

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 the vitamin D receptor (VDR), which functions as a transcription factor to regulate various biological processes including cellular differentiation and immune response. Vitamin D-metabolising enzymes (including CYP24A1 and CYP27B1) and VDR play major roles in exerting and regulating the effects of vitamin D. Preclinical and epidemiological studies have provided evidence for anti-cancer effects of vitamin D (particularly against colorectal cancer), although clinical trials have yet to prove its benefit. In addition, 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. In this study, 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.

Key words: 25-Hydroxyvitamin D: P450 hydroxylases: Vitamin D supplementation

Although a well-recognised physiological role of vitamin D is the regulation of Ca and phosphate metabolism⁽¹⁾, recent studies suggest a much broader range of biological functions of vitamin D, including potential anti-neoplastic effects. Garland & Garland⁽²⁾ discovered in 1980 that colon cancer mortality rates in the USA 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. 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 (MPE)^(5,6), which can bridge the gap between basic science and human population studies of vitamin D and colorectal cancer.

We conducted a literature research in the Web of Science database under the topics 'Vitamin D' AND 'Colorectal Neoplasms', and in the PubMed database using the MeSH terms 'Vitamin D'

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; CDK, cyclin-dependent kinase; miR, microRNA; MPE, molecular pathological epidemiology; VDR, vitamin D receptor.

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AND 'Colorectal Neoplasms', for papers published in English from January 1995 to November 2015. We manually searched for 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 D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol); both can be obtained from the diet and from diet supplements. Vitamin D₃ can also be synthesised in adequate amounts in the skin, under exposure to sunlight⁽⁷⁾. As 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 D₃).

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, cytochrome P450 family 2 subfamily R member 1) 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 – which 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-1 α -hydroxylase (CYP27B1, cytochrome P450 family 27 subfamily B member 1) in 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, cytochrome P450 family 24 subfamily A member 1), which catabolises 25(OH)D and calcitriol into biologically inactive forms (Fig. 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 (VDRE) in the promoter regions of target genes⁽¹³⁾. The conformational change of VDR also recruits the co-activator and detaches the co-repressor to acetylate nucleosome histones and unravel DNA, thus enabling transcription (Fig. 2(a))⁽¹⁴⁾.

Calcitriol-dependent repression of gene transcription is documented for the *CYP27B1*⁽¹⁵⁾ and *PTH* (parathyroid hormone)⁽¹⁶⁾ genes. Haussler *et al.*⁽¹⁷⁾ 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 the co-repressor rather than the co-activator (Fig. 2(b)).

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 Ca²⁺ channels, resulting in the rapid hormonal

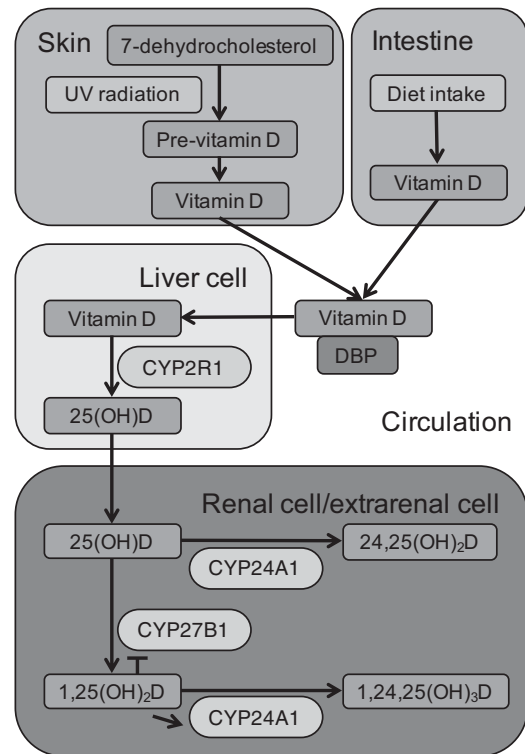


Fig. 1. The metabolism of vitamin D in human body. Vitamin D that is taken up via the diet, or synthesised from 7-dehydrocholesterol by the skin following UV exposure, binds to vitamin D-binding protein (DBP) in the circulation and is transported to the liver. Vitamin D is hydroxylated at C25 by CYP2R1 in the liver to 25-hydroxyvitamin D (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-dihydroxyvitamin D₃ (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.

stimulation of intestinal Ca transport (transcaltachia) within minutes^(19,20). Furthermore, the binding of calcitriol with membrane VDR may engage in cross-talk with the classical VDR pathway to modulate gene expression, possibly through Ca²⁺ influx activation of the Ca²⁺ 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 also on the intracellular concentrations of calcitriol^(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 synthesising enzyme of calcitriol, is normally expressed at low levels in the colon^(25,26). In well-differentiated

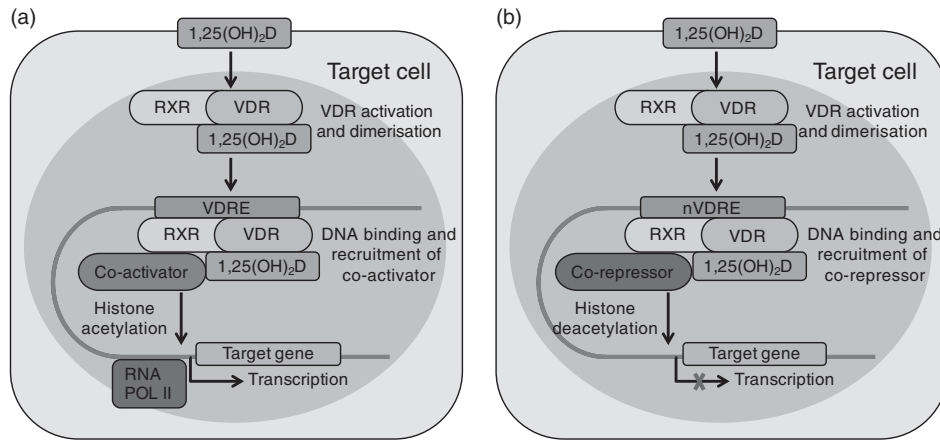


Fig. 2. The mechanism of calcitriol (1,25(OH)₂D) action through vitamin D receptor (VDR). Calcitriol binds and activates nuclear VDR, which then dimerises with retinoid X receptor (RXR). (a) Transcriptional activation involves the VDR–RXR heterodimer binding with vitamin D response element (VDRE) and recruitment of histone acetyltransferase co-activator. (b) Transcriptional depression involves VDR–RXR binding with negative VDRE (nVDRE) and recruitment of histone deacetylase co-repressor. RNA POL II, RNA polymerase II.

and moderately differentiated colorectal cancer samples, expression of CYP27B1 is elevated, whereas in poorly differentiated colorectal cancer samples the expression is repressed^(25–28). Ogunkolade *et al.*⁽²⁹⁾ reported that CYP27B1 mRNA expression levels are similar in colorectal cancer samples and in healthy colon samples, but are decreased in adjacent normal colon mucosa, 10 cm from the tumour border; this finding suggests that CYP27B1 expression in adjacent colon cells is regulated by the tumour, or that low expression of CYP27B1 in the colon is a risk for carcinogenesis. Bareis *et al.*⁽³⁰⁾ 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 up-regulating the expressions of VDR and CYP27B1, whereas highly proliferative, less-differentiated cell lines (Caco-2/AQ, COGA-1A and COGA-1E) show a down-regulation of VDR and CYP27B1 after EGF or calcitriol treatment. Although definite *in vivo* evidence is lacking, local production of calcitriol in the colon has been indirectly suggested by human studies. The serum concentration of 25(OH)D, rather than that of calcitriol, was inversely associated with colonic epithelial cell proliferation in a chemoprevention study⁽³¹⁾. Wagner *et al.*⁽³²⁾ 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 the colon. To summarise, elevated CYP27B1 expression suggests possible benefit from treatment with vitamin D, especially in well-differentiated and moderately differentiated tumours, whereas 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.*⁽³⁴⁾ showed that CYP24A1 mRNA expression is not only significantly up-regulated in human HT29 cells but also profoundly stimulated by calcitriol treatment, abrogating the anti-proliferative effect of calcitriol. Kosa *et al.*⁽³⁵⁾ 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. 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.

Vitamin D receptor

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-differentiated or moderately differentiated colorectal cancer tissues, but is decreased in poorly differentiated tumours, and is negligible in metastatic lymph nodes^(27,37). Palmer *et al.*⁽³⁸⁾ discovered that the transcription factors SNAI1 (snail family zinc finger 1) and SNAI2 (snail family zinc finger 2) repress VDR expression in SW480-ADH cells and block the anti-tumour action of the calcitriol analogue EB1089. RNA expressions of SNAI1 and SNAI2 are up-regulated in human colorectal cancers, and are inversely correlated with VDR mRNA expression^(38,39). These findings suggest that high levels of SNAI1 and SNAI2 are a probable cause for VDR down-regulation and for 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.

Anti-cancer actions of vitamin D on colorectal cancer

The anti-cancer effects of calcitriol are mostly studied *in vitro* by binding to the VDR and causing transcriptional activation and repression of the target genes. Given the pivotal role of nuclear VDR as a transcriptional regulator, researchers have investigated 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 were significantly enriched near colorectal cancer-associated genes identified from genome-wide association studies⁽⁴⁰⁾. Meyer *et al.*⁽⁴¹⁾ performed ChIP-Seq for VDR/RXR on the human colorectal cancer cell LS180, and identified *FOS* (FBJ murine osteosarcoma viral oncogene homolog) and *MYC* (v-myc avian myelocytomatosis viral oncogene homolog) among the target genes. In addition, several transcription factors regulated by calcitriol subsequently amplify and diversify the transcriptional output⁽⁴²⁾. The most studied anti-cancer effects of calcitriol are listed below.

Proliferation

Previous studies have established VDR as a biomarker for 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 analogues (F6-D3, ZK 156718 and BGP-13) increase the expressions of the cyclin-dependent kinase (CDK) inhibitors CDKN1A (cyclin-dependent kinase inhibitor 1A (p21, Cip1)) and CDKN1B (cyclin-dependent kinase inhibitor 1B (p27, Kip1)), which inhibit CDK2 and CDK6, leading to G1 phase arrest^(44–46). Calcitriol also results in the activation of latent transforming growth factor- β 1 (TGFB1) in Caco-2 cells, and sensitises these cells to the growth inhibitory effects of TGFB1⁽⁴⁷⁾. Synthetic low-calcemic vitamin D analogues (EB1089 and CB1093) decrease proliferation of HT29 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, which sequesters IGF2 with high affinity⁽⁴⁸⁾. Calcitriol also counteracts EGF-stimulated Caco-2 cell growth by markedly decreasing epidermal growth factor receptor expression⁽⁴⁹⁾.

Differentiation

Calcitriol has multiple pro-differentiation effects in colorectal cancer cells. The classic marker for differentiation is the 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 analogues (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 *et al.*⁽⁵¹⁾ reported that calcitriol increases alkaline phosphatase activity in Caco-2 cells by stimulating activator protein-1 (FOS/JUN (jun proto-oncogene)) activation, which is accomplished via a protein kinase C α - and mitogen-activated protein kinase (MAPK)-dependent mechanism.

Apart from affecting the expression of alkaline phosphatase, calcitriol also induces the expression of E-cadherin (CDH1,

cadherin 1) and other adhesion proteins, causing β -catenin (CTNNB1, catenin beta 1) 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 has been observed in an *Apc*^{min/+} (adenomatous polyposis coli) 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 (wingless-type MMTV integration site family) antagonist DKK1 (dickkopf WNT signaling pathway inhibitor 1) is induced by calcitriol in association with E-cadherin in SW480-ADH cells⁽⁵⁶⁾. As a result, calcitriol and its analogues 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 up-regulating the pro-apoptotic proteins BAK1 (BCL2-antagonist/killer 1) and BAX (BCL2-associated X protein) and by down-regulating the expressions of anti-apoptotic proteins BAG1, BIRC5 (baculoviral IAP repeat containing 5) and BCL2 (B-cell CLL/lymphoma 2). In two colorectal adenoma and three colorectal cancer cell lines, calcitriol and the vitamin D analogue EB1089 induced p53-independent apoptosis in a dose-dependent manner, and the levels of the pro-apoptotic protein BAK1 were consistently increased in all the cell lines examined⁽⁵⁰⁾. Barnes *et al.*⁽⁵⁷⁾ showed that EB1089 induces apoptosis in a colorectal adenoma S/RG/C2 cell line by re-distributing the anti-apoptotic protein BAG1 from the nucleus to the cytoplasm. Liu *et al.*⁽⁵⁸⁾ 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 Ca-sensing receptor (CASR)-dependent manner, possibly by binding the VDRE in CASR promoters^(59,60). In an *Apc*^{1638N/+} mouse model of intestinal cancer, a western-pattern diet decreased the expression of the pro-apoptotic protein BAX and increased the expression of the anti-apoptotic protein BCL2; treatment with vitamin D and Ca 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 analogues BGP-13 and BGP-15 activated cell apoptosis⁽⁴⁶⁾. However, the pro-apoptotic effect of calcitriol appears to be not always true: Stambolsky *et al.*⁽⁶²⁾ reported that mutant TP53 (tumor protein p53) is recruited to VDR-regulated genes and converts calcitriol into an anti-apoptotic agent in SW480 cells. Thus, TP53 mutation status might be a predictive marker for vitamin D treatment response.

Angiogenesis

Calcitriol also inhibits angiogenesis. Mantell *et al.*⁽⁶³⁾ showed that calcitriol significantly inhibits the sprouting and elongation

of vascular endothelial growth factor A (VEGFA)-induced endothelial cells in a dose-dependent manner. In human colorectal cancer SW480 cells, calcitriol treatment for 24 h at 0.1 and 1 μM decreases the expression of hypoxia-inducible factor-1 α and at 1 μM inhibits the secretion of VEGFA under conditions of hypoxia⁽⁶⁴⁾. However, Fernandez-Garcia *et al.*⁽⁶⁵⁾ reported that calcitriol increases the levels of VEGFA and the anti-angiogenic factor thrombospondin 1, leading to a minimal balanced change in the angiogenic potential of SW480-ADH cells. Calcitriol also represses the expression of DKK4 (dickkopf WNT signaling pathway inhibitor 4) in SW480-ADH cells; DKK4 is induced by the TCF7L2/CTNBN1 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 reduced the incidence of colon tumours and also decreased 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 the expression of the cathelicidin anti-microbial peptide, a major component of the innate immune system, in HT29 cells⁽⁶⁹⁾. Lithocholic acid, a secondary bile acid and a vitamin D analogue, decreases NF- κ B activity via the VDR in colonic cancer cells (Caco-2 and HT29C19A)⁽⁷⁰⁾. *CYP27B1*-knockout mice show increased IL1 and IL17 expressions 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 suppressed MAPK and NF- κ B 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, the *Vdr*-knockout mouse model showed that VDR is required for the maturation and proliferation of intestinal CD8 α ⁺ intra-epithelial 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 the 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 TNF and IL-6 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^(78–80), and vitamin D has been reported to regulate the gut microbiome. In a dextran sodium sulphate-induced colitis model, mice on vitamin D-deficient diet showed 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.*⁽⁸²⁾ showed that *Cyp27b1*-knockout mice had higher concentrations of the *Helicobacter* species in the faeces and more severe symptoms of colitis compared with wild-type littermates. In addition, calcitriol supplementation (1.25 $\mu\text{g}/100\text{ g}$ diet) to

Cyp27b1-knockout mice reduced *Helicobacter* numbers and colitis severity⁽⁸²⁾. Given the data from mouse models, it would be interesting to investigate changes in the human gut microbiome after vitamin D supplementation.

MicroRNA

MicroRNAs (miR) are implicated in the anti-neoplastic influence of vitamin D⁽¹²⁾. Alvarez-Diaz *et al.*⁽⁸³⁾ reported that *miR-22* is induced by calcitriol in a time-, dose- and VDR-dependent manner in multiple human colorectal cancer cell lines. 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.*⁽⁸⁴⁾ found that calcitriol up-regulates *miR-627*, which in turn mediates the anti-growth effect of calcitriol in HT29 cells; they reported that *miR-627* down-regulates the expression of KDM3A (lysine demethylase 3A, a histone demethylase), increases methylation of histone H3K9, and thereby suppresses the expression of proliferative factors such as *GDF15* (growth differentiation factor 15). 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 Ca) induces benign and malignant tumours in various mouse models of intestinal tumourigenesis, and supplementation with vitamin D plus Ca 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 a vitamin D-sufficient diet had smaller tumours than those fed a vitamin D-deficient diet⁽⁸⁸⁾; in nude mice, treatment with vitamin D analogues (BGP-13 and BGP-15) inhibited the growth of human HT29 xenograft⁽⁴⁶⁾. Add-on of the vitamin D analogues PRI-2191 and PRI-2205 showed improved anti-tumour effects compared with chemotherapy alone, which included 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 were fed a western-style diet, adding dietary vitamin D and Ca induced apoptosis of epithelial cells and inhibited tumourigenesis in the intestine⁽⁶¹⁾. A protective effect by vitamin D was 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, showed 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 expressions of Ctnnb1/Tcf7l2 target genes and larger tumours in the intestine⁽⁹³⁾, supporting the anti-neoplastic effect of VDR in the 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 2×2 factorial design, Bostick⁽⁹⁴⁾ and colleagues tested the efficacy of 20 µg of vitamin D and/or 2 g of Ca 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. A statistically significant increase of expressions in the vitamin D group relative to the placebo group was found for BAX (56%)⁽⁹⁵⁾, CDKN1A (142%)⁽⁹⁶⁾, APC (48%), CDH1 (78%)⁽⁹⁷⁾, MSH2 (mutS homolog 2; 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 relationship 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 the 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 <20 ng/ml (50 nmol/l) is considered vitamin D insufficiency⁽¹⁰¹⁾, and 25(OH)D concentration of >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 concentrations⁽¹⁰³⁾, although there is some evidence against this theory⁽¹⁰⁴⁾. Moreover, especially for cohorts, the time of blood sampling 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 sampling may not be feasible in many large-scale cohort studies.

Alternatively, dietary or supplementary intakes of vitamin D can be assessed repeatedly with questionnaires. Nevertheless, recall of diet and supplement use is imprecise. Moreover, as 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 3345 subjects of the Women's Health Initiative (WHI) observational study, total vitamin D intake calculated based on information from questionnaires explained 9% variance in serum 25(OH)D concentrations⁽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.*⁽¹⁰⁸⁾ derived this score based on known determinants of circulating 25(OH)D, including age, race, UV radiation exposure, vitamin D intake, BMI, physical activity, alcohol intake, postmenopausal hormone use and season of blood collection from more than 4500 participants with available blood samples in three US nationwide cohorts. The predicted score explained 25–33% variance in plasma 25(OH)D concentrations 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 concentrations. 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-hydroxyvitamin D and incidence of colorectal cancer

Table 1 summarises previous studies investigating plasma 25(OH)D concentrations and incidence of colorectal cancer with at least 300 cases^(109–122). 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, MPE^(5,6,125,126) research provides new insights into the relationship between vitamin D and colorectal cancer. Jung *et al.*⁽¹⁰⁹⁾ studied the risk of colorectal cancer in relation to the



Table 1. Major studies (no. of cases ≥ 300) investigating plasma 25-hydroxyvitamin D (25(OH)D) concentrations and incidence of colorectal cancer (Odds ratios, hazard ratios (HR) and 95 % confidence intervals)

References	Study name	Design	No. of cases	Follow-up (years)	Association of plasma 25(OH)D and incidence of colorectal cancer	P_{trend}	$P_{\text{heterogeneity}}$
Wactawski-Wende <i>et al.</i> ⁽¹¹⁰⁾	WHI	Nested case-control	322	7	Highest v. lowest quartile OR 0.40 (95 % CI 0.23, 0.67)	0.02	
Wu <i>et al.</i> ⁽¹¹¹⁾	NHS, HPFS	Nested case-control	372	NHS 5.5; HPFS 4.4	Highest v. lowest quintile OR 0.66 (95 % CI 0.42, 1.05)	0.01	
Otani <i>et al.</i> ⁽¹¹²⁾	JPHC Study	Nested case-control	375	11.5	Highest v. lowest quartile Male: OR 0.73 (95 % CI 0.35, 1.5) Female: OR 1.1 (95 % CI 0.50, 2.3)	0.39 0.74	
Jenab <i>et al.</i> ⁽¹¹³⁾	EPIC	Nested case-control	1248	3.8	<25 nmol/l, OR 1.32 (95 % CI 0.87, 2.01) 25.0–49.9 nmol/l, OR 1.28 (95 % CI 1.05, 1.56) 50.0–74.9 nmol/l, Ref. 75.0–99.9 nmol/l, OR 0.88 (95 % CI 0.68, 1.13) ≥ 100.0 nmol/l, OR 0.77 (95 % CI 0.56, 1.06)	<0.001	
Lee <i>et al.</i> ⁽¹¹⁴⁾	N/A	Meta-analysis (prospective studies)	2690	N/A	Highest v. lowest category OR 0.66 (95 % CI 0.54, 0.81) Colon cancer OR 0.77 (95 % CI 0.56, 1.07) Rectal cancer OR 0.50 (95 % CI 0.28, 0.88)	N/A	0.20*
Ma <i>et al.</i> ⁽¹¹⁵⁾	N/A	Meta-analysis (prospective studies)	2767	N/A	Highest v. lowest category OR 0.67 (95 % CI 0.54, 0.80)	N/A	
Chung <i>et al.</i> ⁽¹¹⁶⁾	N/A	Meta-analysis (prospective studies)	1127	N/A	Each 10-nmol/l increase OR 0.94 (95 % CI 0.91, 0.97)	<0.001	
Neuhouser <i>et al.</i> ⁽¹¹⁷⁾	WHI	Nested case-control	310	7	Highest v. lowest quartile OR 0.22 (95 % CI 0.10, 0.51)	0.003	
English <i>et al.</i> ⁽¹¹⁸⁾	MCCS	Case-cohort	563	14	Highest v. lowest quartile OR 0.82 (95 % CI 0.61, 1.10)	0.20	
Jung <i>et al.</i> ⁽¹⁰⁹⁾	NHS, HPFS	Prospective cohort, predicted 25(OH)D, MPE (VDR expression)	1059	22	Highest v. lowest quintile HR 0.52 (95 % CI 0.42, 0.64) VDR (–) HR 0.48 (95 % CI 0.30, 0.78) VDR (+) HR 0.56 (95 % CI 0.42, 0.75)	<0.001 0.004 <0.001	0.22†
Anic <i>et al.</i> ⁽¹¹⁹⁾	ATBC	Nested case-control, male smokers	428	6.1	Highest v. lowest quartile OR 1.35 (95 % CI 0.91, 2.01) DBP low: OR 1.12 (95 % CI 0.65, 1.94) DBP high: OR 1.63 (95 % CI 0.94, 2.83)	0.11 0.98 0.02	0.24‡
Theodoratou <i>et al.</i> ⁽¹²⁰⁾	N/A	Meta-analysis	2764	N/A	Highest v. lowest quartile OR 0.70 (95 % CI 0.58, 0.84)	0.0002	
Weinstein <i>et al.</i> ⁽¹²¹⁾	PLCO	Nested case-control	476	5.6	Highest v. lowest quintile OR 0.59 (95 % CI 0.36, 0.95)	0.02	
Song <i>et al.</i> ⁽¹²²⁾	NHS, HPFS	Nested case-control, MPE, (immune reaction)	318	NHS 20; HPFS 16	Highest v. lowest tertile High reaction: OR 0.10 (95 % CI 0.03, 0.35) Mild reaction: OR 0.98 (95 % CI 0.62, 1.54) Absent reaction: OR 0.71 (95 % CI 0.26, 1.95)	<0.001 0.93 0.55	0.001§

WHI, Women's Health Initiative; 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; Ref., referent values; MCCS, Melbourne Collaborative Cohort Study; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; VDR, vitamin D receptor; DBP, vitamin D-binding protein; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; MPE, molecular pathological epidemiology.

* $P_{\text{heterogeneity}}$ is for colon cancer v. rectal cancer.

† $P_{\text{heterogeneity}}$ is for VDR (–) v. VDR (+).

‡ $P_{\text{heterogeneity}}$ is for low v. high DBP.

§ $P_{\text{heterogeneity}}$ is for high v. mild v. absent reaction.

predicted score for 25(OH)D concentration (with 1059 incident cases during the 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 ($P_{\text{heterogeneity}} = 0.75$). 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 ($P_{\text{trend}} < 0.001$), but not with risk of tumour with lower-level reaction ($P_{\text{trend}} > 0.50$, $P_{\text{heterogeneity}} = 0.001$)⁽¹²²⁾. This statistical analysis of heterogeneity is critical, as 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 levels of active vitamin D^(129–131). Although a replication by additional studies is needed, these findings suggest an interplay of vitamin D status and the immune system in inhibiting the tumorigenesis 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-hydroxyvitamin D concentration and survival of colorectal cancer

Table 2 shows previous studies with at least 300 cases on plasma 25(OH)D concentration and survival of patients with diagnosed colorectal cancer^(133–139). Of note, to reduce potential reverse causation associated with undiagnosed tumours at the time of blood sampling that might lower plasma 25(OH)D concentrations, the Nurses' Health Study^(133,134), the Health Professionals Follow-up Study^(133,134) and the European Prospective Investigation into Cancer and Nutrition 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⁽¹³⁷⁾ and the Cancer and Leukemia Group B/Southwest Oncology Group 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 previous studies exploring the relationship between vitamin D intake and risk of colorectal cancer with at least 500 cases^(113,115,140–150). In contrast to the consistent and strong evidence from the studies measuring plasma 25(OH)D, the

association between vitamin D intake and incidence of colorectal cancer is conflicting. Nevertheless, a 2011 meta-analysis⁽¹¹⁵⁾ of prospective studies reported an inverse association between 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.*⁽¹⁵¹⁾ included 1111 participants in the Cancer Prevention Study II Nutrition Cohort who were diagnosed with invasive, non-metastatic colorectal cancer. The researchers evaluated associations of Ca, 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 Ca 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, which might hopefully conclude 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)^(110,152–154). In a substudy of the WHI, 36 282 postmenopausal women were given 5 µg of vitamin D and 500 mg of Ca twice daily (10 µg of vitamin D and 1000 mg of Ca 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 Ca plus vitamin D and those assigned to placebo (168 *v.* 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 led to only a small rise in plasma 25(OH)D concentrations⁽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 Ca or vitamin D supplements at randomisation, Ca 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 the prevention of colorectal cancer, which has a long natural history and a relatively low incidence.



Table 2. Major studies (no. of cases ≥ 300) investigating plasma 25-hydroxyvitamin D (25(OH)D) concentrations and survival of patients with diagnosed colorectal cancer (CRC) (Hazard ratios (HR) and 95 % confidence intervals)

References	Study name	Design and timing of blood draw	No. of cases	All deaths	CRC deaths	Follow-up (years)	Association of plasma 25(OH)D and mortality of CRC	P_{trend}
Ng <i>et al.</i> ⁽¹³³⁾	NHS, HPFS	Prospective cohort, prediagnosis	304	123	96	6.5	Highest v. lowest quartile CRC specific: HR 0.61 (95 % CI 0.31, 1.19) All-cause: HR 0.52 (95 % CI 0.29, 0.94)	0.23 0.02
Ng <i>et al.</i> ⁽¹³⁴⁾	NHS, HPFS	Prospective cohort, predicted 25(OH)D	1017	283	119	9.7	Highest v. lowest quintile CRC specific: HR 0.50 (95 % CI 0.26, 0.95) All-cause: HR 0.62 (95 % CI 0.42, 0.93)	0.02 0.002
Ng <i>et al.</i> ⁽¹³⁵⁾	NCCTG 9741	Prospective cohort, mCRC postdiagnosis	515	475	N/A	5.1	Highest v. lowest quartile PFS: HR 1.07 (95 % CI 0.81, 1.42) All-cause: HR 0.94 (95 % CI 0.72, 1.23)	0.66 0.55
Fedirko <i>et al.</i> ⁽¹³⁶⁾	EPIC	Prospective cohort, prediagnosis	1202	541	444	6.1	Highest v. lowest quintile CRC specific: HR 0.69 (95 % CI 0.50, 0.93) All-cause: HR 0.67 (95 % CI 0.50, 0.88)	0.04 0.01
Zgaga <i>et al.</i> ⁽¹³⁷⁾	SOCCS	Prospective cohort postdiagnosis	1598	531	363	8.9	Highest v. lowest tertile CRC specific: HR 0.68 (95 % CI 0.50, 0.90) All-cause: HR 0.70 (95 % CI 0.55, 0.89)	0.009 0.003
Maalmi <i>et al.</i> ⁽¹³⁸⁾	N/A	Meta-analysis (prospective studies)	2330	1214	566	N/A	Highest v. lowest category CRC specific: HR 0.65 (95 % CI 0.49, 0.86) All-cause: HR 0.71 (95 % CI 0.55, 0.91)	N/A
Ng <i>et al.</i> ⁽¹³⁹⁾	CALGB/SWOG 80405	Prospective cohort, mCRC postdiagnosis	1043	N/A		7	Highest v. lowest quintile PFS: HR 0.80 (95 % CI 0.64, 1.01) All-cause: HR 0.67 (95 % CI 0.53, 0.86)	0.02 0.002

NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; 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 (no. of cases ≥ 500) investigating vitamin D intake and incidence of colorectal cancer (CRC) (Odds ratios, relative risks (RR) and 95 % confidence intervals)

References	Study name	Design	No. of cases	Follow-up (years)	Association of vitamin D intake and incidence of CRC	P_{trend}
Martinez <i>et al.</i> ⁽¹⁴⁰⁾	NHS	Prospective cohort	501	12	Highest v. lowest quintile RR 0.88 (95 % CI 0.66, 1.16)	0.23
Pritchard <i>et al.</i> ⁽¹⁴¹⁾	Stockholm	Case-control	569	N/A	Highest v. lowest quartile Colon cancer: OR 0.6 (95 % CI 0.4, 1.0) Rectal cancer: OR 0.5 (95 % CI 0.3, 0.9)	0.08 0.08
Marcus <i>et al.</i> ⁽¹⁴⁹⁾	N/A	Case-control	512	N/A	Highest v. lowest quintile Colon cancer: OR 0.7 (95 % CI 0.4, 1.1) Rectal cancer: OR 0.8 (95 % CI 0.5, 1.5)	0.05 0.42
Terry <i>et al.</i> ⁽¹⁴²⁾	SMC	Cohort, women	572	11.3	Highest v. lowest quartile RR 1.05 (95 % CI 0.83, 1.33)	0.73
McCullough <i>et al.</i> ⁽¹⁵⁰⁾	CPS II	Cohort	683	5	Highest v. lowest quartile RR 0.80 (95 % CI 0.62, 1.02)	0.02
Slattery <i>et al.</i> ⁽¹⁴³⁾	N/A	Case-control	2306	N/A	Highest v. lowest quartile Men: OR 1.08 (95 % CI 0.73, 1.60) Women: OR 0.52 (95 % CI 0.32, 0.85)	N/A 0.03 0.80
Park <i>et al.</i> ⁽¹⁴⁴⁾	Multiethnic Cohort Study	Cohort	2100	7.3	Highest v. lowest quintile Men: RR 0.66 (95 % CI 0.54, 0.81) Women: RR 0.81 (95 % CI 0.66, 0.98)	0.03 0.80
Mizoue <i>et al.</i> ⁽¹⁴⁵⁾	Fukuoka CRC Study	Case-control	836	N/A	Highest v. lowest quintile OR 0.79 (95 % CI 0.56, 1.11) Indoor: OR 0.63 (95 % CI 0.36, 1.08) Outdoor: OR 0.94 (95 % CI 0.58, 1.52)*	0.12 0.02 0.86
Ishihara <i>et al.</i> ⁽¹⁴⁶⁾	JPHC Study	Nested case-control	761	7.8	Highest v. lowest quintile Men: OR 0.92 (95 % CI 0.60, 1.42) Women: OR 1.49 (95 % CI 0.86, 2.60)	0.58 0.26
Lipworth <i>et al.</i> ⁽¹⁴⁷⁾	N/A	Case-control	1953	N/A	Highest v. lowest decile Colon cancer: OR 0.69 (95 % CI 0.50, 0.96) Rectal cancer: OR 1.22 (95 % CI 0.82, 1.80)	< 0.0001 0.70
Huncharek <i>et al.</i> ⁽¹⁴⁸⁾	N/A	Meta-analysis (cohorts)	2813	N/A	Highest v. lowest category RR 0.94 (95 % CI 0.83, 1.06)	N/A
Jenab <i>et al.</i> ⁽¹¹³⁾	EPIC	Nested case-control	1248	3.8	Highest v. lowest quintile OR 0.84 (95 % CI 0.60, 1.17)	0.19
Ma <i>et al.</i> ⁽¹¹⁵⁾	N/A	Meta-analysis (prospective studies)	6466	N/A	Highest v. lowest category RR 0.88 (95 % CI 0.80, 0.96)	N/A

NHS, Nurses' Health Study; SMC, Swedish Mammography Cohort; CPS II, Cancer Prevention study II; 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 labour or walking or outdoor physical activity at leisure at least of 120 min/week.

Table 4. Major completed randomised trials ($n \geq 1000$) investigating vitamin D supplementation and cancer (Hazard ratios (HR) and 95% confidence intervals)

References	Study name	No. of subjects	Age (years)	Treatment duration (years)	Vitamin D treatment	End point	No. of cases (treatment/control)	HR	95% CI	P
Trivedi <i>et al.</i> ⁽¹⁵²⁾	N/A	2686	≥ 60	5	2500 $\mu\text{g}/4$ months	Cancer incidence CRC incidence	188/173 28/27	1.09 1.02	0.86, 1.36 0.60, 1.74	0.47 0.94
Wactawski-Wende <i>et al.</i> ⁽¹¹⁰⁾	WHI	36 282	50–79	7	10 $\mu\text{g}/\text{d}$ plus Ca 1000 mg/d	Cancer mortality CRC mortality	1634/1655 168/154	0.86 0.62	0.61, 1.20 0.24, 1.60	0.37 0.33
Lappe <i>et al.</i> ⁽¹⁵³⁾	Nebraska trial	1179	>55	4	27.5 $\mu\text{g}/\text{d}$ plus Ca 1500 mg/d	Cancer incidence CRC mortality	344/382 34/41	0.98 0.82	0.91, 1.05 0.77, 1.03	0.53 0.12
Avenell <i>et al.</i> ⁽¹⁵⁴⁾	RECORD	5292	≥ 70	2–4	20 $\mu\text{g}/\text{d}$	Cancer incidence CRC incidence	13/20 1/2	0.40 N/A	0.52, 1.29 0.20, 0.82	0.39 0.01
Baron <i>et al.</i> ⁽¹⁵⁷⁾	Vitamin D/ Calcium Polyp Prevention	2259	45–75	3–5	25 $\mu\text{g}/\text{d}$	Cancer mortality Adenoma incidence Cancer incidence CRC incidence	338/315 41/30 151/178 20/13	1.07 N/A 0.85 N/A	0.92, 1.25 N/A 0.68, 1.06 N/A	0.38 N/A 0.16 N/A
							438/442 47/61 3/2	0.99 N/A N/A	0.89, 1.09 N/A N/A	0.98 0.17 1.00

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

A second completed randomised trial was carried out in the UK, with 2686 participants (2037 men and 649 women)⁽¹⁵²⁾. An oral supplement of 2500 μg vitamin D or a matching placebo was given every 4 months for 5 years. Over the 5-year period, twenty-eight and twenty-seven 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 *v.* 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 period.

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

In the recently published Vitamin D/Calcium Polyp Prevention trial (Table 4)⁽¹⁵⁷⁾, patients with recently diagnosed adenomas were randomly assigned 25 μg of vitamin D 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 concentrations of 7.83 ng/ml, relative to participants given placebo. Overall, 43% of the participants had one or more adenomas diagnosed during follow-up, and the adjusted risk ratio for recurrent adenoma was 0.99 (95% CI 0.89, 1.09) with vitamin D *v.* no vitamin D.

Two points are worth noting for comparison of this null finding with preexisting epidemiological evidence. First, as the authors admitted, the vitamin D dose in the Polyp Prevention trial (25 μg 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 with a generally >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. Second, 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 fibre intake was associated with reduced risk of incident colorectal adenoma and colorectal cancer (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 generalised to persons without a recent history of colorectal adenoma. On the basis of 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 the metastatic stage or adenoma recurrence.

Table 5. Major ongoing randomised trials ($n \geq 1000$) investigating vitamin D supplementation and cancer

Study name	Location	No. of subjects	Age (years)	Treatment duration (years)	Vitamin D treatment	Primary end point	Trial registry no.	Estimated completion
VITAL	USA	25 874	Men, ≥ 50 ; women, ≥ 55	5	50 $\mu\text{g}/\text{d}$	Cancer, CVD	NCT01169259	2017
D-Health	Australia	21 000	60–84	5	1500 $\mu\text{g}/\text{month}$	Total mortality, cancer	ACTRN12613000743763	2020
Vitamin D/Calcium Polyp Prevention	USA	2813, with colorectal adenoma removed within 120 d	45–75	1–5	25 $\mu\text{g}/\text{d}$	Colorectal adenoma, colorectal cancer	NCT00153816	2016
FIND	Finland	2495	Men, ≥ 60 ; women, ≥ 65	5	40 or 80 $\mu\text{g}/\text{d}$	Cancer, CVD	NCT01463813	2020
CAPS	USA	2332 postmenopausal women	≥ 55	5	50 $\mu\text{g}/\text{d}$ plus Ca 1600 mg/d	Cancer	NCT01052051	2015
VIDAL	UK	Pilot, 1600; main, 20 000	65–84	Pilot, 2; main, 5	2500 $\mu\text{g}/\text{month}$	Total mortality, cancer	ISRCTN46328341	Pilot, 2013; main, N/A

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

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 are applying higher dosages of vitamin D than previous trials, and are measuring baseline and/or follow-up plasma 25(OH)D concentrations. For example, the VITamin D and Omega-3 Trial study collects baseline blood samples on 17 000 participants and follow-up samples on 6000⁽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 randomising them into two arms. Together with the standard chemotherapy with FOLFOX and bevacizumab, arm 1 gets 10 $\mu\text{g}/\text{d}$ of vitamin D, whereas arm 2 gets a loading dose of 200 $\mu\text{g}/\text{d}$ for 2 weeks followed by a maintenance dose of 100 $\mu\text{g}/\text{d}$. Although the sample size is small, the study does collect plasma 25(OH)D concentrations, and thus 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 to vitamin D status might have an impact on the risk of colorectal cancer. A genome-wide association study of circulating 25(OH)D concentrations in 33 996 individuals has identified SNP loci near four genes including *GC* (group-specific component, which encodes vitamin D-binding protein), *DHCR7* (7-dehydrocholesterol reductase, which 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.*⁽¹⁶⁷⁾ 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. 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⁾. As the SNP identified by Wang *et al.*⁽¹⁶⁶⁾ can explain only a small variation (1–4%) in 25(OH)D concentrations, the reduction in overall colorectal cancer risk by

increased vitamin D levels due to the SNP might be too small to be detectable. In addition to genes related to vitamin D metabolism, *VDR* polymorphism has also been studied with regard to 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* (rs1544410) (relative risk (RR) 0.57; 95% CI 0.36, 0.89 for *BB v. bb*)⁽¹⁷¹⁾ and *TaqI* (rs731236) (OR 1.43; 95% CI 1.30, 1.58 for *tt v. TT*)⁽¹⁷²⁾, respectively.

As one future direction, the MPE approach may link vitamin-D-related SNP to specific subtypes of colorectal cancer. Another future direction is to investigate interactions between SNP of the 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 SNP 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 & Garland⁽²⁾ proposed vitamin D for colon cancer prevention 25 years ago, functional studies on vitamin D or its analogues 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 miR regulation are involved in the anti-tumour effect of vitamin D. Recent studies also have explored the local expressions and impacts of vitamin D-metabolising enzymes and *VDR*, which may lead to the 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 the following reasons: the slow development of colorectal cancer, the confounding effects caused by sunlight exposure, outdoor physical activity, BMI, dairy and Ca 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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