**Name of journal: World Journal of Gastrointestinal Oncology**

**ESPS Manuscript NO: 11452**

**Columns: Minireviews**

**Vitamin D and colon cancer**

Klampfer L. Vitamin D and colon cancer

Lidija Klampfer

**Lidija Klampfer,** Southern Research Institute, Birmingham, AL 35205, United States

**Author contributions:** Klampfer L solely contributed to this paper.

**Correspondence to: Lidija Klampfer, PhD,** Southern Research Institute, 2000 9th Avenue, Birmingham, AL 35205, United States. klampfer@southernresearch.org

**Telephone:** +1-205-5812731

**Received:** May 22, 2014 **Revised:** July 31, 2014

**Accepted:** September 23, 2014

**Published online:**

**Abstract**

Calcitriol, 1,25 (OH)2D3 (1α, 25-dihydroxyvitamin D3), the most active form of vitamin D, is a pleotropic hormone with a wide range of biological activities. Due to its ability to regulate calcium and phosphate metabolism, 1,25D3 plays a major role in bone health. In addition, 1,25D3 binds to the vitamin D receptor (VDR) and thereby regulates the expression of a number of genes which control growth, differentiation and survival of cancer cells. In agreement, the levels of vitamin D3 appear to be an essential determinant for the development and progression of colon cancer and supplementation with vitamin D3 is effective in suppressing intestinal tumorigenesis in animal models. Vitamin D3 has been estimated to lower the incidence of colorectal cancer by 50%, which is consistent with the inverse correlation between dietary vitamin D3 intake or sunlight exposure and human colorectal cancer. Several studies confirmed that increasing vitamin D3 lowers colon cancer incidence, reduces polyp recurrence, and that sufficient levels of vitamin D3 are associated with better overall survival of colon cancer patients. Vitamin D regulates the homeostasis of intestinal epithelium by modulating the oncogenic Wnt signaling pathway and by inhibiting tumor-promoting inflammation. Both activities contribute to the ability of 1,25D3 toprevent the development and progression of colon cancer.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Colon cancer; Vitamin D; Wnt signaling; Inflammation; Chemoprevention

**Core tip:** Epidemiological studies suggest that deficiency of vitamin D increases the incidence of colon cancer and also has a negative impact on the survival of colon cancer patients. The ability of 1,25D3 to interfere with Wnt signaling and to ameliorate inflammation is likely to contribute to its anticancer activity.

Klampfer L.Vitamin D and colon cancer. *World J Gastrointest Oncol* 2014; In press

**INTRODUCTION**

The biologically active form of vitamin D3,1α,25(OH)2D3 (1,25D3), is obtained by 25-hydroxylation of vitamin D3 in the liver and 1α-hydroxylation in the kidney, liver or other tissues. Hydroxylation of 25(OH)D3 by CYP27B1 yields the hormonally active form 1,25(OH)2D3, which is metabolized to less active metabolites by CYP24A1 (reviewed in[[1](#_ENREF_1)]). While the levels of CYP21B1 have been shown to be reduced in some cancers, the levels of CYP24A1 are increased in cancer cells, which may contribute to the resistance of some tumors to 1,25D3[[2](#_ENREF_2)].

 1,25D3 exerts most of its biological activity through binding to a specific vitamin D3 receptor (VDR), a member of the nuclear receptor superfamily[[1](#_ENREF_1)]. VDR binds to retinoid X receptor (RXR), and the VDR-RXR heterodimers bind to a vitamin D response element (VDRE), activating or repressing gene expression, which contribute to the anti-neoplastic activity of vitamin D. VDR associates with other transcription factors, such as SP1 and β-catenin[[3](#_ENREF_3)] and thereby also regulates the expression of genes that do not harbor the consensus VDRE. A number of cancer cell lines, including colon cancer cell lines tested in our laboratory, display a limited response to vitamin D3 *in vitro* [[4](#_ENREF_4)] and the expression of VDR is downregulated in late stages of colon cancer[[5](#_ENREF_5)] (Figure 1), suggesting that vitamin D3 may exert some of its biological activities in a VDR- independent manner, or that it targets cells in the tumor microenvironment. VDR-/- mice display hyper-proliferation and have elevated levels of c-myc in both skin and colon, and VDR suppresses c-myc expression *in vitro* and *in vivo* in the absence of 1,25D3[[6](#_ENREF_6)]. However, 1,25D3 triggers association of VDR with c-myc and thereby promotes turnover of c-myc protein[[6](#_ENREF_6)], indicating that vitamin D signaling suppresses transcription of c-myc and also inhibits c-myc stability. In addition to its ability to inhibit c-Myc, 1,25D3 induces the expression of its antagonist Mxd1/Mad1, suggesting that 1,25D3 can exert its chemopreventive activity through regulation of the c-myc/Mxd1 network[[6](#_ENREF_6)].

 The focus of this report is to discuss the role of vitamin D in colon cancer, however the beneficial effects of vitamin D have been noted in other malignancies. Reduced serum levels of vitamin D were found in stage IV melanoma patients and it has been shown that melanoma patients with low serum levels of vitamin D developed metastasis earlier than patients with high levels of vitamin D[[7](#_ENREF_7)]. Similarly, chemopreventive activity of vitamin D has been observed in breast, ovarian, pancreatic and prostate cancer patients[[8](#_ENREF_8)].

 In addition to its chemopreventive activity, 1,25D3 or its analogues have been tested for their ability to improve the response to anticancer agents. Vitamin D and its derivatives have been shown to enhance the anticancer activity of 5FU, irinotecan and oxaliplatin both *in vitro* and *in vivo*[[9](#_ENREF_9),[10](#_ENREF_10)]. Although the therapeutic use of 1,25D3 is restricted by its hypercalcemic activity, several 1,25D3 analogues that retain the antitumor activity while being devoid of hypercalcemic effects, are currently being tested in clinical trials for a variety of malignancies.

**VITAMIN D AND COLON CANCER**

Recent case-controlled studies have established that there is an inverse correlation between serum levels of vitamin D and the incidence of polyps and adenomas in the colon[[11-13](#_ENREF_11)], consistent with the inverse correlation between dietary vitamin D3 intake or sunlight exposure and human colorectal cancer[[14-17](#_ENREF_14)]. This is significant because a large segment of the human population suffers from vitamin D3 insufficiency or deficiency[[18](#_ENREF_18)], which is particularly prevalent among colon cancer patients. Indeed, numerous studies have suggested that higher vitamin D3 levels are associated with lower colon cancer incidence, reduced polyp recurrence and better overall survival of colon cancer patients[[19-22](#_ENREF_19)].

 Vitamin D and its analogues reduce the growth of colon cancer xenografts and inhibit tumorigenesis in several genetic models of intestinal cancer. In agreement, dietary initiation of colon cancer in rodents, a model of sporadic colon cancer, has been shown to be prevented by supplementation with vitamin D3 and Ca[[23](#_ENREF_23),[24](#_ENREF_24)].

 Despite the established chemopreventive activity of vitamin D3, its targets and the molecular basis for its antitumor activity remain poorly understood. 1,25D3 inhibits growth of tumor cells by inducing the expression of cyclin-dependent kinase inhibitors, such as p21, p27, and cystatin D, and by inhibiting the expression of pro-proliferative genes, including c-my and cyclin D1. In addition, 1,25D3 has been shown to upregulate miR-627, which targets the histone demethylase JMJD1A, and thereby inhibits proliferation of colon cancer cells *in vitro* and *in vivo* through epigenetic regulation[[25](#_ENREF_25)]. By increasing the expression of alkaline phosphatase, maltase, E-cadherin and cell adhesion proteins, vitamin D promotes differentiation. In a cell-type specific manner, vitamin D promotes apoptosis by regulating the expression of BCL-2 family members. Thus, due to its ability to affect multiple signaling pathways and to regulate many target genes, 1,25D3 controls a variety of biological processes. Although 1,25D3 has also been shown in preclinical studies to inhibit invasiveness of tumor cells and to reduce their ability to metastasize, clinical trials suggest that while vitamin D is effective in early stages of cancer, it appears to have limited activity in advanced, aggressive malignancies.

 Important mechanisms whereby 1,25D3 regulates the homeostasis of intestinal epithelium and exerts its anti-neoplastic activity is through its ability to interfere with Wnt/β-catenin signaling[[3](#_ENREF_3),[26](#_ENREF_26),[27](#_ENREF_27)] and to inhibit inflammation. Because inflammation can fuel Wnt signaling in colon cancer cells, the two activities may be coupled, suggesting that 1,25D3 might exert chemopreventive activity by interrupting the link between inflammation and cancer. However, large clinical trials are required to firmly establish the preventive and therapeutic value of vitamin D in colon cancer. Such trials are complicated by the necessity of maintaining and monitoring vitamin D levels as well as clinical outcome in a large number of patients over a long period of time.

**INHIBITION OF WNT SIGNALING BY VITAMIN D**

The Wnt/β-catenin signaling pathway regulates the intracellular levels of β-catenin and controls the expression of β-catenin/TCF4 target genes. In normal cells, β-catenin is sequestered in a large cytoplasmic protein complex, called the β-catenin destruction box, which includes Axin and Apc and the GSK3β and CK1 kinases[[28](#_ENREF_28),[29](#_ENREF_29)]. Due to mutations in the tumor suppressor Apc, or less frequently in Axin or β-catenin, the oncogenic Wnt/β-catenin signaling pathway is abnormally activated in over 90% of colon cancers[[30](#_ENREF_30)].

Theβ-catenin destruction complex promotes β-catenin phosphorylation and its subsequent degradation. Wnt activation of its receptors, Frizzled and LRP5/6, inhibits the destruction complex and results in accumulation ofβ-catenin, both in the cytoplasm and in the nucleus, where it acts as a co-activator of LEF/TCF and regulates the expression of a variety of genes. Wnt/β-catenin signaling activates genes, such as c-myc and cyclin D and thereby promotes proliferation of tumor cells. Activation of Wnt signaling also induces the expression of COX2 and survivin which increases the survival of intestinal epithelial cells. Wnt signaling has been shown to promote transcription, protein stability and to regulate nuclear localization of Snail, a transcription factor that mediates epithelial mesenchymal transition[[31](#_ENREF_31),[32](#_ENREF_32)]. In turn, Snail interacts withβ-catenin and increases the expression of Wnt target genes[[33](#_ENREF_33)]. We showed that inflammation-induced stabilization of Snail contributes to Wnt signaling in colon cancer cells and creates a positive feedback loop initiated, and propagated, by macrophage-derived IL-1β[[34](#_ENREF_34)].IL1β was sufficient to increase the levels of Snail in colon cancer cells[[35](#_ENREF_35)], and the levels of both IL1β and Snail are increased in colon cancer patients (Figure 1). Importantly, Snail1 and Slug (Snail2) have been shown to inhibit the expression of VDR and to inhibit the activity of 1,25D3[[5](#_ENREF_5),[36-38](#_ENREF_36)]. Wnt-dependent stabilization of Snail is likely to contribute to reduced expression of VDR in colon cancer patients (Figure 1).

1,25D3 has been shown, in a VDR-dependent manner, to antagonize Wnt signaling through a variety of mechanisms. These include sequestration of β-catenin through a direct VDR/β-catenin interaction and induction of nuclear export of β-catenin. 1,25D3 also enhances the expression of DKK1, which is an endogenous inhibitor of Wnt signaling. Furthermore, cystatin D, whose expression is strongly upregulated by 1,25D3, inhibits Wnt signaling and the expression of its target genes, including Snail (Figure 2). Cystatin D inhibits migration and anchorage- independent growth of colon cancer cells and its silencing abrogates the anti-proliferative activity of 1,25D3 and increases the expression of c-Myc[[39](#_ENREF_39)]. A comprehensive review of the mechanisms whereby vitamin D represses Wnt signaling has been published recently[[40](#_ENREF_40)].

 Wnt activity in primary human tumors is heterogeneous, and it has been demonstrated that its activity is regulated by factors from the tumor microenvironment. Although loss of Apc occurs early in adenoma development in the colon, *in* *vivo* progression from micro-adenomas to macroscopic tumors in *ApcMin/+*mice is associated with further elevation of canonical Wnt signaling and increased expression of Wnt target genes[[41](#_ENREF_41)]. This suggests that enhancement of Wnt signaling beyond a threshold level sufficient for tumor initiation may be required for tumor progression and metastatic spread. Often factors from the tumor microenvironment provide signals that regulate the extent of oncogenic signaling in tumor cells. We and others have demonstrated that tumor-associated macrophages promote Wnt signaling in colon cancer cells *via* IL1β and TNF[[34](#_ENREF_34),[42](#_ENREF_42)]. Fibroblasts have also been shown to enhance Wnt signaling through HGF[[43](#_ENREF_43)], confirming the role of inflammatory factors in Wnt signaling and in maintenance of cancer stem cells (see below). Leukotriene D4, which can be produced and secreted by stromal cells in the local tumor microenvironment, promotes the expression and nuclear translocation of β-catenin and thus enhances the growth of colon cancer cells[[44](#_ENREF_44)]. Indeed, β-catenin translocation is often detected at the invasive front of tumors[[45](#_ENREF_45),[46](#_ENREF_46)], consistent with the interpretation that stromal tissue at the invasion front provides signals to tumor cells that promote nuclear translocation of β-catenin and thus drive tumor progression. It is therefore likely that 1,25D3 regulates Wnt signaling by targeting both the tumor microenvironment as well as the tumor cells themselves. Indeed, we have shown that vitamin D interrupts signaling between tumor cells and macrophages and thereby decreases the intensity of Wnt signaling in HCT116 colon cancer cells which are themselves unresponsive to direct effect of vitamin D[[34](#_ENREF_34)]. We demonstrated that this mechanism involved 1,25D3 inhibition of STAT1 activity in macrophages, blocking the release of IL1 and thereby restoring the sensitivity of colon cancer cells to TRAIL-induced apoptosis[[35](#_ENREF_35)]. This is in line with the concept that the tumor microenvironment represents an important target of chemopreventive and chemotherapeutic agents[[47](#_ENREF_47)].

 The ability of vitamin D to regulate Wnt signaling has been confirmed in animal models. Vitamin D and its analogues reduced the number of tumors in ApcMin/+ mice [[48](#_ENREF_48)], associated with decreased nuclear β-catenin and reduced expression of β-catenin target genes[[49](#_ENREF_49)]. Likewise, dietary induction of colon tumors in mice, a model of sporadic colon cancer, accompanied by functional enrichment of Wnt signaling, is reversed by supplementation with vitamin D and Ca[[24](#_ENREF_24)]. ApcMin/+ mice lacking VDR have an increased number of aberrant crypt foci (ACF) and both ACFs and tumors in ApcMin/+/ VDR-/- mice display increased nuclear β-catenin and elevated expression of β-catenin/TCF target genes[[50](#_ENREF_50)]. While the number of adenomas and carcinomas was not affected by the inactivation of VDR, tumors that developed in the ApcMin+/VDR-/- mice were significantly larger, consistent with increased growth dues to enhanced Wnt signaling. We recently confirmed that while targeted inactivation of VDR in intestinal cells did not alter tumor multiplicity in ApcMin/+ mice, inactivation of VDR in macrophages substantially reduced ApcMin/+ tumors (submitted), confirming the important role of VDR signaling in the tumor microenvironment.

Consistent with these *in vitro* data and with studies in mice, dietary supplementation with 1,25D3 decreased the levels ofβ-catenin and increased the expression of E-cadherin in normal mucosa of colon cancer patients[[51](#_ENREF_51)].

**ANTI-INFLAMMATORY PROPERTIES OF VITAMIN D**

Chronic inflammation has been shown to predispose to development of tumors, a striking example being inflammatory bowel disease, which is associated with elevated risk of colon cancer[[52](#_ENREF_52)]. Moreover, it appears that colon cancers that are not linked to inflammatory bowel disease are also driven by inflammation; it has been shown that regular use of NSAIDs lowers the mortality from sporadic colon cancer and inhibits adenomas in FAP patients, who inherit a mutation in the *Apc* gene[[53](#_ENREF_53)]. The mechanisms whereby anti-inflammatory agents inhibit progression of tumors that are not associated with overt inflammation are not fully understood. However, it has been established that cancer and several other chronic diseases are associated with para-inflammation, a low grade inflammation that is coupled to a persistent activation of the DNA damage response[[54](#_ENREF_54)] and the induction of DNA damage- induced soluble factors, including major pro-inflammatory cytokines, chemokines and growth factors. It is possible that anti-inflammatory agents exert their chemopreventive activity by ameliorating the pro-tumorigenic activity of para-inflammation that is associated with aging and that is observed in colon cancer patients.

 Inflammatory bowel disease (IBD) is among the three most prevalent high risk conditions for colon cancer[[52](#_ENREF_52)]. The risk for colorectal cancer increases with the duration and the extent of the disease, consistent with a direct connection between inflammation and colon cancer development. Patients with intestinal inflammatory conditions such as ulcerative colitis (UC) and Crohn’s disease (CD) have a high incidence of vitamin D insufficiency and deficiency[[55](#_ENREF_55)] and show reduced levels of VDR in intestinal epithelium[[56](#_ENREF_56)]. Likewise, higher levels of vitamin D have been shown to lower the risk of Crohn’s disease[[57](#_ENREF_57)]. Overexpression of VDR in intestinal cells inhibits the colitis-associated increase in proinflammatory cytokines, such as TNF, IL1 and CCL2, and protects mice from developing colitis[[56](#_ENREF_56)]. Finally, a vitamin D analogue has been shown to inhibit colon carcinogensis in the AOM/DSS model of ulcerative colitis[[58](#_ENREF_58)], suggesting that VDR signaling may avert the conversion of the inflammatory stimuli into a tumor promoting signal.

VDR knockout mice exhibit a proinflammatory phenotype associated with increased NF-κB activity in intestine, consistent with the ability of VDR signaling to inhibit NF-κB activation[[59](#_ENREF_59)]. TNFα is a major proinflammatory cytokine that activates the NF-κB signaling pathway in tumor cells and thereby regulates their growth and survival. Human colon cancers are infiltrated by inflammatory cells which secrete a variety of proinflammatory factors, including TNFα[[60](#_ENREF_60)]. Likewise, polyps arising in ApcΔ468 mice, a genetic model for intestinal cancer, showed infiltration with mast cells, and depletion of mast cells or anti-TNFα treatment significantly suppressed polyposis in ApcΔ468 mice[[60](#_ENREF_60)]. Etanercept, a specific antagonist of TNFα, also reduced the number and the size of tumors in the AOM/DSS model, confirming a role of TNFα in inflammation-promoted intestinal tumorigenesis. More intriguing was the observation that inhibition of TNFα blocks the accumulation of β-catenin mutations in intestinal cells, suggesting a mutagenic role of TNFα[[61](#_ENREF_61)]. Pharmacological inhibition of TNFα by neutralizing TNFα antibodies is very effective in alleviating inflammation in IBD patients[[62](#_ENREF_62)] and inhibitors of TNFα have also been tested as potential agents for the treatment of colon cancer. Unfortunately, TNFα inhibitors have been linked to a broad range of infections and to the development of lymphomas and skin and lung cancer, limiting their clinical utility.

 An alternative approach to targeting TNF/NF-κB-mediated inflammation and interrupting the link between inflammation and cancer may be offered by vitamin D. 1,25D3 inhibits the interaction of peripheral blood mononuclear cells and colon cancer cells and inhibits the production of TNF[[63](#_ENREF_63)] and blocks NF-κB signaling, a major TNF signaling pathway. VDR physically interacts with IKKβ[[59](#_ENREF_59)] and vitamin D downregulates the expression of NFB target genes, such as Puma[[56](#_ENREF_56)], which play a major role in the survival of cancer cells. In addition, 1,25D3 has been shown to downregulate the expression of Toll–like receptors 2 and 4 (TLR2 and TLR4) on human monocytes, resulting in hyporesponsiveness to TLR activating ligands[[64](#_ENREF_64),[65](#_ENREF_65)]. Inhibition of TLR signaling by vitamin D3 has been suggested to reduce AOM/DSS- induced colon cancer[[66](#_ENREF_66)], pointing to a convergence of the chemopreventive and anti-inflammatory properties of vitamin D3.

NF-κB is not the only oncogenic signaling pathway activated in tumor cells by inflammatory factors. We have shown that TNF enhances Wnt signaling in β-catenin mutant colon cancer cells[[34](#_ENREF_34)], and established that macrophage-derived factors activate Wnt signaling in colon cancer cells through NF-κB signaling[[42](#_ENREF_42)]. Oguma *et al*[[67](#_ENREF_67)]demonstrated that TNFβ promotes Wnt signaling also in gastric cancer cells, which was independent of NF-κB in this tissue.

The HCT116 colon cancer cells have a functional VDR, but do not respond to 1,25D3 treatment with growth arrest, apoptosis or differentiation. However, we demonstrated that in the presence of macrophages, 1,25D3 reduced Wnt signaling in these seemingly vitamin D unresponsive cells by interrupting signaling between tumor cells and macrophages. 1,25D3 inhibits STAT1 activity and prevents tumor cell-induced release of IL1 from macrophages and thereby prevents inflammation-induced Wnt signaling in colon cancer cells[[34](#_ENREF_34)] (Figure 2). Accordingly, 1,25D3 inhibits the ability of macrophages to increase proliferation and survival of colon cancer cells. Among genes that were repressed by 1,25D3 in tumor cells in a macrophage-dependent manner were cyclin D1 and c-myc, consistent with the finding that 1,25D3 prevented macrophage-induced clonogenic growth of HCT116 cells. Therefore, 1,25D3 can exert its tumor-preventive activity by normalizing the tumor microenvironment, and it can inhibit inflammation through a variety of mechanisms.

 Diet-induced obesity, a risk factor for colon cancer, is also associated with increased expression of TNFβ in the intestine. In this settings, TNFβ has also been shown to be coupled to inactivation of GSK3β and increased expression of β-catenin and c-myc, suggesting that obesity increases the risk of colorectal cancer by promoting inflammation[[68](#_ENREF_68)]. Indeed, western style diet (WSD), sufficient to initiate intestinal tumorigenesis in mice[[24](#_ENREF_24)], has been shown to trigger an inflammatory response in mice, accompanied by the accumulation of macrophages in intestinal mucosa and increased levels of circulating proinflammatory cytokines, including IL1β, CCL5 and CCL2[[69](#_ENREF_69)]. Importantly, dietary supplementation with vitamin D and Ca prevents WSD-induced increases in inflammatory markers and inhibits intestinal tumorigensis[[24](#_ENREF_24),[69](#_ENREF_69)]. Dietary supplementation with 1,25D3 reduced markers of inflammation, including C-reactive protein (CRP), TNF, IL1β, IL6 and IL8 also in colon cancer patients[[70](#_ENREF_70)], strongly suggesting that 1,25D3 protects from colon cancer, at least in part, by decreasing inflammation.

**CONCLUSION**

Calcitriol, the most active form of vitamin D3, acts as a potent steroid hormone that binds to VDR and thereby alters the expression of a variety of genes that regulate growth, differentiation and survival of epithelial cells. Epidemiological studies suggest that deficiency of vitamin D increases the incidence of colon cancer and also has a negative impact on the survival of colon cancer patients. The ability of 1,25D3 to interfere with Wnt signaling and to ameliorate inflammation is likely to contribute to its anticancer activity. The optimal form and adequate concentration of vitamin D that have cancer preventive activity should be established, and randomized clinical trials are needed to confirm that 1,25D3 alone, or in combination with other cytotoxic agents, offers therapeutic benefits.

**ACKNOWLEDGMENTS**

I am grateful to Hans-Georg Wisniewski and Len Augenlicht for reading the manuscript and for their helpful suggestions.

**REFERENCES**

1 **Deeb KK**, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer* 2007; **7**: 684-700 [PMID: 17721433 DOI: 10.1038/nrc2196]

2 **Anderson MG**, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of VDR and CYP24A1 mRNA in human tumors. *Cancer Chemother Pharmacol* 2006; **57**: 234-240 [PMID: 16180015 DOI: 10.1007/s00280-005-0059-7]

3 **Pálmer HG**, González-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M, Cano A, de Herreros AG, Lafarga M, Muñoz A. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J Cell Biol* 2001; **154**: 369-387 [PMID: 11470825 DOI: 10.1083/jcb.200102028]

4 **Kumagai T**, O'Kelly J, Said JW, Koeffler HP. Vitamin D2 analog 19-nor-1,25-dihydroxyvitamin D2: antitumor activity against leukemia, myeloma, and colon cancer cells. *J Natl Cancer Inst* 2003; **95**: 896-905 [PMID: 12813173]

5 **Pálmer HG**, Larriba MJ, García JM, Ordóñez-Morán P, Peña C, Peiró S, Puig I, Rodríguez R, de la Fuente R, Bernad A, Pollán M, Bonilla F, Gamallo C, de Herreros AG, Muñoz A. The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. *Nat Med* 2004; **10**: 917-919 [PMID: 15322538 DOI: 10.1038/nm1095]

6 **Salehi-Tabar R**, Nguyen-Yamamoto L, Tavera-Mendoza LE, Quail T, Dimitrov V, An BS, Glass L, Goltzman D, White JH. Vitamin D receptor as a master regulator of the c-MYC/MXD1 network. *Proc Natl Acad Sci USA* 2012; **109**: 18827-18832 [PMID: 23112173 DOI: 10.1073/pnas.1210037109]

7 **Nürnberg B**, Gräber S, Gärtner B, Geisel J, Pföhler C, Schadendorf D, Tilgen W, Reichrath J. Reduced serum 25-hydroxyvitamin D levels in stage IV melanoma patients. *Anticancer Res* 2009; **29**: 3669-3674 [PMID: 19667163]

8 **Feldman D**, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer* 2014; **14**: 342-357 [PMID: 24705652 DOI: 10.1038/nrc3691]

9 **Milczarek M**, Psurski M, Kutner A, Wietrzyk J. Vitamin D analogs enhance the anticancer activity of 5-fluorouracil in an in vivo mouse colon cancer model. *BMC Cancer* 2013; **13**: 294 [PMID: 23777514 DOI: 10.1186/1471-2407-13-294]

10 **Milczarek M**, Rosinska S, Psurski M, Maciejewska M, Kutner A, Wietrzyk J. Combined colonic cancer treatment with vitamin D analogs and irinotecan or oxaliplatin. *Anticancer Res* 2013; **33**: 433-444 [PMID: 23393334]

11 **Moon M**, Song H, Hong HJ, Nam DW, Cha MY, Oh MS, Yu J, Ryu H, Mook-Jung I. Vitamin D-binding protein interacts with Aβ and suppresses Aβ-mediated pathology. *Cell Death Differ* 2013; **20**: 630-638 [PMID: 23257976 DOI: 10.1038/cdd.2012.161]

12 **Jacobs ET**, Hibler EA, Lance P, Sardo CL, Jurutka PW. Association between circulating concentrations of 25(OH)D and colorectal adenoma: a pooled analysis. *Int J Cancer* 2013; **133**: 2980-2988 [PMID: 23754630 DOI: 10.1002/ijc.28316]

13 **Gandini S**, Boniol M, Haukka J, Byrnes G, Cox B, Sneyd MJ, Mullie P, Autier P. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int J Cancer* 2011; **128**: 1414-1424 [PMID: 20473927 DOI: 10.1002/ijc.25439]

14 **Garland CF**, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet* 1989; **2**: 1176-1178 [PMID: 2572900]

15 **Kampman E**, Slattery ML, Caan B, Potter JD. Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). *Cancer Causes Control* 2000; **11**: 459-466 [PMID: 10877339]

16 **Newmark HL**, Lipkin M. Calcium, vitamin D, and colon cancer. *Cancer Res* 1992; **52**: 2067s-2070s [PMID: 1544142]

17 **Robsahm TE**, Tretli S, Dahlback A, Moan J. Vitamin D3 from sunlight may improve the prognosis of breast-, colon- and prostate cancer (Norway). *Cancer Causes Control* 2004; **15**: 149-158 [PMID: 15017127]

18 **Kremer R**, Campbell PP, Reinhardt T, Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *J Clin Endocrinol Metab* 2009; **94**: 67-73 [PMID: 18984659 DOI: jc.2008-1575]

19 **Gorham ED**, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, Newmark HL, Giovannucci E, Wei M, Holick MF. Vitamin D and prevention of colorectal cancer. *J Steroid Biochem Mol Biol* 2005; **97**: 179-194 [PMID: 16236494]

20 **Grau MV**, Baron JA, Sandler RS, Haile RW, Beach ML, Church TR, Heber D. Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. *J Natl Cancer Inst* 2003; **95**: 1765-1771 [PMID: 14652238]

21 **Ng K**, Meyerhardt JA, Wu K, Feskanich D, Hollis BW, Giovannucci EL, Fuchs CS. Circulating 25-hydroxyvitamin d levels and survival in patients with colorectal cancer. *J Clin Oncol* 2008; **26**: 2984-2991 [PMID: 18565885 DOI: 10.1200/JCO.2007.15.1027]

22 **Freedman DM**, Looker AC, Chang SC, Graubard BI. Prospective study of serum vitamin D and cancer mortality in the United States. *J Natl Cancer Inst* 2007; **99**: 1594-1602 [PMID: 17971526 DOI: 10.1093/jnci/djm204]

23 **Newmark HL**, Yang K, Kurihara N, Fan K, Augenlicht LH, Lipkin M. Western-style diet-induced colonic tumors and their modulation by calcium and vitamin D in C57Bl/6 mice: a preclinical model for human sporadic colon cancer. *Carcinogenesis* 2009; **30**: 88-92 [PMID: 19017685 DOI: 10.1093/carcin/bgn229]

24 **Yang K**, Kurihara N, Fan K, Newmark H, Rigas B, Bancroft L, Corner G, Livote E, Lesser M, Edelmann W, Velcich A, Lipkin M, Augenlicht L. Dietary induction of colonic tumors in a mouse model of sporadic colon cancer. *Cancer Res* 2008; **68**: 7803-7810 [PMID: 18829535 DOI: 10.1158/0008-5472.CAN-08-1209]

25 **Padi SK**, Zhang Q, Rustum YM, Morrison C, Guo B. MicroRNA-627 mediates the epigenetic mechanisms of vitamin D to suppress proliferation of human colorectal cancer cells and growth of xenograft tumors in mice. *Gastroenterology* 2013; **145**: 437-446 [PMID: 23619147 DOI: 10.1053/j.gastro.2013.04.012]

26 **Shah S**, Hecht A, Pestell R, Byers SW. Trans-repression of beta-catenin activity by nuclear receptors. *J Biol Chem* 2003; **278**: 48137-48145 [PMID: 12972427]

27 **Shah S**, Islam MN, Dakshanamurthy S, Rizvi I, Rao M, Herrell R, Zinser G, Valrance M, Aranda A, Moras D, Norman A, Welsh J, Byers SW. The molecular basis of vitamin D receptor and beta-catenin crossregulation. *Mol Cell* 2006; **21**: 799-809 [PMID: 16543149]

28 **Burgess AW**, Faux MC, Layton MJ, Ramsay RG. Wnt signaling and colon tumorigenesis--a view from the periphery. *Exp Cell Res* 2011; **317**: 2748-2758 [PMID: 21884696 DOI: 10.1016/j.yexcr.2011.08.010]

29 **Schepers A**, Clevers H. Wnt signaling, stem cells, and cancer of the gastrointestinal tract. *Cold Spring Harb Perspect Biol* 2012; **4**: a007989 [PMID: 22474007 DOI: 10.1101/cshperspect.a007989]

30 Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

31 **Bachelder RE**, Yoon SO, Franci C, de Herreros AG, Mercurio AM. Glycogen synthase kinase-3 is an endogenous inhibitor of Snail transcription: implications for the epithelial-mesenchymal transition. *J Cell Biol* 2005; **168**: 29-33 [PMID: 15631989]

32 **Zhou BP**, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung MC. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. *Nat Cell Biol* 2004; **6**: 931-940 [PMID: 15448698]

33 **Stemmer V**, de Craene B, Berx G, Behrens J. Snail promotes Wnt target gene expression and interacts with beta-catenin. *Oncogene* 2008; **27**: 5075-5080 [PMID: 18469861]

34 **Kaler P**, Augenlicht L, Klampfer L. Macrophage-derived IL-1beta stimulates Wnt signaling and growth of colon cancer cells: a crosstalk interrupted by vitamin D3. *Oncogene* 2009; **28**: 3892-3902 [PMID: 19701245 DOI: 10.1038/onc.2009.247]

35 **Kaler P**, Galea V, Augenlicht L, Klampfer L. Tumor associated macrophages protect colon cancer cells from TRAIL-induced apoptosis through IL-1beta-dependent stabilization of Snail in tumor cells. *PLoS One* 2010; **5**: e11700 [PMID: 20661477 DOI: 10.1371/journal.pone.0011700]

36 **Larriba MJ**, Bonilla F, Muñoz A. The transcription factors Snail1 and Snail2 repress vitamin D receptor during colon cancer progression. *J Steroid Biochem Mol Biol* 2010; **121**: 106-109 [PMID: 20138990 DOI: 10.1016/j.jsbmb.2010.01.014]

37 **Larriba MJ**, Martín-Villar E, García JM, Pereira F, Peña C, de Herreros AG, Bonilla F, Muñoz A. Snail2 cooperates with Snail1 in the repression of vitamin D receptor in colon cancer. *Carcinogenesis* 2009; **30**: 1459-1468 [PMID: 19502595 DOI: 10.1093/carcin/bgp140]

38 **Larriba MJ**, Muñoz A. SNAIL vs vitamin D receptor expression in colon cancer: therapeutics implications. *Br J Cancer* 2005; **92**: 985-989 [PMID: 15770204 DOI: 10.1038/sj.bjc.6602484]

39 **Alvarez-Díaz S**, Valle N, García JM, Peña C, Freije JM, Quesada V, Astudillo A, Bonilla F, López-Otín C, Muñoz A. Cystatin D is a candidate tumor suppressor gene induced by vitamin D in human colon cancer cells. *J Clin Invest* 2009; **119**: 2343-2358 [PMID: 19662683]

40 **Larriba MJ**, González-Sancho JM, Barbáchano A, Niell N, Ferrer-Mayorga G, Muñoz A. Vitamin D Is a Multilevel Repressor of Wnt/b-Catenin Signaling in Cancer Cells. *Cancers (Basel)* 2013; **5**: 1242-1260 [PMID: 24202444 DOI: 10.3390/cancers5041242]

41 **Oyama T**, Yamada Y, Hata K, Tomita H, Hirata A, Sheng H, Hara A, Aoki H, Kunisada T, Yamashita S, Mori H. Further upregulation of beta-catenin/Tcf transcription is involved in the development of macroscopic tumors in the colon of ApcMin/+ mice. *Carcinogenesis* 2008; **29**: 666-672 [PMID: 18204079]

42 **Kaler P**, Godasi BN, Augenlicht L, Klampfer L.. The NF-kappaB/AKT-dependent Induction of Wnt Signaling in Colon Cancer Cells by Macrophages and IL-1beta. *Cancer Microenviron* 2009 [PMID: 19779850 DOI: 10.1007/s12307-009-0030-y]

43 **Vermeulen L**, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, Sprick MR, Kemper K, Richel DJ, Stassi G, Medema JP. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; **12**: 468-476 [PMID: 20418870 DOI: 10.1038/ncb2048]

44 **Salim T**, Sand-Dejmek J, Sjölander A. The inflammatory mediator leukotriene D₄ induces subcellular β-catenin translocation and migration of colon cancer cells. *Exp Cell Res* 2014; **321**: 255-266 [PMID: 24211746 DOI: 10.1016/j.yexcr.2013.10.021]

45 **Brabletz T**, Jung A, Hermann K, Günther K, Hohenberger W, Kirchner T. Nuclear overexpression of the oncoprotein beta-catenin in colorectal cancer is localized predominantly at the invasion front. *Pathol Res Pract* 1998; **194**: 701-704 [PMID: 9820866]

46 **Brabletz T**, Jung A, Reu S, Porzner M, Hlubek F, Kunz-Schughart LA, Knuechel R, Kirchner T. Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc Natl Acad Sci U S A* 2001; **98**: 10356-10361 [PMID: 11526241]

47 **Albini A**, Sporn MB. The tumour microenvironment as a target for chemoprevention. *Nat Rev Cancer* 2007; **7**: 139-147 [PMID: 17218951]

48 **Huerta S**, Irwin RW, Heber D, Go VL, Koeffler HP, Uskokovic MR, Harris DM. 1alpha,25-(OH)(2)-D(3) and its synthetic analogue decrease tumor load in the Apc(min) Mouse. *Cancer Res* 2002; **62**: 741-746 [PMID: 11830528]

49 **Xu H**, Posner GH, Stevenson M, Campbell FC. Apc(MIN) modulation of vitamin D secosteroid growth control. *Carcinogenesis* 2010; **31**: 1434-1441 [PMID: 20488884 DOI: 10.1093/carcin/bgq098]

50 **Larriba MJ**, Ordóñez-Morán P, Chicote I, Martín-Fernández G, Puig I, Muñoz A, Pálmer HG. Vitamin D receptor deficiency enhances Wnt/β-catenin signaling and tumor burden in colon cancer. *PLoS One* 2011; **6**: e23524 [PMID: 21858154 DOI: 10.1371/journal.pone.0023524]

51 **Ahearn TU**, Shaukat A, Flanders WD, Rutherford RE, Bostick RM. A randomized clinical trial of the effects of supplemental calcium and vitamin D3 on the APC/β-catenin pathway in the normal mucosa of colorectal adenoma patients. *Cancer Prev Res (Phila)* 2012; **5**: 1247-1256 [PMID: 22964475 DOI: 10.1158/1940-6207.CAPR-12-0292]

52 **Itzkowitz SH**, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G7-17 [PMID: 15194558]

53 **Oshima M**, Taketo MM. COX selectivity and animal models for colon cancer. *Curr Pharm Des* 2002; **8**: 1021-1034 [PMID: 11945149]

54 **Medzhitov R**. Origin and physiological roles of inflammation. *Nature* 2008; **454**: 428-435 [PMID: 18650913 DOI: 10.1038/nature07201]

55 **Driscoll RH**, Meredith SC, Sitrin M, Rosenberg IH. Vitamin D deficiency and bone disease in patients with Crohn's disease. *Gastroenterology* 1982; **83**: 1252-1258 [PMID: 6982188]

56 **Liu W**, Chen Y, Golan MA, Annunziata ML, Du J, Dougherty U, Kong J, Musch M, Huang Y, Pekow J, Zheng C, Bissonnette M, Hanauer SB, Li YC. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *J Clin Invest* 2013; **123**: 3983-3996 [PMID: 23945234 DOI: 10.1172/JCI65842]

57 **Ananthakrishnan AN**, Khalili H, Higuchi LM, Bao Y, Korzenik JR, Giovannucci EL, Richter JM, Fuchs CS, Chan AT. Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. *Gastroenterology* 2012; **142**: 482-489 [PMID: 22155183 DOI: 10.1053/j.gastro.2011.11.040]

58 **Fichera A**, Little N, Dougherty U, Mustafi R, Cerda S, Li YC, Delgado J, Arora A, Campbell LK, Joseph L, Hart J, Noffsinger A, Bissonnette M. A vitamin D analogue inhibits colonic carcinogenesis in the AOM/DSS model. *J Surg Res* 2007; **142**: 239-245 [PMID: 17574271 DOI: 10.1016/j.jss.2007.02.038]

59 **Chen Y**, Zhang J, Ge X, Du J, Deb DK, Li YC. Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein. *J Biol Chem* 2013; **288**: 19450-19458 [PMID: 23671281 DOI: 10.1074/jbc.M113.467670]

60 **Gounaris E**, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, Lee DM, Zhang G, Glickman JN, Shin K, Rao VP, Poutahidis T, Weissleder R, McNagny KM, Khazaie K. Mast cells are an essential hematopoietic component for polyp development. *Proc Natl Acad Sci U S A* 2007; **104**: 19977-19982 [PMID: 18077429]

61 **Popivanova BK**, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest* 2008; **118**: 560-570 [PMID: 18219394]

62 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095]

63 **Bessler H**, Djaldetti M. 1α,25-Dihydroxyvitamin D3 modulates the interaction between immune and colon cancer cells. *Biomed Pharmacother* 2012; **66**: 428-432 [PMID: 22795808 DOI: 10.1016/j.biopha.2012.06.005]

64 **Sadeghi K**, Wessner B, Laggner U, Ploder M, Tamandl D, Friedl J, Zügel U, Steinmeyer A, Pollak A, Roth E, Boltz-Nitulescu G, Spittler A. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. *Eur J Immunol* 2006; **36**: 361-370 [PMID: 16402404 DOI: 10.1002/eji.200425995]

65 **Khoo AL**, Chai LY, Koenen HJ, Oosting M, Steinmeyer A, Zuegel U, Joosten I, Netea MG, van der Ven AJ. Vitamin D(3) down-regulates proinflammatory cytokine response to Mycobacterium tuberculosis through pattern recognition receptors while inducing protective cathelicidin production. *Cytokine* 2011; **55**: 294-300 [PMID: 21592820 DOI: 10.1016/j.cyto.2011.04.016]

66 **Murillo G**, Nagpal V, Tiwari N, Benya RV, Mehta RG. Actions of vitamin D are mediated by the TLR4 pathway in inflammation-induced colon cancer. *J Steroid Biochem Mol Biol* 2010; **121**: 403-407 [PMID: 20214986 DOI: 10.1016/j.jsbmb.2010.03.009]

67 **Oguma K**, Oshima H, Aoki M, Uchio R, Naka K, Nakamura S, Hirao A, Saya H, Taketo MM, Oshima M. Activated macrophages promote Wnt signalling through tumour necrosis factor-alpha in gastric tumour cells. *EMBO J* 2008; **27**: 1671-1681 [PMID: 18511911 DOI: 10.1038/emboj.2008.105]

68 **Liu Z**, Brooks RS, Ciappio ED, Kim SJ, Crott JW, Bennett G, Greenberg AS, Mason JB. Diet-induced obesity elevates colonic TNF-α in mice and is accompanied by an activation of Wnt signaling: a mechanism for obesity-associated colorectal cancer. *J Nutr Biochem* 2012; **23**: 1207-1213 [PMID: 22209007 DOI: 10.1016/j.jnutbio.2011.07.002]

69 **Bastie CC**, Gaffney-Stomberg E, Lee TW, Dhima E, Pessin JE, Augenlicht LH. Dietary cholecalciferol and calcium levels in a Western-style defined rodent diet alter energy metabolism and inflammatory responses in mice. *J Nutr* 2012; **142**: 859-865 [PMID: 22437564 DOI: 10.3945/jn.111.149914]

70 **Hopkins MH**, Owen J, Ahearn T, Fedirko V, Flanders WD, Jones DP, Bostick RM. Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: a randomized, controlled clinical trial. *Cancer Prev Res (Phila)* 2011; **4**: 1645-1654 [PMID: 21724580 DOI: 10.1158/1940-6207.CAPR-11-0105]

71 **Skrzypczak M**, Goryca K, Rubel T, Paziewska A, Mikula M, Jarosz D, Pachlewski J, Oledzki J, Ostrowski J. Modeling oncogenic signaling in colon tumors by multidirectional analyses of microarray data directed for maximization of analytical reliability. *PLoS One* 2010; **5**: [PMID: 20957034 DOI: 10.1371/journal.pone.0013091]

**P-Reviewer:** Barni S, Wang ZH **S-Editor:** Song XX **L-Editor:** **E-Editor:**

****

**Figure 1 The expression levels of IL1β and Snail are increased and the levels of vitamin D receptor decreased in colon cancer patients (Skrypziack, PLOS ONE 2010, [**[**71**](#_ENREF_71)**]).**

****

**Figure 2 The multiple mechanisms whereby vitamin D inhibits Wnt signaling**: **1,25D3 acts on both tumor cells and tumor-associated macrophages (and potentially on other stromal cells).** In tumor cells, 1,25D3 promotes VDR/β-catenin binding and thus inhibits nuclear translocation of β-catenin. It also induces the expression of E-cadherin (CDH1), Dickkopf1 (DKK1), Dickkopf4 (DKK4) and cystatin 5 (CST5), antagonizingβ-catenin/TCF transcriptional activity. As a result, the expression of several Wnt target genes, such as Snail, CD44, Myc, Axin2 (in red) is downregulated by 1,25D3. These activities require VDR expression in tumor cells. In addition, vitamin D also acts on cells in the tumor microenvironment. We demonstrated that 1,25D3 inhibits STAT1 activity in tumor-associated macrophages and prevents the release of IL1β, which in a paracrine manner promotes Wnt signaling in cancer cells. 1,25D3 can thereby regulate Wnt signaling in tumor cells that do not respond directly to 1,25D3. VDR: Vitamin D receptor.