has been associated with epithelial-mesenchymal transition (EMT) of the cell induced by TGF-β1/β2 (Transforming Growth Factor Beta-1/Beta-2). They found that administration of 1,25$(OH)_2D_3$ attenuates invasion and migration of colon cancer cells through several paths. First, 1,25$(OH)_2D_3$ prevents the transition of E-cadherin to N-cadherin that is induced by TGF-β. E-cadherin increases cell-to-cell adhesion and prevent tumor detachment. Second, 1,25$(OH)_2D_3$ inhibits transcription of EMT-related factors such as Snail or Slug via VDR-mediated suppression. Finally, 1,25$(OH)_2D_3$ suppresses the production of MMPs (matrix metalloproteinases) -2 and -9 that aided the tumor in detaching from its extracellular matrix. This study has shown that vitamin D could impair tumor invasion and migration by suppressing TGF-β1/β2 pathway-induced EMT.

DISCUSSION

Our review seeks to build a picture of vitamin D molecular mechanisms in colorectal cancer based on various studies that have been published. We found that vitamin D acts on colorectal carcinogenesis by suppressing proliferation, inflammation, angiogenesis, and metastatic potential while inducing cellular differentiation and apoptosis. The colonic epithelial neoplastic transformation has previously been described to be initiated by mutations of the APC tumor suppressor gene [34]. This gene encodes an APC protein that functions to control the Wnt/β-catenin signaling pathway by binding and degrading catenin in the cytoplasm. APC mutations lead to overactivation of the Wnt/β-catenin pathway, and we found that the anti-tumor function of vitamin D in colorectal cancer acts through regulation of this pathway. This indicates that the anti-carcinogenic function of vitamin D has begun at the beginning of the colonic epithelial change into adenoma. Vitamin D synthetic analog such as EB1089 or Ro26-2198 were used in some of these studies andable to confer the same benefit as calcitriol with the added benefit of less hypercalcemic side effects. Moreover, combining calcium or agents that improve calcium function, such as vanadium, with vitamin D treatment is shown to synergistically increase the anti-tumor effects of vitamin D.

We compared our findings with other reviews regarding the molecular mechanism of vitamin D for different cancers. A systematic review on ovarian cancer by Dovnik *et al*. [35] shows a similar working of vitamin D anti-tumor activity. Still, they also discover other targets not reported in colorectal cancer studies before such as downregulation of telomerase transcription to induce apoptosis and suppression of DDX4 (DEAD-box helicase 4) expression to inhibit cancer invasion. On prostate cancer, reviews by Moreno *et al*. [36] and Krishnan *et al*. [37] evaluating the molecular mechanism of vitamin D actions reveal that, besides the mechanism found in our study, calcitriol is capable of modulating androgen metabolism to suppress prostate cancer growth. Similarly, two studies on breast cancer by Krishnan *et al*. [38,39] reported that calcitriol could suppress tumor growth by inhibiting estrogen synthesis and downregulating estrogen receptor α that mediates estrogen activity. These findings suggest that vitamin D has broad working pathways in preventing carcinogenesis despite the difference in cancerous tissue origin.

Several clinical trials have proven the mechanism of colorectal cancer carcinogenesis prevention by vitamin D that we found. Studies by Grau *et al*. [40] that provide calcium and/or vitamin D supplementation in postoperative colorectal adenoma patients found that the combination of the two substances was reported to significantly reduce the risk of adenoma recurrence. Another study by Lappe *et al*. [41] providing postmenopausal women with calcium and/or vitamin D supplementation also found that subjects receiving a combination of vitamin D and calcium had a lower incidence of cancer and that calcium and vitamin D levels were significant predictors of cancer risk.

Despite having extensive anti-tumor functions, vitamin D has also been reported to undergo metabolic dysregulation in various types of cancer. Studies by Bises *et al*. [42] and Matusiak *et al*. [43] have observed that the expression of VDR and calcitriol-synthesizing enzyme CYP27B1 in colorectal cancer was progressively decreased as the tumor turns into a more undifferentiated state. CYP24A1, an enzyme that catabolizes calcitriol that is generally not expressed in healthy colonic tissue, was observed to be increased in the cancer cell [44]. This pattern was not unique to colorectal cancer as various studies have shown similar findings in breast cancer [45], prostate cancer [46], and ovarian cancer [47], among others. The alterations of vitamin D metabolism in cancers have been proposed to be caused by various genome mutations such as Snail [48], P53 [49], and miR-125b [50].

CONCLUSION

Based on published *in vitro* and *in vivo* studies, Vitamin D has the potential to prevent the development of colorectal cancer through suppressing tumor growth, maintaining cell differentiation, induction of apoptosis, inhibition of angiogenesis, inactivation of the inflammatory pathway, and reducing the metastatic potential of cancer cells. Additionally, calcium and calcium-enhancing agents could amplify the anti-tumoral effects of vitamin D through cross-talk between both of its signaling pathways. Further research is needed to confirm whether these potentials could be applied as a novel or supplementary therapy for colorectal cancer patient's treatment.

FUTURE PERSPECTIVES

As vitamin D has been shown in various studies to affect the development of colorectal cancers, further action would be to test it in the clinical setting. Some of the research questions it could answer would be whether the supplementation of vitamin D in colorectal cancer patients undergoing therapies would improve the treatment effect, or whether vitamin D supplementation in colorectal cancer patients helps increase their survival rate. Due to the dysregulation of vitamin D by cancer, any experiments inquiring into the usage of vitamin D as colorectal cancer therapy or as a combination with pre-existing treatment need to take these changes into account to develop a better research plan.

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ETHICAL CONSIDERATION

Ethical issues (including plagiarism, data fabrication, etc) have been completely observed by the authors.

CONFLICT OF INTEREST

The author(s) declared that there is no conflict of interest in this review.

AUTHORS' CONTRIBUTIONS

Herdian F: principal investigator, conducting the study, collecting data, analyzing and interpreting the data, preparing the draft, and reviewing the manuscript; Radityamurti F: assisting the data collection, reviewing the manuscript; Permata TBM: advising on the data analysis and reviewing the manuscript; Handoko: advising on the data analysis and reviewing the manuscript; Kodrat H: reviewing the manuscript; Nuryadi E: reviewing the manuscript; Wibowo H: reviewing the manuscript; Gondhowiardjo S: conceptualized and designed the study, advising on the data analysis and interpretation, assisting the draft's preparation, and reviewing the manuscript.

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