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Vitamin D and Colorectal Cancer: Molecular, Epidemiological, and Clinical Evidence

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Abstract

In many cells throughout the body, vitamin D is converted into its active form calcitriol, and binds to vitamin D receptor (VDR), which functions as a transcription factor to regulate various biological processes including cellular differentiation and immune response. Vitamin D metabolizing enzymes (including CYP24A1 and CYP27B1) and VDR play major roles in exerting and regulating effects of vitamin D. Preclinical and epidemiological studies provide evidence for anticancer effects of vitamin D (in particular, against colorectal cancer), though clinical trials have

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Use of standardised official symbols: We use HUGO (Human Genome Organisation)-approved official symbols for genes and gene products, including BAG1, BAK1, BAX, BCL2, BIRC5, CAMP, CASR, CDH1, CDK2, CDK6, CDKN1A, CDKN1B, CTNNB1, CYP2R1, CYP24A1, CYP27B1, DHCR7, DKK1, DKK4, EGF, EGFR, FOS, GC, GDF15, HIF1A, IGF2, IGFBP6, IL6, JUN, KDM3A, MAPK, MSH2, PRKCA, PTH, SNAI1, SNAI2, TCF7L2, TGFB1, THBS1, TNF, VDR, VEGFA, WNT; all of which are described at www.genenames.org. Gene names are italicised, and gene product names are non-italicised. Human genes and gene products are written with all capital letters, while non-human genes and gene products are written in small letters except for the first capital letter.

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yet to prove its benefit. Additionally, molecular pathological epidemiology research can provide insights into the interaction of vitamin D with tumour molecular and immunity status. Other future research directions include genome-wide research on VDR transcriptional targets, gene-environment interaction analyses, and clinical trials on vitamin D efficacy in colorectal cancer patients. Here we review the literature on vitamin D and colorectal cancer from both mechanistic and population studies, and discuss the links and controversies within and between the two parts of evidence.

Keywords

25-hydroxyvitamin D; P450 hydroxylases; vitamin D supplementation

Introduction

Although a well-recognised physiological role of vitamin D is the regulation of calcium and phosphate metabolism[1], recent studies suggest a much broader range of biological functions of vitamin D, including potential anti-neoplastic effects. Garland *et al.* discovered in 1980 that colon cancer mortality rates in the U.S. were highest in places where populations were exposed to the least amount of sunlight, and proposed that vitamin D might be a protective factor against colon cancer[2]. Since then, extensive studies have reported anti-neoplastic actions of vitamin D, particularly in colorectal cancer[3; 4]. If adequate vitamin D does have a protective effect, ensuring that people have sufficient vitamin D can be an effective way to reduce cancer incidence and mortality[4]. In this review, we discuss relevant basic science and preclinical studies, which examined the mechanisms including the regulation of proliferation, differentiation, apoptosis, angiogenesis, and immunity. We also discuss epidemiological and human intervention studies, and address possible reasons why evidence for an effect of vitamin D supplementation remains inconclusive. In addition, we remark on molecular pathological epidemiology[5; 6], which can bridge the gap between basic science and human population studies of vitamin D and colorectal cancer.

We conducted the literature research in the Web of Science database under the topics of “Vitamin D” AND “Colorectal Neoplasms”, and in the PubMed database using the MeSH terms of “Vitamin D” AND “Colorectal Neoplasms”, for papers published in English from January 1995 till November 2015. We manually searched references cited in the chosen articles and in published reviews.

Source and metabolism of vitamin D

Vitamin D belongs to a group of steroids known as secosteroids. In humans, the most common forms of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol); both can be ingested from the diet and as diet supplements. Vitamin D_3 can also be synthesised in adequate amounts in the skin, under exposure to sunlight[7]. Since vitamin D can be produced in the human body, strictly speaking it is not a vitamin *per se*, but rather is the precursor to the potent steroid hormone calcitriol [also known as 1,25-dihydroxyvitamin D_3, or $1,25(OH)_2D$].

Vitamin D from the skin and diet is activated to calcitriol by two cytochrome P450-mediated hydroxylation steps. The first step takes place mostly in the liver, where the enzyme vitamin D-25-hydroxylase (predominantly CYP2R1) catalyses the first hydroxylation of vitamin D at C25. This reaction yields 25-hydroxyvitamin D [25(OH)D], the circulating form with a half-life of 2 weeks that is used to determine an individual's vitamin D status[7; 8]. In the second step, 25(OH)D is metabolised by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) at the kidneys and certain extrarenal sites, to yield the active form calcitriol[9]. Calcitriol then performs its biological functions, inhibits CYP27B1 activity[10], and induces expression of the enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which catabolizes 25(OH)D and calcitriol into biologically inactive forms (Figure 1)[11].

Mechanism of calcitriol action

Calcitriol exerts its biological effects by binding and activating the nuclear vitamin D receptor (VDR) and regulating gene expression[3; 12]. The binding of calcitriol induces a conformational change in VDR that allows the receptor to dimerise with the retinoid X receptor (RXR); this heterodimer specifically docks on vitamin D response elements (VDREs) in the promoter regions of target genes[13]. The conformational change of VDR also recruits co-activator and detaches co-repressor to acetylate nucleosome histones and unravel DNA, thus enabling transcription (Figure 2A)[14].

Calcitriol-dependent repression of gene transcription is documented for the *CYP27B1*[15] and *PTH*[16] genes. Haussler *et al.* postulated that VDR-mediated repression initiates with the docking of liganded VDR-RXR on a negative VDRE in the promoter regions of target genes, which then conforms liganded VDR such that it binds co-repressor rather than co-activator (Figure 2B)[17].

In addition to its genomic actions that occur over a period of hours or days, calcitriol also rapidly initiates many biological responses[18]. For instance, calcitriol can bind with a plasma membrane VDR of the intestinal epithelial cells and cause the coupled opening of Ca^{2+} channels, resulting in the rapid hormonal stimulation of intestinal calcium transport (transcaltachia) within minutes[19; 20]. Furthermore, the binding of calcitriol with membrane VDR may engage in crosstalk with the classical VDR pathway to modulate gene expression, possibly through Ca^{2+} influx activation of Ca^{2+} messenger system such as protein kinase C[3].

Vitamin D metabolism in colorectal cancer

The response of cancer cells to calcitriol depends not only on VDR expression, but on the intracellular concentrations of calcitriol as well[21; 22]. Intracellular calcitriol concentrations are determined by the circulating concentrations of 25(OH)D and calcitriol, and by the activity of CYP27B1 and CYP24A1 within the cell. CYP27B1 and CYP24A1 were previously known as enzymes within the kidney, but are now also found in extrarenal sites including the colon[23; 24]. As described below, the levels of CYP27B1, CYP24A1, and VDR in colorectal cancer cells are studied in relation to differentiation and response to treatment.

CYP27B1

CYP27B1, as the synthesizing enzyme of calcitriol, is normally expressed at low levels in the colon[25; 26]. In well and moderately differentiated colorectal cancer samples, expression of CYP27B1 is elevated, whereas in poorly differentiated colorectal cancer samples the expression is repressed[25; 27; 28]. Ogunkolade *et al.* reported that *CYP27B1* mRNA expression levels are similar in colorectal cancer samples and in healthy colons, but are decreased in adjacent normal colon mucosa 10 cm from the tumour border[29]; this finding suggests that *CYP27B1* expression in adjacent colon is regulated by the tumour, or that low expression of *CYP27B1* in the colon is a risk for carcinogenesis. Bareis *et al.* showed that the slowly dividing, highly differentiated colorectal cancer cell line Caco-2/15 responds in a dose-dependent manner to epidermal growth factor (EGF) or calcitriol by upregulating expression of VDR and CYP27B1, whereas highly proliferative, less differentiated cell lines (Caco-2/AQ, COGA-1A and COGA-1E) show a downregulation of VDR and CYP27B1 after EGF or calcitriol treatment[30]. Although definite *in vivo* evidence is lacking, local production of calcitriol in colon has been indirectly suggested by human studies. The serum concentration of 25(OH)D, rather than of calcitriol, is inversely associated with colonic epithelial cell proliferation in a chemoprevention study[31]. Wagner *et al.* showed a positive correlation between serum and colon calcitriol concentrations ($r = 0.58$, $P = 0.0008$), with a positive colon calcitriol intercept (21.5 pmol/kg, $P < 0.001$) at zero serum calcitriol, supporting the notion of synthesis of calcitriol within colon[32]. To summarize, elevated CYP27B1 expression suggests possible benefit from treatment with vitamin D, especially in well and moderately differentiated tumours, while the relatively low expression of CYP27B1 in poorly differentiated colorectal cancer indicates a mechanism of resistance of the cancer cells to calcitriol actions.

CYP24A1

As the main enzyme determining the biological half-life of calcitriol, CYP24A1 is found in low levels in normal human colon mucosa and in colorectal adenomas, but in elevated levels in the majority of adenocarcinomas[33]. *CYP24A1* mRNA expression is also increased in poorly differentiated and late-stage colorectal cancers, compared with well-differentiated, early stage tumours[28]. Anderson *et al.* showed that *CYP24A1* mRNA expression is not only significantly upregulated in human HT-29 cells, but also profoundly stimulated by calcitriol treatment, abrogating the anti-proliferative effect of calcitriol[34]. Kosa *et al.* also observed that *CYP24A1* mRNA is induced by calcitriol treatment in Caco-2, a human colon adenocarcinoma cell line. Cell viability and proliferation are not influenced by calcitriol alone, but are markedly reduced when calcitriol is co-administered with KD-35, a CYP24A1 inhibitor[35]. Together, these findings suggest that CYP24A1 exhibits a potent negative feedback effect, and that inhibition of CYP24A1 may be a good strategy for enhancing the anti-tumour effect of calcitriol.

VDR

As the major receptor to mediate the biological effects of calcitriol, VDR is present in most cells of the human body, and is especially abundant in intestinal epithelial cells[36]. VDR expression is increased in adenoma, and in well or moderately differentiated colorectal

cancer tissues, but is decreased in poorly differentiated tumours, and negligible in metastatic lymph nodes[27; 37]. Palmer *et al.* discovered that the transcription factors SNAI1 and SNAI2 (snails) repress VDR expression in SW480-ADH cells, and block the anti-tumour action of the calcitriol analog EB1089[38]. RNA expression of *SNAI1* and *SNAI2* is upregulated in human colorectal cancers, and is inversely correlated with *VDR* mRNA expression[38, 39]. These findings suggest that high levels of SNAI1 and SNAI2 are a probable cause of VDR downregulation and of vitamin D unresponsiveness in advanced colorectal cancer, and that vitamin D therapy may not be a good treatment choice for patients who overexpress SNAI1 and SNAI2.

Anticancer actions of vitamin D on colorectal cancer

The anticancer effects of calcitriol are mostly studied *in vitro* by binding to the VDR and causing transcriptional activation and repression of target genes. Given the pivotal role of nuclear VDR as a transcriptional regulator, researchers investigate the genome-wide targets of calcitriol-stimulated VDR in human cells by chromatin immunoprecipitation-sequencing (ChIP-Seq). In one such study profiling human lymphoblastoid cells, VDR binding sites are significantly enriched near colorectal cancer associated genes identified from genome-wide association studies[40]. Meyer *et al.* performed ChIP-Seq for VDR/RXR on human colorectal cancer cell LS180, and identified *FOS* and *MYC* among the target genes[41]. In addition, several transcription factors regulated by calcitriol subsequently amplify and diversify the transcriptional output[42]. The most studied anticancer effects of calcitriol are listed below.

Proliferation

Early studies established VDR as a biomarker for the vitamin D-mediated inhibition of human colon cancer cell growth[43]. The anti-proliferative effect of vitamin D on colorectal cancer involves multiple pathways. In Caco-2 cells, calcitriol and its analogs (F6-D3, ZK 156718 and BGP-13) increase expression of the cyclin-dependent kinase (CDK) inhibitors CDKN1A and CDKN1B, which inhibit CDK2 and CDK6, leading to G1 phase arrest[44, 45; 46]. Calcitriol also results in activation of latent transforming growth factor-β1 (TGFB1) in Caco-2 cells, and sensitises these cells to the growth inhibitory effects of TGFB1[47]. Synthetic low-calcemic vitamin D analogs (EB1089 and CB1093) decrease proliferation of HT-29 human cancer cells by inhibiting the secretion of insulin-like growth factor 2 (IGF2), and by inducing the insulin-like growth factor-binding protein-6 (IGFBP6), which sequesters IGF2 with high affinity[48]. Calcitriol also counteracts EGF-stimulated Caco-2 cell growth by markedly decreasing epidermal growth factor receptor (EGFR) expression[49].

Differentiation

Calcitriol has multiple pro-differentiation effects in colorectal cancer cells. The classic marker for differentiation is expression of alkaline phosphatase, which is found along the brush border of the colon mucosa but is poorly expressed in proliferating colorectal cancer cells. Calcitriol and its analogs (ZK 156718 and EB1089) increase the activity of alkaline phosphatase in colorectal adenoma cell lines (RG/C2 and AA/C1) and colorectal cancer cells (Caco-2, PC/JW, HT29 and SW620)[45; 50]. Chen *el al.* reported that calcitriol increases

alkaline phosphatase activity in Caco-2 cells by stimulating activator protein-1 (JUN/FOS) activation, which is accomplished via a protein kinase C alpha (PRKCA) and mitogen-activated protein kinase (MAPK)-dependent mechanism[51].

Apart from affecting the expression of alkaline phosphatase, calcitriol also induces the expression of E-cadherin (CDH1) and other adhesion proteins, causing β-catenin (CTNNB1) to translocate from the nucleus to E-cadherin complexes at the plasma membrane in the human colon cancer SW480-ADH cell line[52, 53]; similar effect on Cdh1 is observed in an *Apc*$^{min/+}$ mouse model[54]. Meanwhile, ligand-activated VDR competes with the T cell-specific transcription factor 7-like 2 (TCF7L2) for CTNNB1 binding and represses downstream gene expression in SW480-ADH cells[52]. Calcitriol-VDR also inhibits CTNNB1 activity in Caco-2 cells, and the inhibition is enhanced by wild-type APC[55]. Finally, the WNT antagonist DKK1 is induced by calcitriol in association with E-cadherin in SW480-ADH cells[56]. As a result, calcitriol and its analogs inhibit the WNT/CTNNB1 pathway and the activation of its target genes in colorectal cancer cells; this in turn contributes to the inhibition of cell proliferation, and to the maintenance of the differentiated phenotype.

Apoptosis

Calcitriol induces apoptosis in colorectal adenoma and colorectal cancer by upregulating the pro-apoptotic proteins BAK1 and BAX, and by downregulating the anti-apoptotic proteins BAG1, BIRC5, and BCL2. In two colorectal adenoma and three colorectal cancer cell lines, calcitriol and vitamin D analog EB1089 induce p53-independent apoptosis in a dose-dependent manner, and levels of the pro-apoptotic protein BAK1 are consistently increased in all cell lines examined[50]. Barnes *et al.* showed that EB1089 induces apoptosis in a colorectal adenoma S/RG/C2 cell line by redistributing the anti-apoptotic protein BAG1 from the nucleus to the cytoplasm[57]. Liu *et al.* discovered that calcitriol suppresses the expression of BIRC5 (survivin), and promotes a cytotoxic response to 5-fluorouracil in human colon cancer cells (CBS, Moser, Caco-2 and HCT116) in a calcium-sensing receptor (CASR)-dependent manner[58], possibly by binding the VDREs in CASR promoters[59, 60]. In an Apc$^{1638N/+}$ mouse model of intestinal cancer, a western-pattern diet decreases expression of the pro-apoptotic protein BAX, and increases expression of the anti-apoptotic protein BCL2; treatment with vitamin D and calcium reverses these effects of the western-style diet, and markedly inhibits tumour growth[61]. In a human colorectal cancer xenograft model in nude mice, treatment with the vitamin D analogs BGP-13 and BGP-15 activates cell apoptosis[46]. However, the pro-apoptotic effect of calcitriol appears not always true: Stambolsky *et al.* reported that mutant TP53 is recruited to VDR-regulated genes, and converts calcitriol into an anti-apoptotic agent in SW480 cells[62]. Thus, *TP53* mutation status might be a predictive marker for vitamin D treatment response.

Angiogenesis

Calcitriol also inhibits angiogenesis. Mantell *et al.* showed that calcitriol significantly inhibits the sprouting and elongation of vascular endothelial growth factor A (VEGFA)-induced endothelial cells in a dose-dependent manner[63]. In human colorectal cancer SW480 cells, calcitriol treatment for 24 hours at 0.1 and 1 SM decreases expression of

hypoxia-inducible factor-1α (HIF1A), and at 1 SM inhibits the secretion of VEGFA under conditions of hypoxia[64]. However, Fernandez-Garcia *et al.* reported that calcitriol increases the levels of VEGFA and the anti-angiogenic factor thrombospondin 1 (THBS1), leading to a minimal balanced change in the angiogenic potential of SW480-ADH cells[65]. Calcitriol also represses expression of DKK4 in SW480-ADH cells; DKK4 is induced by the TCF7L2/CTNNB1 pathway and enhances the migratory, invasive and pro-angiogenic potential of colorectal cancer[66]. In a rat model of colon tumourigenesis induced by azoxymethan, intraperitoneal administration of calcitriol significantly reduces the incidence of colon tumours, and also decreases the level of VEGFA and microvessel counts in tumours, suggesting that anti-angiogenesis is a mechanism for the anti-tumourigenic effect of vitamin D[67].

Immune modulation

Calcitriol modulates innate and adaptive immunity in the colon[68]. Calcitriol induces expression of the cathelicidin antimicrobial peptide (CAMP), a major component of the innate immune system, in HT29 cells[69]. Lithocholic acid, a secondary bile acid and a vitamin D analog, decreases nuclear factor-κB activity via the VDR in colonic cancer cells (Caco-2 and HT29C19A)[70]. *CYP27B1* knockout mice show increased IL1 and IL17 expression in the colon and are more susceptible to colitis, compared with heterozygote controls[71]. In a *Smad3*$^{-/-}$ mouse model of bacteria-induced colitis, increased dietary vitamin D suppresses MAPK and nuclear factor-κB activation, severity of colitis, and incidence of intestinal cancer[72]. In addition, calcitriol has effects on several immune cell types, including dendritic cells, B cells, and T cells, throughout the human body[73]. Specifically, *Vdr* knockout mouse model shows that VDR is required for the maturation and proliferation of intestinal CD8αα^{+} intraepithelial lymphocytes[74], which might have a regulatory role within the gut[75]. On the other hand, the effect of calcitriol, and the level of expression of VDR, may both be affected by the immune environment of colon: in human colon ductal epithelium, VDR expression is considerably decreased in patients with ulcerative colitis, and is even lower in patients with colitis-associated colorectal cancer[76]. In line with this, treatment with tumour necrosis factor (TNF) and interleukin 6 (IL6) leads to decreased expression of CYP27B1 in colonic epithelial COGA-1A cells[77].

Recent studies have shown interactions between gut microbiota and immunity in colon carcinogenesis[78, 79, 80], and vitamin D has been reported to regulate the gut microbiome. In a dextran sodium sulfate-induced colitis model, mice on vitamin D-deficient diet show more prominent symptoms of colitis and elevated concentrations of bacteria compared with mice on vitamin D-sufficient diet[81]. Similarly, in the same colitis model, Ooi *et al.* showed that *Cyp27b1* knockout mice have higher concentrations of the *Helicobacter* species in the faeces and more severe symptoms of colitis compared with wild-type littermates[82]. In addition, calcitriol supplementation (1.25 Sg/100 g diet) to *Cyp27b1* knockout mice reduces *Helicobacter* numbers and colitis severity[82]. Given the data from mouse models, it would be interesting to investigate changes of the human gut microbiome after vitamin D supplementation.

MicroRNA

MicroRNAs (miRs) are implicated in the antineoplastic influence of vitamin D[12]. Alvarez-Diaz *et al.* reported that *miR-22* is induced by calcitriol in a time-, dose- and VDR-dependent manner in multiple human colorectal cancer cell lines[83]. Specifically, in SW480-ADH and HCT116 cells that express VDR, *miR-22* is required for the anti-proliferative and anti-migratory effects of calcitriol, and regulates the expression of several target genes of calcitriol. Consistently, *miR-22* expression is associated with *VDR* expression in human colorectal cancer samples, suggesting that *miR-22* has a role in the VDR mediated anti-tumour effect of vitamin D.

Padi *et al.* found that calcitriol upregulates *miR-627*, which in turn mediates the anti-growth effect of calcitriol in HT-29 cells; they reported that *miR-627* downregulates the expression of KDM3A (which encodes a histone demethylase), increases methylation of histone H3K9, and thereby suppresses expression of proliferative factors such as *GDF15*[84]. This same effect of *miR-627* is also found in the HCT116 xenograft model of nude mice[84]. Collectively, these findings suggest that enhancing the effect of *miR-627*, or suppressing its target KDM3A, has the same anti-tumour effect as does vitamin D, and may bypass the side effects of hypercalcaemia.

Vitamin D in animal models of colorectal cancer

Studies in various animal models of colorectal cancer support a protective role of vitamin D. A western-style diet (high in fat and low in vitamin D and calcium) induces benign and malignant tumours in various mouse models of intestinal tumourigenesis, and supplementation with vitamin D plus calcium produces a significant decrease in the incidence and multiplicity of colon tumours[85]. In murine models of colorectal carcinogenesis induced by exogenous carcinogens, administration of calcitriol or vitamin D also impedes the neoplastic process[67; 86; 87].

Tumour cells implanted into mice are commonly used to evaluate anti-cancer treatments. In a human colorectal cancer (MC26) xenograft model, mice fed on a vitamin D-sufficient diet have smaller tumours than those fed on a vitamin D-deficient diet[88]; in nude mice, treatment with vitamin D analogs (BGP-13 and BGP-15) inhibits the growth of human HT29 xenograft[46]. Add-on of the vitamin D analogs PRI-2191 and PRI-2205 shows improved anti-tumour effects compared with chemotherapy alone, which includes 5-fluorouracil, capecitabine, irinotecan or oxaliplatin[89; 90].

Mouse models of intestinal cancer are also generated by introducing specific germ line mutations. The $Apc^{+/min}$ mice develop more than 100 intestinal tumours per animal, and calcitriol significantly decreases the surface area with polyps in the gastrointestinal tract[54; 91]. In the $Apc^{+/1638N}$ mouse model of intestinal cancer, when the animals are fed on a western-style diet, adding dietary vitamin D and calcium induces apoptosis of epithelial cells and inhibits tumourigenesis in the intestine[61]. A protective effect by vitamin D is also observed in Smad3$^{-/-}$ mice, a model of bacteria-driven colitis and colon cancer when infected with *Helicobacter bilis*[72]. Finally, a *Vdr* knockout mouse model, compared with wild-type and heterozygote mice, has shown increased markers of cell proliferation and

oxidative stress in the colon descendens[92]. Compared with *Apc*$^{+/min}$ *Vdr*$^{+/+}$ mice, *Apc*$^{+/min}$ *Vdr*$^{-/-}$ mice have increased nuclear Ctnnb1, higher expression of Ctnnb1/Tcf7l2 target genes, and larger tumors in the intestine[93], supporting the anti-neoplastic effect of VDR in colon.

Vitamin D action in human colon and rectum

Beyond cell lines and animal models, researchers have studied the effects of supplemental vitamin D in the colon and rectum of humans. In a randomised, double-blinded, controlled trial of 2 X 2 factorial design, Bostick[94] and colleagues tested the efficacy of 800 IU of vitamin D and/or 2 g of calcium daily for 6 months on subjects with recently diagnosed colorectal adenoma. Normal-appearing rectal mucosa was biopsied, and immunohistochemistry was performed for markers of differentiation and proliferation. Statistically significant increase of expression in the vitamin D group relative to the placebo group was found in BAX (56%)[95], CDKN1A (142%)[96], APC (48%), CDH1 (78%)[97], MSH2 (169%)[98], CASR (39%), and CYP27B1 (159%)[99]. These findings, in line with preclinical studies, indicate that supplemental vitamin D can favourably modulate multiple biomarkers of colorectal cancer risk in normal colon tissues.

Epidemiological studies of vitamin D and colorectal cancer

Epidemiological studies have extensively investigated the relation between vitamin D status and colorectal cancer, not only on the incidence of the disease, but also on the survival of its patients. Regarding the surrogates for vitamin D status, the evidence of association is strong for plasma 25(OH)D concentration, but less so for vitamin D intake. For a better interpretation of the data, the strengths and weaknesses of the surrogates are discussed in the context of study design.

Measurement of vitamin D in human populations

Determination of vitamin D status of individuals in population-based studies needs a consideration of both biology and logistics. The plasma concentration of total 25(OH)D, the major circulating metabolite of vitamin D, is commonly used to determine vitamin D status[100]. For instance, a 25(OH)D concentration of less than 20 ng/mL (50 nmol/L) is considered vitamin D insufficiency[101], and 25(OH)D concentration of greater than 150 ng/ml (375 nmol/L) may cause vitamin D intoxication[100]. However, the association of 25(OH)D with colorectal cancer may be confounded by other risk factors. For example, both obesity and low physical activity have been associated with lower plasma 25(OH)D concentrations, as well as with increased colorectal cancer risk[102]. Inflammation has been postulated as another confounder based on the assumption that inflammation reduces 25(OH)D concentration[103], although there is some evidence against this theory[104]. Moreover, especially for cohorts, the time of blood drawing will likely precede the diagnosis of colorectal cancer for a variety of years for different patients, and it might be helpful to have an additional 25(OH)D measurement that is within a comparable time from diagnosis among all patients[105; 106]. However, serial blood drawing may not be feasible in many large-scale cohort studies.

Alternatively, dietary or supplementary intake of vitamin D can be assessed repeatedly with questionnaires. Nevertheless, recall of diet and supplement use is imprecise. Moreover, since skin exposed to sunlight also produces vitamin D, vitamin D intake does not necessarily represent overall vitamin D status, or the plasma concentration of 25(OH)D. In 3,345 subjects of the Women's Health Initiative (WHI) observational study, total vitamin D intake calculated based on information from questionnaires explains 9% variance in serum 25(OH)D concentration[107].

Recently, a predicted 25(OH)D score using dietary and lifestyle information collected from questionnaires has been used as a surrogate of vitamin D status[108, 109]. Using multivariate linear regression, Bertrand *et al.* derived this score based on known determinants of circulating 25(OH)D, including age, race, ultraviolet radiation exposure, vitamin D intake, BMI, physical activity, alcohol intake, post-menopausal hormone use, and season of blood draw, from more than 4,500 participants with available blood samples in three U.S. nationwide cohorts[108]. The predicted score explains 25% to 33% variance in plasma 25(OH)D concentration in different cohorts. This approach of using information from questionnaires estimates vitamin D status data in cohorts where plasma concentrations are not available, and incorporates not only dietary vitamin D intake but also non-dietary exposures which are associated with increased plasma 25(OH)D concentration. Of note, the predicted score was derived from the original cohorts, and its application to other cohorts will require further validation.

Plasma concentrations of 25(OH)D and incidence of colorectal cancer

Table 1 summarises the previous studies investigating plasma 25(OH)D concentration and incidence of colorectal cancer with at least 300 cases[109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122]. Evidence for the association of plasma 25(OH)D concentration or 25(OH)D score with lower colorectal cancer incidence is quite strong. To further support this, two meta-analyses reported inverse associations between plasma 25(OH)D concentration and risk of colorectal adenoma, a well-established precancerous lesion for colorectal cancer[123, 124].

By integrating exposure data such as vitamin D status and tumour molecular/immune features of colorectal cancer tissue, molecular pathological epidemiology (MPE)[5, 6, 125, 126] research provides new insights into the relationship between vitamin D and colorectal cancer. Jung *et al.* studied the risk of colorectal cancer in relation to predicted score for 25(OH)D concentration (with 1,059 incident cases during follow-up of 140,418 participants). A higher predicted 25(OH)D score was inversely associated with colorectal cancer risk ($P < 0.001$), regardless of VDR expression levels in tumour cells ($P_{heterogeneity} = 0.75$)[109]. Considering the role of vitamin D in the immune system, another MPE study showed that high plasma 25(OH)D concentration was associated with lower risk of colorectal cancer with high-level immune reaction ($P_{trend} < 0.001$), but not with risk of tumour with lower-level reaction ($P_{trend} > 0.50$, $P_{heterogeneity} = 0.001$)[122]. This statistical analysis of heterogeneity is critical, since the hypotheses address differential effects of vitamin D on subtypes[127, 128]. These data support the hypothesis that effect of vitamin D might be strong in tumours enriched with immune cells[122] because immune cells in tumour

can activate vitamin D and thereby increase local level of active vitamin D[129; 130; 131]. Although a replication by additional studies is needed, these findings suggest an interplay of vitamin D status and the immune system in inhibiting the tumourigenesis of colorectal cancer. In addition, a possible interaction may exist between vitamin D status and tumour immunity status in colorectal cancer patient survival analyses, requiring further investigation. With complex immune and inflammatory processes suggested to be involved in colorectal cancer progression and regulated by vitamin D, it has been recommended that future epidemiological studies should measure both vitamin D and inflammatory markers, preferably multiple times, and perform mediation analysis[132] to study the role of inflammation as a mediator between vitamin D and colorectal cancer[68].

Plasma 25(OH)D concentration and survival of colorectal cancer

Table 2 shows the previous studies with at least 300 cases on plasma 25(OH)D concentration and survival of patients with diagnosed colorectal cancer[133; 134; 135; 136; 137; 138; 139]. Of note, to reduce potential reverse causation associated with undiagnosed tumours at the time of blood draw that might lower plasma 25(OH)D concentration, the Nurses' Health Study (NHS)[133; 134], the Health Professionals Follow-up Study (HPFS)[133; 134], and the European Prospective Investigation into Cancer and Nutrition (EPIC) study[136] measured plasma 25(OH)D concentration before diagnosis of colorectal cancer, and excluded cases diagnosed within 2 years after blood collection. In contrast, the Study of Colorectal Cancer in Scotland (SOCCS)[137] and the CALGB/SWOG 80405[139] studies measured 25(OH)D shortly after diagnosis, a timing more subject to reverse causation. Despite the different timing of blood collection, there is a consistent prognostic association of plasma 25(OH)D concentration with colorectal cancer patient survival.

Vitamin D intake and incidence of colorectal cancer

Table 3 lists the previous studies exploring the relationship between vitamin D intake and risk of colorectal cancer with at least 500 cases[113; 115; 140; 141; 142; 143; 144; 145; 146; 147; 148; 149; 150]. In contrast to the consistent and strong evidence from the studies measuring plasma 25(OH)D, the association of vitamin D intake and incidence of colorectal cancer is conflicting. Nevertheless, a 2011 meta-analysis[115] of prospective studies reported an inverse association of vitamin D intake and colorectal cancer incidence.

Vitamin D intake and survival of colorectal cancer

Observational studies on the impact of vitamin D intake in patients with diagnosed colorectal cancer are limited. In a paper published in 2014, Yang *et al.* included 1,111 participants in the Cancer Prevention Study II Nutrition Cohort who were diagnosed with invasive, non-metastatic colorectal cancer. The researchers evaluated associations of calcium, vitamin D, and diary product intakes after colorectal cancer diagnosis with all-cause and colorectal cancer-specific mortality. After a mean follow-up of 7.6 years, both calcium and milk intakes were inversely associated with all-cause mortality and colorectal cancer-specific mortality, but vitamin D intake was not associated with either mortality outcomes[151].

Randomised controlled trials

Randomised placebo-controlled trials are the “gold standard” in establishing causal association; however, such evidence to date has been inconclusive on the effect of vitamin D on colorectal cancer. The findings and limitations of completed clinical trials are discussed, with a preview of ongoing trials that might hopefully bring a conclusion to the controversy.

Completed clinical trials of vitamin D intake and incidence of colorectal cancer

To date, four completed randomised controlled trials of vitamin D have a reasonable number of cancer cases (Table 4)[110, 152, 153, 154]. In a substudy of the Women’s Health Initiative (WHI), 36,282 postmenopausal women were given 200 IU of vitamin D and 500 mg of calcium twice daily (400 IU of vitamin D and 1000 mg of calcium daily), or a matching placebo, for an average of 7 years[110]. The incidence of invasive colorectal cancer in this study did not differ significantly between women assigned to calcium plus vitamin D and those assigned to placebo (168 versus 154 cases, hazard ratio = 1.08, 95% CI: 0.86–1.34, $P = 0.51$), and tumour characteristics were similar in the two groups. This study has several limitations. First, the modest dose of vitamin D used in the trial leads to only a small rise in plasma 25(OH)D concentration[155], which was measured only in a small sample of the study population. Second, the limited compliance in the treatment group and the allowance for the placebo group to take supplements could have further reduced the actual contrast of 25(OH)D between groups. In fact, as shown in a *post hoc* analysis of WHI, in 15,646 women (43%) who were not taking personal calcium or vitamin D supplement at randomization, calcium and vitamin D treatment non-significantly reduced the risk of colorectal cancer by 17%[156]. Third, the 7-year follow-up may not be sufficient to show a benefit for prevention of colorectal cancer, which has a long natural history and a relatively low incidence.

A second completed randomised trial was carried out in the United Kingdom, with 2686 participants (2037 men and 649 women)[152]. An oral supplement of 100,000 IU vitamin D, or a matching placebo, was given every 4 months for 5 years. Over the 5-year period, 28 and 27 cases of colon cancer were documented in the treatment and control group, respectively, with no association with vitamin D treatment (relative risk = 1.02, 95% CI: 0.60–1.74, $P = 0.94$). This study applied a dosage of vitamin D that had a moderate effect upon the measured plasma 25(OH)D concentration (74.3 nmol/L in the treatment group vs. 53.4 nmol/L in the control group, $P < 0.001$); nevertheless, it was limited by the small sample size and the short follow-up.

Two other studies have investigated the association of vitamin D and calcium supplement intake with cancer incidence. The Nebraska trial[153] detected lower incidence of cancer in patients treated with vitamin D plus calcium than with placebo ($P < 0.03$), whereas the RECORD trial[154] found no association. However, neither study was designed to detect the association of supplement use with colorectal cancer incidence as the primary endpoint.

In the recently published Vitamin D/Calcium Polyp Prevention trial (Table 4)[157], patients with recently diagnosed adenomas were randomly assigned vitamin D 1000 IU daily or no vitamin D in a factorial design. After 3 or 5 years of treatment, participants given vitamin D

had a mean net increase in serum 25(OH)D concentration of 7.83 ng/ml, relative to participants given placebo. Overall, 43% of participants had one or more adenoma diagnosed during follow-up, and the adjusted risk ratio for recurrent adenoma was 0.99 (95% CI, 0.89–1.09) with vitamin D versus no vitamin D.

Two points are worth noting for comparison of this null finding with preexisting epidemiological evidence. Firstly, as the authors admitted, the vitamin D dose in the Polyp Prevention Trial (1000 IU daily) was lower than the dose many experts now recommend[158; 159], and it was used for a limited time[157]. This resulted in a net increase of 7.83 ng/ml of serum 25(OH)D, in contrast to a generally more than 20 ng/ml difference between the high and low quartiles or quintiles of 25(OH)D in observational studies[160]. Thus, the moderate dose of vitamin D might not cause a change in adenoma incidence that was detectable by the power of this trial. Secondly, the risk of incidence for recurrent adenoma is not a direct translation of the risk for incident adenoma or colorectal cancer. For instance, in a colorectal cancer screening trial, elevated dietary fiber intake was associated with reduced risk of incident colorectal adenoma and colorectal cancer [odds ratio (OR) = 0.76 and 0.85, respectively], but not with the risk of recurrent adenoma (OR = 1.08)[161]. Similarly, a meta-analysis has also shown different associations of higher serum 25(OH)D with incident or recurrent colorectal adenoma (OR = 0.82 or 0.87 for a 20 ng/ml increase, respectively)[124]. Therefore, the null finding should not be generalized to persons without a recent history of colorectal adenoma. Based on the clinical literature included in this review, high vitamin D status might have the greatest anti-neoplastic effects early in colorectal carcinogenesis and later in disease progression, but less so in metastatic stage or adenoma recurrence.

Ongoing clinical trials of vitamin D intake and incidence of colorectal cancer

Several randomised controlled trials are under way to study whether vitamin D supplementation reduces the risk of cancer (Table 5)[162]. These trials apply higher dosages of vitamin D than previous trials, and measure baseline and/or follow-up plasma 25(OH)D concentrations. For example, the VITAL study collects baseline blood samples on 17,000 participants and follow-up samples on 6,000[163]. In aggregate, these trials have already recruited over 53,000 participants, and the first results are expected to be available in 2015.

Clinical trial of vitamin D intake and survival of colorectal cancer

Accumulating evidence of the involvement of vitamin D in cancer progression demands clinical trials for patients diagnosed with colorectal cancer. The study of mortality, rather than incidence, of colorectal cancer will likely require fewer subjects and shorter follow-up. To date, only one clinical trial is registered on ClinicalTrials.gov addressing this question (NCT01516216); it is recruiting 120 participants with previously untreated metastatic colorectal cancer and randomizing them to 2 arms. Together with the standard chemotherapy with FOLFOX and bevacizumab, Arm 1 gets vitamin D 400 IU/day, whereas Arm 2 gets a loading dose of 8000 IU/day for 2 weeks followed by a maintenance dose of 4000 IU/day. Although the sample size is small, the study does collect plasma 25(OH)D concentration, so analyses of the relationships between high dose vitamin D treatment, 25(OH)D status, and prognosis are possible.

Genetic variation, vitamin D status, and colorectal cancer

Heritable factors explain approximately 35% of the risk of colorectal cancer[164], and contribute substantially to the variability of vitamin D status[165]. Thus, genetic variation related with vitamin D status might have impact on the risk of colorectal cancer. A genome-wide association study of circulating 25(OH)D concentrations in 33,996 individuals has identified single nucleotide polymorphism (SNP) loci near four genes, including *GC* (which encodes vitamin D binding protein), *DHCR7* (which encodes 7-dehydrocholesterol reductase that can remove the substrate from vitamin D synthesis in skin), *CYP2R1*, and *CYP24A1*[166]. To gain insight into the genetic link between vitamin D status and colorectal cancer, Hiraki *et al.* investigated these four SNP loci in 10,061 colorectal cancer cases and 12,768 controls, but found no significant association between the loci and risk of colorectal cancer[167]. A similar null finding was reported in another cohort containing 438 colorectal cancer cases[168]. Moreover, the four loci do not overlap with the risk variants identified from previous genome-wide association studies for colorectal cancer[169]. Because the SNPs identified by Wang *et al.* can explain only a small variation (1% – 4%) of 25(OH)D concentrations[166], the reduction in overall colorectal cancer risk by increased vitamin D levels due to the SNPs might be too small to be detectable. In addition to genes related to vitamin D metabolism, *VDR* polymorphism has also been studied for risk of colorectal cancer, although most results are inconclusive[170]. Nevertheless, two meta-analyses have shown significant associations of risk for colorectal cancer with two *VDR* polymorphisms, *BsmI* (RR = 0.57, 95% CI: 0.36–0.89 for BB vs. bb)[171] and *TaqI* (OR = 1.43, 95% CI: 1.30–1.58 for tt vs. TT)[172], respectively.

As one future direction, the MPE approach may link vitamin-D-related SNPs to specific subtype of colorectal cancer. Another future direction is to investigate interactions between SNPs of vitamin D pathway genes and vitamin D status variables in analyses of colorectal cancer incidence and mortality[173]. In addition to such a candidate gene approach, analyses of genome-wide gene-environment interactions with vitamin D status variables may enable us to discover potentially important SNPs and pathways for colorectal cancer[169]. Next generation sequencing technologies, with greater depth and finer resolution, will draw a broader picture for the targets and interacting factors of vitamin D and VDR, and relate them with specific diseases including colorectal cancer[174].

Conclusion

Since Garland *et al.*[2] proposed vitamin D for colon cancer prevention 25 years ago, functional studies on vitamin D or its analogs have provided supportive evidence for its anti-tumour effect in colorectal cancer. Evidence from both *in vitro* and *in vivo* experiments suggests that anti-proliferation, pro-differentiation, pro-apoptosis, anti-angiogenesis, immune modulation, and microRNA regulation are involved in the anti-tumour effect of vitamin D. Recent studies also explore the local expression and impact of vitamin D metabolizing enzymes and VDR, which may lead to discovery of predictive biomarkers for vitamin D treatment response.

Epidemiological studies have consistently demonstrated a strong inverse association of plasma 25(OH)D concentration with colorectal cancer incidence and mortality. The MPE approach is valuable in generating hypotheses on potential mechanisms of the observed protective effect of vitamin D, and in identifying molecular pathological signatures as predictive markers for benefit from vitamin D. On the other hand, the effect of vitamin D intake on colorectal cancer prevention is controversial, largely due to three reasons: the slow development of colorectal cancer, the confounding effects caused by sunlight exposure, outdoor physical activity, body mass index, dairy and calcium intakes, etc. in observational studies, and the suboptimal dosage of vitamin D applied in previous clinical trials. Ongoing large randomised controlled trials with high dose vitamin D treatment are promising to tackle these problems and decide the value of vitamin D supplementation. Meanwhile, clinical trials of vitamin D on colorectal cancer survival are scarce and logistically more feasible, suggesting a new direction for future studies. Finally, next generation sequencing and studies of genome-wide gene-environment interactions will likely shed more light on the mechanisms of association between vitamin D and colorectal cancer.

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Abbreviations

1,25(OH)$_2$D	1,25-dihydroxyvitamin D3
25(OH)D	25-hydroxyvitamin D
CI	confidence interval
CRC	colorectal cancer
HPFS	Health Professionals Follow-up Study
MPE	molecular pathological epidemiology
NHS	Nurses' Health Study
OR	odds ratio
RXR	retinoid X receptor
SD	standard deviation
SNP	single nucleotide polymorphism
VDR	vitamin D receptor

VDRE	vitamin D response elements
VEGFA	vascular endothelial growth factor A
WHI	Women's Health Initiative
VITAL	VITamin D and OmegA-3 TriaL

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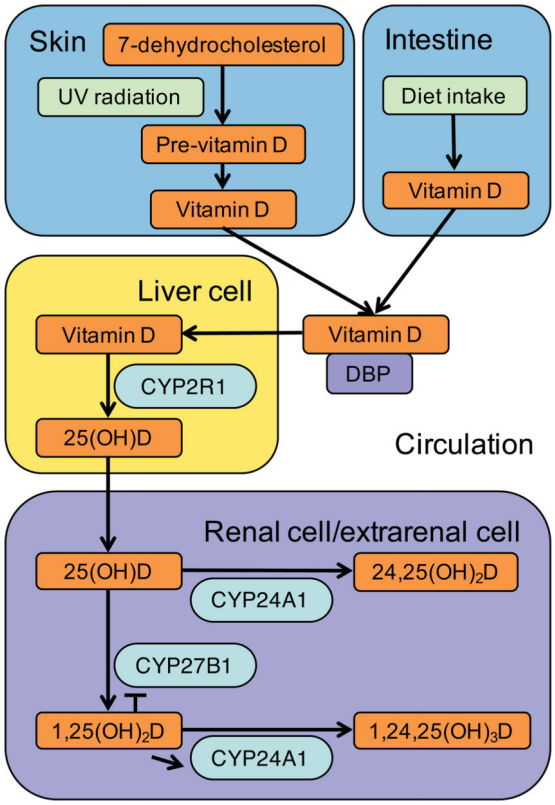

Figure 1.

The metabolism of vitamin D in human body. Vitamin D that is taken up in the diet, or synthesized from 7-dehydrocholesterol by skin following UV exposure, binds to DBP in the circulation and is transported to the liver. Vitamin D is hydroxylated at C25 by CYP2R1 in the liver to 25(OH)D, the major circulating form of vitamin D in the human body. In the kidney and some extrarenal sites, 25(OH)D is further hydroxylated at C1 by CYP27B1 into 1,25(OH)2D (calcitriol), the bioactive form. Both 25(OH)D and 1,25(OH)2D are deactivated by CYP24A1 through additional hydroxylation at C24. Both CYP27B1 and CYP24A1 are regulated by calcitriol. UV, ultraviolet. DBP, vitamin D binding protein.

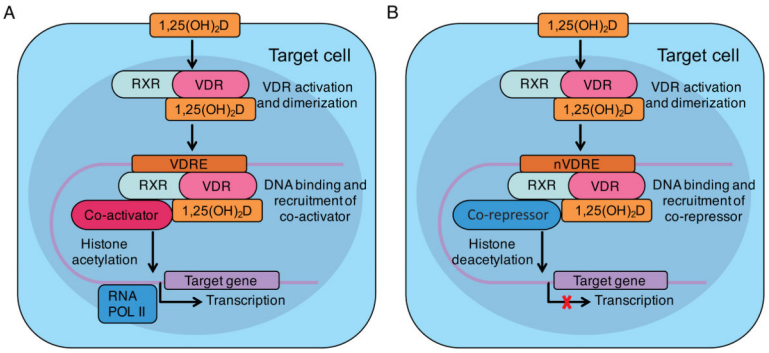

Figure 2.

The mechanism of calcitriol [1,25(OH)$_2$D] action through VDR. Calcitriol binds and activates nuclear VDR, which then dimerises with RXR. (A) Transcriptional activation involves VDR-RXR heterodimer binding with VDRE and recruitment of histone acetyltransferase co-activator. (B) Transcriptional depression involves VDR-RXR binding with nVDRE and recruitment of histone deacetylase co-repressor. nVDRE, negative VDRE; RNA POL II, RNA polymerase II; RXR, retinoid X receptor; VDR, vitamin D receptor; VDRE, vitamin D response element.

Table 1

Major studies (N of cases ≥ 300) investigating plasma 25(OH)D concentration and incidence of colorectal cancer

Author (year)	Study name	Design	N of cases	Follow-up, y	Association of plasma 25(OH)D and incidence of colorectal cancer (95% CI)	P_{trend}	$P_{heterogeneity}$
Wactawski-Wende (2006)[110]	WHI	Nested case-control	322	7	Highest vs. lowest quartile:	0.02	
					OR = 0.40 (0.23–0.67)		
Wu *et al.* (2007)[111]	NHS, HPFS	Nested case-control	372	NHS 5.5; HPFS 4.4	Highest vs. lowest quintile:	0.01	
					OR = 0.66 (0.42–1.05)		
Otani *et al.* (2007)[112]	JPHC Study	Nested case-control	375	11.5	Highest vs. lowest quartile:		
					Male OR = 0.73 (0.35–1.5);	0.39	
					Female OR = 1.1 (0.50–2.3)	0.74	
Jenab *et al.* (2010)[113]	EPIC	Nested case-control	1,248	3.8	<25 nmol/L, OR = 1.32 (0.87–2.01);	<0.001	
					25.0–49.9 nmol/L, OR = 1.28 (1.05–1.56);		
					50.0–74.9 nmol/L, referent;		
					75.0–99.9 nmol/L, OR = 0.88 (0.68–1.13);		
					≥100.0 nmol/L, OR = 0.77 (0.56–1.06)		
Lee *et al.* (2011)[114]	N/A	Meta-analysis (prospective studies)	2,690	N/A	Highest vs. lowest category:	N/A	
					OR = 0.66 (0.54–0.81);		
					Colon cancer OR = 0.77 (0.56–1.07);		0.20 *
					Rectal cancer OR = 0.50 (0.28–0.88)		
Ma *et al.* (2011)[115]	N/A	Meta-analysis (prospective studies)	2,767	N/A	Highest vs. lowest category:	N/A	
					OR = 0.67 (0.54–0.80)		
Chung *et al.* (2011)[116]	N/A	Meta-analysis (prospective studies)	1,127	N/A	Each 10-nmol/L increase:		
					OR = 0.94 (0.91–0.97)	<0.001	
Neuhouser *et al.* (2012)[117]	WHI	Nested case-control	310	7	Highest vs. lowest quartile:		
					OR = 0.22 (0.10–0.51)	0.003	
English *et al.* (2013)[118]	MCCS	Case-cohort	563	14	Highest vs. lowest quartile:		
					OR = 0.82 (0.61–1.10)	0.20	
Jung *et al.* (2014)[109]	NHS, HPFS	Prospective cohort, predicted 25(OH)D, MPE (VDR expression)	1,059	22	Highest vs. lowest quintile:		
					HR = 0.52 (0.42–0.64);	<0.001	
					VDR (−) HR = 0.48 (0.30–0.78);	0.004	0.22 †
					VDR (+) HR = 0.56 (0.42–0.75)	<0.001	

Author (year)	Study name	Design	N of cases	Follow-up, y	Association of plasma 25(OH)D and incidence of colorectal cancer (95% CI)	P_{trend}	$P_{heterogeneity}$
Anic *et al.* (2014)[119]	ATBC	Nested case-control, male smokers	428	6.1	Highest vs. lowest quartile:		
					OR = 1.35 (0.91–2.01)	0.11	
					DBP low OR = 1.12 (0.65–1.94)	0.98	0.24‡
					DBP high OR = 1.63 (0.94–2.83)	0.02	
Theodoratou *et al.* (2014)[120]	N/A	Meta-analysis	2,764	N/A	Highest vs. lowest quartile:		
					OR = 0.70 (0.58–0.84)	0.0002	
Weinstein *et al.* (2015)[121]	PLCO	Nested case-control	476	5.6	Highest vs. lowest quintile:		
					OR = 0.59 (0.36–0.95)	0.02	
Song *et al.* (2015)[122]	NHS, HPFS	Nested case-control, MPE (immune reaction)	318	NHS 20; HPFS 16	Highest vs. lowest tertile:		
					High reaction OR = 0.10 (0.03–0.35);	<0.001	0.001§
					Mild reaction OR = 0.98 (0.62–1.54);	0.93	
					Absent reaction OR = 0.71 (0.26–1.95)	0.55	

Abbreviations: CI, confidence interval; WHI, Women's Health Initiative; OR, odds ratio; NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; JPHC Study, Japan Public Health Center-based Prospective Study; EPIC, European Prospective Investigation into Cancer and Nutrition; MCCS, Melbourne Collaborative Cohort Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; DBP, vitamin D binding protein; MPE, molecular pathological epidemiology; VDR, vitamin D receptor; HR, hazard ratio; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.

*, †, ‡, § $P_{heterogeneity}$ is for colon cancer vs. rectal cancer (*), VDR (-) vs. VDR (+) (†), low vs. high DBP (‡), and high vs. mild vs. absent reaction (§), respectively.

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Table 2

Major studies (N of cases ≥ 300) investigating plasma 25(OH)D concentration and survival of patients with diagnosed colorectal cancer

Author (year)	Study name	Design and timing of blood draw	N of cases	All deaths (CRC deaths)	Follow-up, y	Association of plasma 25(OH)D and mortality of CRC (95% CI)	P_{trend}
Ng *et al.* (2008)[133]	NHS, HPFS	Prospective cohort, pre-diagnosis	304	123 (96)	6.5	Highest vs. lowest quartile:	
						CRC-specific HR = 0.61 (0.31–1.19);	0.23
						All-cause HR = 0.52 (0.29–0.94)	0.02
Ng *et al.* (2009)[134]	NHS, HPFS	Prospective cohort, predicted 25(OH)D	1,017	283 (119)	9.7	Highest vs. lowest quintile:	
						CRC-specific HR = 0.50 (0.26–0.95);	0.02
						All-cause HR = 0.62 (0.42–0.93)	0.002
Ng *et al.* (2011)[135]	NCCTG 9741	Prospective cohort, mCRC post-diagnosis	515	475 (N/A)	5.1	Highest vs. lowest quartile:	
						PFS HR = 1.07 (0.81–1.42)	0.66
						All-cause HR = 0.94 (0.72–1.23)	0.55
Fedirko *et al.* (2012)[136]	EPIC	Prospective cohort, pre-diagnosis	1,202	541 (444)	6.1	Highest vs. lowest quintile:	
						CRC-specific HR = 0.69 (0.50–0.93);	0.04
						All-cause HR = 0.67 (0.50–0.88)	0.01
Zgaga *et al.* (2014)[137]	SOCCS	Prospective cohort post-diagnosis	1,598	531 (363)	8.9	Highest vs. lowest tertile:	
						CRC-specific HR = 0.68 (0.50–0.90);	0.009
						All-cause HR = 0.70 (0.55–0.89)	0.003
Maalmi *et al.* (2014)[138]	N/A	Meta-analysis (prospective studies)	2,330	1,214 (566)	N/A	Highest vs. lowest category:	N/A
						CRC-specific HR = 0.65 (0.49–0.86)	
						All-cause HR = 0.71 (0.55–0.91)	
Ng *et al.* (2015)[139]	CALGB/SWOG 80405	Prospective cohort, mCRC post-diagnosis	1,043	N/A	7	Highest vs. lowest quintile:	
						PFS HR = 0.80 (0.64–1.01)	0.02
						All-cause HR = 0.67 (0.53–0.86)	0.002

Abbreviations: CRC, colorectal cancer; CI, confidence interval; NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; NCCTG, North Central Cancer Treatment Group; mCRC, metastatic colorectal cancer; PFS, progression-free survival; EPIC, European Prospective Investigation into Cancer and Nutrition; SOCCS, Study of Colorectal Cancer in Scotland; CALGB, Cancer and Leukemia Group B; SWOG, Southwest Oncology Group.

Table 3

Major studies (N of cases ≥ 500) investigating vitamin D intake and incidence of colorectal cancer

Author (year)	Study name	Design	N of cases	Follow-up, y	Association of vitamin D intake and incidence of CRC (95% CI)	P_{trend}
Martinez *et al.* (1996)[140]	NHS	Prospective cohort	501	12	Highest vs. lowest quintile:	
					RR = 0.88 (0.66–1.16)	0.23
Pritchard *et al.* (1996)[141]	Stockholm	Case-control	569	N/A	Highest vs. lowest quartile:	
					Colon cancer OR = 0.6 (0.4–1.0)	0.08
					Rectal cancer OR = 0.5 (0.3–0.9)	0.08
Marcus *et al.* (1998)[149]	N/A	Case-control	512	N/A	Highest vs. lowest quintile:	
					Colon cancer OR = 0.7 (0.4–1.1)	0.05
					Rectal cancer OR = 0.8 (0.5–1.5)	0.42
Terry *et al.* (2002)[142]	SMC	Cohort, women	572	11.3	Highest vs. lowest quartile:	
					RR = 1.05 (0.83–1.33)	0.73
McCullough *et al.* (2003)[150]	CPS II	Cohort	683	5	Highest vs. lowest quartile:	
					RR = 0.80 (0.62–1.02)	0.02
Slattery *et al.* (2004)[143]	N/A	Case-control	2,306	N/A	Highest vs. lowest quartile:	N/A
					Men OR = 1.08 (0.73–1.60);	
					Women OR = 0.52 (0.32–0.85)	
Park *et al.* (2007)[144]	Multiethnic Cohort Study	Cohort	2,100	7.3	Highest vs. lowest quintile:	
					Men RR = 0.66 (0.54–0.81);	0.03
					Women RR = 0.81 (0.66–0.98)	0.80
Mizoue *et al.* (2008)[145]	Fukuoka CRC Study	Case-control	836	N/A	Highest vs. lowest quintile:	
					OR = 0.79 (0.56–1.11);	0.12
					Indoor OR = 0.63 (0.36–1.08);	0.02
					Outdoor OR = 0.94 (0.58–1.52)*	0.86
Ishihara *et al.* (2008)[146]	JPHC Study	Nested case-control	761	7.8	Highest vs. lowest quintile:	
					Men OR = 0.92 (0.60–1.42);	0.58
					Women OR = 1.49 (0.86–2.60)	0.26
Lipworth *et al.* (2009)[147]	N/A	Case-control	1,953	N/A	Highest vs. lowest decile:	
					Colon cancer OR = 0.69 (0.50–0.96);	<0.0001
					Rectal cancer OR = 1.22 (0.82–1.80)	0.70

Author (year)	Study name	Design	N of cases	Follow-up, y	Association of vitamin D intake and incidence of CRC (95% CI)	P_{trend}
Huncharek *et al.* (2009)[148]	N/A	Meta-analysis (cohorts)	2,813	N/A	Highest vs. lowest category:	N/A
					RR = 0.94 (0.83–1.06)	
Jenab et al. (2010)[113]	EPIC	Nested case-control	1,248	3.8	Highest vs. lowest quintile:	
					OR = 0.84 (0.60–1.17)	0.19
Ma *et al.* (2011)[115]	N/A	Meta-analysis (prospective studies)	6,466	N/A	Highest vs. lowest category:	N/A
					RR = 0.88 (0.80–0.96)	

Abbreviations: CRC, colorectal cancer; CI, confidence interval; NHS, Nurses' Health Study; RR, relative risk; SMC, Swedish Mammography Cohort; CPS II, Cancer Prevention study II; OR, odds ratio; JPHC Study, Japan Public Health Center-based Prospective Study; EPIC, European Prospective Investigation into Cancer and Nutrition.

*Indoor: subjects engaged in sedentary or standing work (including no job) and no outdoor physical activity at leisure. Outdoor: subjects engaged in work with labor or walking or outdoor physical activity at leisure at least 120 min/wk.

Table 4

Major completed randomised trials (N ≥ 1,000) investigating vitamin D supplementation and cancer

Author (year)	Study name	N of subjects	Age, y	Treatment duration, y	Vitamin D treatment	Endpoint	N of cases (treat/ctrl)	Hazard ratio (95% CI)	*P*
Trivedi, *et al.* (2003)[152]	N/A	2,686	≥60	5	100,000 IU/4 mo	Cancer incidence	188/173	1.09 (0.86–1.36)	0.47
						CRC incidence	28/27	1.02 (0.60–1.74)	0.94
						Cancer mortality	56/59	0.86 (0.61–1.20)	0.37
						CRC mortality	7/7	0.62 (0.24–1.60)	0.33
Wactawski-Wende, *et al.* (2006)[110]	WHI	36,282	50–79	7	400 IU/d plus calcium 1,000 mg/d	Cancer incidence	1634/1655	0.98 (0.91–1.05)	0.53
						CRC incidence	168/154	1.08 (0.86–1.34)	0.51
						Cancer mortality	344/382	0.89 (0.77–1.03)	0.12
						CRC mortality	34/41	0.82 (0.52–1.29)	0.39
Lappe, *et al.* (2007)[153]	Nebraska trial	1,179	>55	4	1,100 IU/d plus calcium 1,500 mg/d	Cancer incidence	13/20	0.40 (0.20–0.82)	0.01
						CRC incidence	1/2	N/A	N/A
Avenell, *et al.* (2012)[154]	RECORD	5,292	≥70	2–4	800 IU/d	Cancer incidence	338/315	1.07 (0.92–1.25)	0.38
						CRC incidence	41/30	N/A	N/A
						Cancer mortality	151/178	0.85 (0.68–1.06)	0.16
						CRC mortality	20/13	N/A	N/A
Baron, *et al.* (2015)[157]	Vitamin D/ Calcium Polyp Prevention	2,259	45–75	3–5	1000 IU/d	Adenoma incidence	438/442	0.99 (0.89–1.09)	0.98
						Cancer incidence	47/61	N/A	0.17
						CRC incidence	3/2	N/A	1.00

Abbreviations: CI, confidence interval; CRC, colorectal cancer; WHI, Women's Health Initiative; RECORD, Randomised Evaluation of Calcium Or vitamin D.

Table 5

Major ongoing randomised trials (N ≥ 1,000) investigating vitamin D supplementation and cancer

Study name	Location	N of subjects	Age, y	Treatment duration, y	Vitamin D treatment	Primary Endpoint	Trial Registry No.	Estimated completion
VITAL	United States	25,874	Men, ≥50; women, ≥55	5	2,000 IU/d	Cancer, CVD	NCT01169259	2017
D-Health	Australia	21,000	60–84	5	60,000 IU/mo	Total mortality, cancer	ACTRN12613000743763	2020
Vitamin D/ Calcium Polyp Prevention	United States	2,813, with colorectal adenoma removed within 120 days	45–75	1–5	1,000 IU/d	Colorectal adenoma, colorectal cancer	NCT00153816	2016
FIND	Finland	2,495	Men, ≥60; women, ≥65	5	1,600 IU/d or 3,200IU/d	Cancer, CVD	NCT01463813	2020
CAPS	United States	2,332 post-menopausal women	≥55	5	2,000 IU/d plus calcium 1,600 mg/d	Cancer	NCT01052051	2015
VIDAL	United Kingdom	Pilot, 1,600; main, 20,000	65–84	Pilot, 2; main, 5	100,000 IU/mo	Total mortality, cancer	ISRCTN46328341	Pilot, 2013; main, N/A

Abbreviations: VITAL, Vitamin D and Omega-3 Trial; CVD, cardiovascular diseases; FIND, Finnish Vitamin D Trial; CAPS, Clinical Trial of Vitamin D3 to Reduce Cancer Risk in Postmenopausal Women; VIDAL, Vitamin D and Longevity Trial.