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Modulating the activity of fluoropyrimidines against colorectal cancer by Vitamin D and its analogs

Michał Jurczyk[1], Aleksandra Midro[1], Magdalena Król[1], Weronika Olesiak[1], Dariusz Stąpor[1], Anna Gil[1, 2], Krzysztof Gil[1]

[1]Department of Pathophysiology, Jagiellonian University Medical College, Kraków, Poland
[2]Department of Anatomy, Jagiellonian University Medical College, Kraków, Poland

Corresponding author: Michał Jurczyk, M.D.
Department of Pathophysiology, Jagiellonian University Medical College
ul. Czysta 18, 31-121 Kraków, Poland
Phone: +48 12 633 39 47; E-mail: michal.jurczyk@uj.edu.pl

Abstract: Colorectal cancer (CRC) is the third most common malignancy worldwide and the second most deadly cancer. Scientists have projected that by 2040, the prevalence will reach up to 3.2 million new cases annually due to population aging, disadvantageous diet transformations, and elevated exposure to risk factors. In the past decades, the five-year survival rate in colorectal cancer has significantly increased to 65% due to the development of an early endoscopic diagnosis and new chemotherapeutic approaches. Fluoropyrimidines, such as 5-fluorouracil or capecitabine, are commonly used to treat CRC. One of the most fundamental mechanisms of 5-FU is based on the inhibition of thymidylate synthase. This action is responsible for the therapeutic, but also toxic, effects of the drug. In this short review, we discuss the possible effects of vitamin D activity on colorectal cancer cells in relation to fluoropyrimidines.
PubMed, Embase, and Web of Science databases were searched up to January 2022 for studies on vitamin D and 5-fluorouracil interaction mechanisms. Original studies, case reports, and review articles were included.
Vitamin D or its analogs target multiple biochemical pathways and modulate numerous pathophysiological mechanisms in the course of colon cancer, including those related to the pharmacological sites of fluoropyrimidines. However, the available data concerning vitamin D–fluoropyrimidine pharmacological interactions are limited, especially regarding patients suffering from colon cancer and being treated with fluoropyrimidines.

Keywords: Vitamin D, colorectal cancer, 5-fluorouracil, fluoropyrimidines.

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Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide and the second most deadly cancer. In 2020, there were approximately 1.9 million incidences and 0.9 million deaths, representing 10% of all cancer incidences and 9.4% of deaths [1, 2]. Scientists have projected that by 2040, the prevalence will reach up to 3.2 million new cases annually due to population aging, disadvantageous diet transformations, and elevated exposure to risk factors [2]. In the past decades, the five-year survival rate in colorectal cancer has significantly increased to 65% due to the development of an early endoscopic diagnosis as well as treatments with new chemotherapeutic approaches, radiation therapy, and immunotherapy [3, 4]. CRC is a significant problem in young adults, in both males and females (<40 years old) [4]. The survival rate primarily depends on the stage of CRC, ethnicity, cancer subtype, genetic abnormalities, and lifestyle (low physical activity, excessive caloric intake, gut microbiota abnormalities, smoking, and alcohol overuse) [2, 4].

Various chemotherapeutic agents have been clinically proven for their effectiveness in the therapy of solid tumors. Fluoropyrimidines, such as 5-fluorouracil or capecitabine, are commonly used to treat CRC as well as skin and breast cancers [5]. The anti-tumor activity of 5-fluorouracil (5-FU) was described for the first time in 1954 and approved by the Food and Drug Administration (FDA) to treat adenocarcinomas of the gastrointestinal tract in 1962 [6, 7]. The drug is metabolized in a similar manner to other pyrimidines. One of the most fundamental mechanisms of 5-FU is based on the inhibition of thymidylate synthase. This action is responsible for the therapeutic, but also the toxic, effects of the drug. 5-FU may interfere with cellular membranes, leading to surface charges and potential alterations to transmembranes. Despite its many advantages, 5-FU belongs to the most cardiotoxic drugs following anthracyclines and can result in symptoms such as chest pain, myocardial infarctions, or arrhythmias [8]. Although a treatment with 5-fluorouracil is associated with cardiotoxicity and other side effects, 5-FU is still widely used in numerous therapeutic regimens. The frequency of cardiotoxicity remains debatable; studies have estimated that up to 68% of patients treated with 5-FU suffer from asymptomatic cardiotoxicity [9, 10]. Many possible mechanisms of cardiotoxicity have been described, including coronary vasospasms, endothelial dysfunctions, increased thrombogenicity, excessive oxidative stress, mitochondrial disturbances, Krebs cycle abnormalities, and the accumulation of highly toxic metabolites [11–18].

Ergosterol, also called pro-vitamin D_2 (pro-D_2), is the main dietary form of vitamin D (VD). Pro-D_2 is transformed into pre-vitamin D_2 (pre-D_2) by UVB radiation and further into ergocalciferol–vitamin D_2 (D_2) by thermo-sensitive isomerization. The skin provides the significant cholecalciferol (vitamin D_3) localization for the metabolism, where 7-dehydrocholesterol (pro-D_3) is transformed into pre-vitamin

D_3 (pre-D_3) via UVB radiation, which causes a break in the B ring. Pre-D_3 then undergoes thermo-induced isomerization into vitamin D_3. Various enzymes hydroxylate vitamin D_2 and D_3 into 25(OH)D_2 and 25(OH)D_3. The most important is CYP2R1, followed by CYP3A4 and CYP2J2. 25(OH)D_2 and 25(OH)D_3 are processed into 1, 25$(OH)_2D_2$ and 1, 25$(OH)_2D_3$ (calcitriol) by CYP27B1 localized in the kidneys and other tissues, including colon cancer cells. The catabolic pathway is associated with the 24-hydroxylation of 25(OH)D_3 or 1, 25$(OH)_2D_3$ by CYP27B1 or CYP24A1. An overexpression of CYP24A1 may be observed in several subtypes of colorectal cancer. The concentration of circulating 25(OH)D is validated as the marker of the vitamin D status, both produced in the skin and food-derived [19–28].

As a hydrophobic molecule, VD is directly transferred into cells and then into the nucleus where the vitamin D receptors (VDRs) are localized. The VDR is the transcription factor belonging to the steroid hormone nuclear receptor family. The VDR is preferentially heterodimerized with retinoid X receptor (RXR), creating a VDR–XDR complex; however, this may also occur with the all-trans retinoic acid receptor (RAR) or thyroid hormone receptor (T3R). It can also act as a homodimer. The VDR complex interferes with the target genes in DNA (the vitamin D response element (VDRE)). It possesses a specific nucleotide sequence, leading to the promotion or repression of transcription [19, 29, 30].

Methods

PubMed, Embase, and Web of Science databases were searched up to January 2022 for studies on vitamin D and 5-fluorouracil interaction mechanisms published in English. We used the following terms: (vitamin D, cholecalciferol, or alfacalcidol) and (5-FU, 5-fluorouracil, fluorouracil, or fluoropyrimidine). Five reviewers independently screened the titles and abstracts, including appropriate studies on the mechanisms. Any disagreements in rating the studies were resolved by a discussion until a consensus was reached. Full-text articles were acquired based on the study relevance; nevertheless, the classification procedure was not methodically proven. Original studies, case reports, and review articles were included. References to included studies were also checked for further appropriate articles.

Results

Vitamin D receptors

The colon is one of the VD target organs due to the presence of VDRs in colorectal tissue and also in colorectal cancer cells [31, 32]. In comparison with normal colon tissue, the expression of VDRs is increased in hyperplastic polyps and the early stages

of cancer. It has been shown that functional p-53 increases the expression of VDR: Maruyama *et al.* established a family of p-53 proteins (p-53, p-63, and p-73) that increased the transcription of VDRs from 4- to 10-fold [33]. However, the expression of VDRs decays with the progression of the malignancy and is even absent in metastases [34, 35]. This phenomenon may be explained by a p-53 dysfunction commonly acquired during cancerogenesis, which causes a loss of the transcriptional function of p-53 in VDR genes [33]. In advanced colon cancer, SNAI1 and SNAI2 gene activity increases SNAIL1/SNAIL2 transcriptional repressors and VDR cell expression [36]. In a mouse model of colon cancer loss, VDRs facilitated cancerogenesis [37]. Contrariwise, VDR expression may be increased by a diversity in growth factors, including Sp1, WT1, and VD. The expression of VDRs is not essential for downstream signaling associated with mineral homeostasis (CYP24A1), but it is crucial for downstream signaling inducing cell cycle arrest (e.g., CDKN1A) [33].

Vitamin D concentration

Ng *et al.* proved that pre-diagnosing a high VD concentration reduces colorectal cancer incidence and overall mortality [38]. Markotic *et al.* described the role of postoperative VD concentrations in facilitating overall survival [39]. Dietary VD decreased vulnerability to carcinogens and diminished tumorigenesis incidence in an animal model [40, 41]. In a mouse model, the VD level decreased the concentration of oncogenic proteins [37]. An increased VD concentration decreased the risk of inflammatory bowel disease [42].

Apoptosis

Vitamin D and its analogs cause p-53-independent apoptosis. This pro-apoptotic mechanism of action of VD and VDA is crucial because up to 80% of advanced colorectal cancers lose their p-53 activity during the disease progression [43–45]. Diaz *et al.* proved that changes in Bcl-2 family proteins exposed to VD and VDA vary between the cell lines. A VD-induced increase in the activity of pro-apoptotic Bak was observed in the cell lines of both colon adenomas and colon cancer. However, increases in the Bak activity varied in-between. On the contrary, the VD effect on anti-apoptotic Bcl was inconsistent between the cell lines. Bcl-2 downregulation does not appear to be essential for apoptosis because in the adenoma cell line AA/C1 without a Bcl-2 expression, VD still induced apoptosis [43].

Anti-proliferative effects

VD and its analogs cause G1 cell arrest in both colorectal carcinomas and adenoma cell lines, with a stronger expression in adenoma cell lines AA/C1 and RG/C2 [43]. Milczarek *et al.* demonstrated an increased percentage of cells in the G0/G1 phase in the MC38 cell line after VDA PRI2191 whereas 5-FU + PRI2205 increased the percentage of cells in the S phase [46].

TGF-β inhibits growth in colon cells, but cancer cells alter this pathway via a decreasing expression of TGF-β receptor mutations or disabled intracellular mediators (smad2 and smad4). VD exposure significantly increased the TGF-β expression in a rat model [47].

VD administration caused a decrease in Id2 expression, leading to an anti-proliferative effect in [48]. Vitamin D decreased the activity of c-myc and TCF-4/β-catenin; both were upregulated by the Id2 expression [49–51]. Vitamin D significantly increased cyclin-dependent kinase inhibitors (CDKIs) p-21 and p-27 [34, 48]. 5-FU administration increased the level and activity of p-51, which induced p-21. The p-21 gene in the VDR responding region is essential in p-53-deficient cancers. Vitamin D reduced phosphorylated ERK1/2 (p-ERK1/ERK2), leading to a decrease in thymidylate synthase expression [34]. Vitamin D supplementation increased the activity of the Id1 (a member of the transcriptional regulator family) promoter, Id1 RNA transcription, and protein concentration in human cancer cell lines [48].

Similarly, vitamin D decreased the risk of forming aberrant crypt foci (ACF), potential preneoplastic conditions, thus reducing the risk of colon cancer. ACF is often associated with a p-53 mutation. Reducing the genesis of ACF confirmed vitamin D p-53-independent activity [52]. Diaz *et al.* confirmed that vitamin D promotes colon adenomas and differentiation in cancer cells [43].

Another mechanism concerns the CaSR-regulating intra- and extracellular calcium balance, which increases after VDR activation or increases the Ca concentration [37]. The CaSR expression decreases with the progression of cancerogenesis, which is the same as VDRs. CaSR activation lowered the activity of thymidylate synthase and the presence of survivin [37, 53]. A calcium influx induces a pro-apoptotic cascade through the CAM/CAMKII pathway as well as pro-apoptotic BAX protein, a cytochrome C influx from the mitochondria, and caspase activity [37]. VD activity in cells resembles the activity of CaSR, which regulates proliferation and differentiation in both standard and neoplastic colon cells [53]. Both promoters of CaSR contain a VD response element and VD upregulates CaSR. CaSR knockout colon cancer cells are resistant to VD and Ca anti-proliferative activity. A calcium influx activates calpain, causing focal adhesive kinase (FAK) proteolysis; this decreases PI3K and Akt activity, which is crucial for cancer cell survival [54].

Activating invasion

Vitamin D increased the cell membrane expression of vinculin located and acting in a tight junction, an adherence junction, and focal adhesion plaques [50, 55]. E-cadherin is a transmembrane protein belonging to the cell adhesion molecule family and is essential for the function of the intercellular adherent junction, which is considered to be a tumor-suppressor gene [56, 57]. E-cadherin is also responsible for maintaining the adhesive and polarized phenotypes of epithelial cells. The losing function of this protein is an essential moment in the transition to cancer, facilitating an invasive capacity and disturbing the epithelial phenotype [58–60]. In 2013, Milczarek *et al.* suggested that the induction of E-cadherin may be an important mechanism underlying the chemopreventive effect of Ca2+ and VD in colon cancer. VD and VDA administration in HT-29 colon cancer cell lines increased the expression of E-cadherin. A VDA analog combined with 5-FU decreased metastasis in the lymph nodes of mice transplanted with MC38 colon cancer cells. Similarly, data from clinical studies have shown that the E-cadherin expression is significantly downregulated in gastrointestinal tumors with distant metastases [61]. β-catenin, considered to be a proto-oncogene, encodes a protein mainly connected to β-cadherin in the epithelial cells. Unrelated β-catenin levels in the cytosol are catabolized via phosphorylation by glycogen synthase kinase-3β (GSK-3β). The interaction of the Wnt ligand with the Wnt membrane receptor reduced the activity of GSK-3β, leading to an increased level of free cytosol β-catenin [50, 62]. Free β-catenin accumulates in the nucleus associated with TCF-4β, promoting the activation of c-myc, c-jun, and cyclin D1, thus increasing proliferation. VD increases the E-cadherin level, promoting the intercellular adherent junction and preventing phenotype changes. VD significantly relocates β-catenin from the nucleus to the E-cadherin complex in the cell membrane. Ligand-activated VDRs compete with TCF-4β for β-catenin, enhancing a decreased signaling of the β-catenin/TCF-4β pathway.

Angiogenesis

Angiogenesis facilitates cancer growth, invasion, and metastasis. Iseki *et al.* observed that vitamin D decreased the vascular endothelial growth factor concentration and reduced microvessel counts in an animal model of colon cancer [63]. Normal endothelial cells and vascular smooth muscle cells express VDRs; the stimulation leads to angiogenesis [64, 65]. On the contrary, cancer angiogenesis is inhibited by VD [66–69]. This phenomenon is explained by VDRs inhibiting the pro-angiogenic factors of tumors [70]. VD decreases Wnt β-catenin signaling, which promotes angiogenesis. Raafat *et al.* [47] hypothesized that vitamin D could promote the efficacy of 5-FU and colorectal cancer regression by downregulating the expression of iNOS and COX-2, leading to the inhibition of angiogenesis and damaging DNA [37].

Oxidative stress

Cell metabolism leads to the genesis of free radicals, which are balanced by antioxidants. In 2021, Boakye *et al.* claimed that VD participates in the detoxification and elimination of free radicals from the cells. The total thiol concentration (TTC) is one of the best-established biomarkers of antioxidant capacity. In groups of low and moderate TTC activity, there was an inverse correlation between the VD concentration and mortality [71]. In another study, Wimalawansa *et al.* observed that VD increased the antioxidant capacity and facilitated the proper function of the mitochondria. A decreased concentration of VD is associated with an increased accumulation of mitochondrial reactive oxygen species. VD reduces inflammation, decreasing the formation of oxidative stress [72, 73].

Summing up, we can conclude:

1. Vitamin D or its analogs target multiple biochemical pathways and modulate numerous pathophysiological mechanisms in the course of colon cancer, including those related to the pharmacological sites of fluoropyrimidines (Fig. 1).

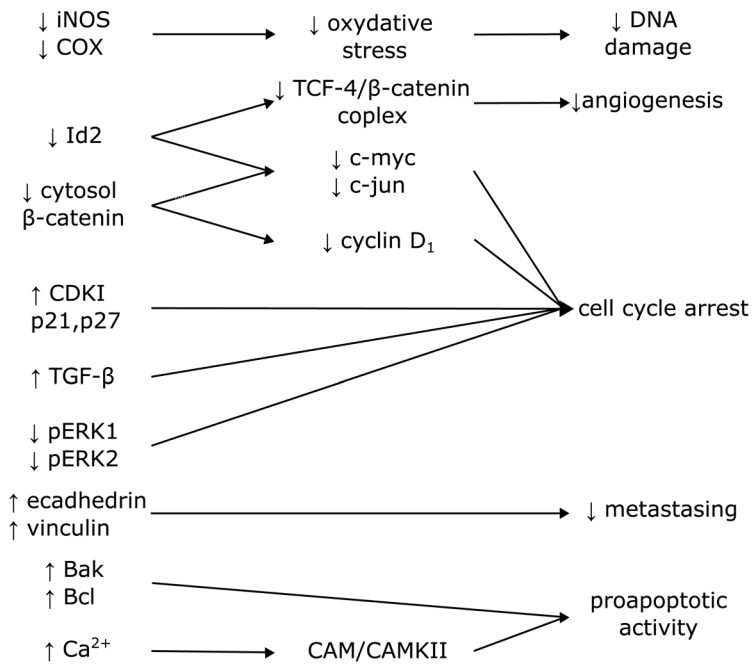

Fig. 1. Mechanisms of vitamin D's antitumor action.

2. Vitamin D administration in colon cancer patients exerts predominantly positive results, influencing oxidative stress and modulating angiogenesis, apoptosis, and cell invasion as well as promoting anti-proliferative effects.
3. We are aware that available data concerning vitamin D — fluoropyrimidine pharmacological interactions are scarce, especially regarding patients suffering from colon cancer and being treated with fluoropyrimidines. This is a limitation of this study.

Conflict of interest

None declared.

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