Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Vitamin D Inhibits colorectal cancer cell proliferation, migration and invasion via downregulating the Notch1 pathway

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ARTICLE INFO

Original paper

Article history:
Received: April 23, 2023
Accepted: June 24, 2023
Published: July 31, 2023

Keywords:

Vitamin D, colorectal cancer, proliferation, metastasis, Notch1

ABSTRACT

Due to its high incidence and mortality rates, colorectal cancer (CRC) has become the focus of research. Vitamin D has anticancer functions in a variety of cancers, but the relationship between vitamin D status and CRC risk and survival is inconclusive. The purpose of this study was to explore the effect of vitamin D on the proliferation and invasion of CRC cells to find an effective way to treat CRC. The results showed that vitamin D could inhibit SW480 cell progression in vitro. The results showed that vitamin D significantly blocked the Notch1 pathways by decreasing NOTCH1 protein expression. In addition, we found that Notch1 overexpression could significantly reverse the inhibitory effect of vitamin D on SW480 cells. These results reveal novel insights into the mechanisms of vitamin D anticancer activity in CRC development and progression.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.7.26

Introduction

Colorectal cancer (CRC), a tumor in the digestive system, ranks as the third most prevalent malignant neoplasm and the second leading cause of cancer-related mortality worldwide (1). According to the latest data released by the International Agency for Research on Cancer, more than 1.9 million new cases of CRC (10.0% of all malignant tumours) and 935,000 deaths (9.4% of all malignant tumour deaths) were estimated to occur globally in 2020 (1). Although remarkable progress has been made in CRC treatment, the prognosis of CRC patients is still poor, with overall 5-year survival below 15% in advanced disease (2). Aging, obesity, smoking, drinking, and ethnic background are widely accepted risk factors for sporadic CRC (2). Accumulating evidence supports that the risk of developing CRC is influenced by diet and lifestyle factors (3). Insufficient intake of calcium and inadequate levels of vitamin D are believed to heighten the susceptibility to the disease (4).

Over the past few decades, the association between vitamin D and CRC has been extensively explored. However, the relationship between vitamin D levels and CRC risk has shown several conflicts. Several studies have reported that lower vitamin D levels are associated with a high risk of CRC (5, 6). However, others have not confirmed the benefit of vitamin D supplementation on the primary endpoint of CRC incidence (7). Therefore, enhanced comprehension of the impact of vitamin D on CRC progression is imperative to expedite the exploration of novel therapeutic targets and the formulation of efficacious treatment strategies for CRC.

Preclinical studies have suggested that it may function as a cancerogenesis inhibitor, retarding tumor progression by inducing cellular differentiation and inhibiting the proliferation of malignant cells (8). Calcitriol, the active metabolite of vitamin D, exerts its antiproliferative and pro-differentiation effects on colorectal cancer cells through the activation of the vitamin D receptor signaling pathway (9, 10). Multilevel suppression of the Wnt/-catenin signaling pathway, whose aberrant activity in colon epithelial cells originates and promotes CRC (11), is a crucial mechanism driving this impact. It has been demonstrated that Wnt and Notch signaling pathways are crucial for intestinal development and homeostasis (12-15). According to Fre et al., Notch1 activation may be a crucial first step in the development of CRC. (16).

In prostate epithelial cells and breast cancer cells, calcitriol administration dramatically decreased NOTCH1 mRNA levels, demonstrating an inhibitory action of vitamin D on Notch signaling (17, 18). However, calcitriol had no antiproliferative effects in these cells and did not disrupt Notch signaling in brain cancer cell lines (19). These findings imply that the tissue and cellular environment may affect how vitamin D affects Notch signaling. Recent studies have shown that an appropriate amount of vitamin C combined with vitamin D3 can regulate claudin-2 expression by regulating Notch-1, alleviate the destruction of the intestinal mucosal barrier, and promote repair of cellular mucosal barrier damage (20). These results indicate that vitamin D may affect Notch signaling in intestinal cells. Accordingly, the aim of this study was to investigate whether vitamin D supplementation could directly interfere with the proliferation, migration, and invasion of SW480

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CRC cells and whether vitamin D suppressed the malignant progression of CRC by downregulating the Notch1 signaling pathway.

Materials and Methods

Cell culture

Procell Life Science & Technology Co., Ltd. (Wuhan, China) produced the human colorectal cancer cell line SW480. The cell line was grown in L15 medium from Procell (Wuhan, China) together with 10% fetal bovine serum (FBS) from Yeasen (Shanghai, China) and 1% penicillin-streptomycin solution from Procell (Wuhan, China) at a temperature of 37°C.

Cell viability assay

Cultured SW480 cells were seeded at a density of 1 × 10^4 cells/well in 96-well plates, and they were then given either vehicle control or 100 nM vitamin D. Using the cell counting kit-8 (CCK-8) assay, cell viability was evaluated (Beyotime, Shanghai, China). At 37°C for two hours, cells were treated with 10 L of CCK-8 solution. Using a Diatek DR-200Bs, the mixture's absorbance was calculated at 450 nm.

Cell apoptosis assay

SW480 cells were treated with vehicle control or 100 nM vitamin D (Solarbio, Beijing, China). Cells were collected after 48 hours, quantified using an Annexin V-FITC kit (Sungenebiotech, Shanghai, China), and then subjected to flow cytometric analysis (Beckman CytoFLEX, Detroit, MI, USA).

Wound healing assay

SW480 cells were treated with vehicle control or 100 nM vitamin D. Six-well plates were seeded with 5 × 10^5 cells per well and cultivated to 90% confluency after 48 hours. Then, to create a consistent wound, 200 L pipette tips were used to scratch the cell monolayers. The plates were grown in RPMI-1640 medium without FBS after being washed with PBS. An Olympus (Tokyo, Japan) IX51 inverted microscope was used to take pictures of the cells at the appropriate time intervals. The wound area at 0 h was set to 100% when the wound area was computed using ImageJ software.

Transwell migration assays

A 24-well Corning (Corning, NY, USA) plate was used to assess cell migration. Vitamin D 100 nM or vehicle control was applied to SW480 cells. After 48 hours, complete media was added to the lower compartment, and a suspension of 2 × 10^4 cells in serum-free medium was added to the upper compartment. The membranes were fixed with paraformaldehyde for 20 minutes and stained with crystal violet solution for 10 minutes after incubation for 24 hours. The remaining cells on the opposite side of the filter were then counted using an Olympus IX51 inverted microscope after the cells on the upper filter surface had been removed with a cotton swab.

Western blot analysis

100 nM vitamin D or vehicle control was applied to SW480 cells at the times specified. Each group's total protein content was extracted, and the bicinchoninic acid (BCA) protein reagent kit (ASPEN, China) was used to measure the protein concentration. The ratio of the target band to the glyceraldheyde 3-phosphate dehydrogenase (GAPDH) band was used to determine the protein expression level. Three repetitions of each protein sample were used.

Statistical analysis

GraphPad Prism 8.0 software (La Jolla, CA, USA) was used to compare the data using a t-test. The mean and standard deviation (SD) are used to represent the data. Statistics were judged significant at P <0.05.

Results

Vitamin D inhibits the proliferation, migration and invasion of colorectal cancer cells

To investigate the mechanism underlying vitamin D's inhibitory effect on CRC cells, we first performed assays to evaluate cell proliferation and apoptosis. In the CCK8 cell viability test, we found that cells treated with vitamin D showed lower viability than the control group (Figure 1A), which indicated that vitamin D inhibited cell proliferation in vitro. Next, we tested apoptosis in SW480 cells with different treatments. The percentage of Annexin V-positive apoptotic cells treated with vitamin D significantly increased compared to control cells (Figure 1B). These results indicated that vitamin D inhibited CRC cells by inhibiting proliferation and promoting apoptosis.

We also evaluated the effects of vitamin D on the migration activities of CRC cells in vitro. The wound healing assay showed that vitamin D supplementation in SW480 cells significantly inhibited tumor cell migration capacity (Figure 2A). The invasion assays suggested that vitamin D treatment significantly inhibited the invasiveness of SW480 cell lines (Figure 2B). These data suggest that vitamin D inhibits CRC invasion and migration.

Vitamin D inhibits the Notch1 signaling pathway

The Notch1 signaling pathway plays an important role in CRC development by promoting cell proliferation (21). Therefore, we asked whether vitamin D antitumour activity was mediated by Notch1 signaling. Western blot analysis showed that the expression levels of Notch1, cleaved Notch1 and the Notch1 target gene Hes1 were decreased after vitamin D treatment (Figure 3A). The results showed that vitamin D inhibited CRC cell proliferation by inhibiting Notch1 signaling pathways.

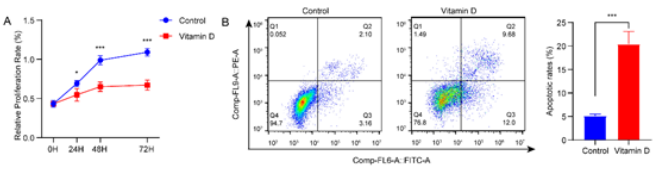

Figure 1. Vitamin D inhibits the proliferation and promotes the apoptosis of colorectal cancer cells. (A) CCK-8 assay was used to detect the effect of vitamin D (100 nM) on the viability of SW480 cells; (B) Cell apoptosis was analysed by flow cytometric analysis and quantitative analysis of the cell apoptosis rate in SW480 cells in all groups. *P<0.05, ***P<0.001.

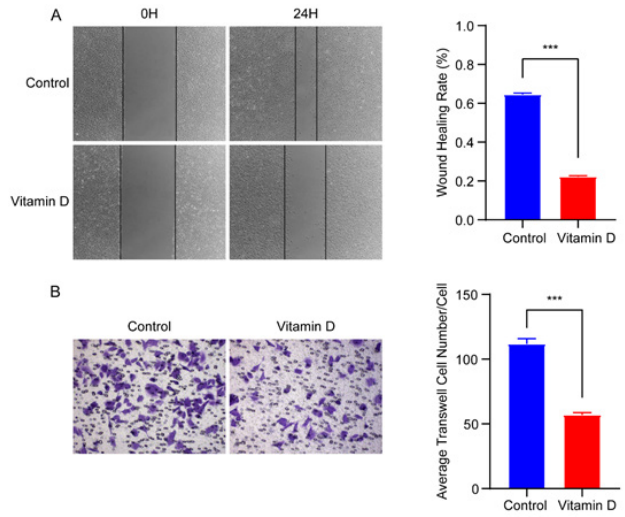

Figure 2. Vitamin D inhibits the migration and invasion of colorectal cancer cells. (A) A wound healing assay was used to detect the effect of vitamin D (100 nM) on the cell migration ability of SW480 cells. Wound closure images were taken at 0 and 24 hours after scratching the cell layer. (B) Transwell assays were used to detect the effect of vitamin D (100 nM) on the invasion ability of SW480 cells. Cell invasion curves were drawn using the average cell number in three different areas. ***P<0.001.

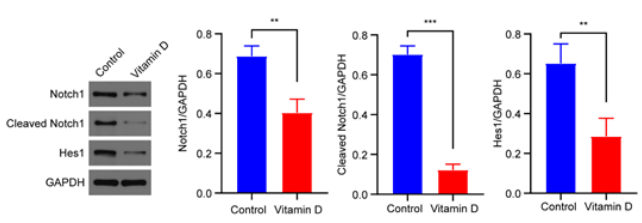

Figure 3. Vitamin D inhibits the Notch1 signaling pathway. The protein expression of Notch1, cleaved Notch1 and Hes1 was detected by western blotting, and quantitative analysis of the protein expression levels in SW480 cells in all groups was performed. **P<0.01, ***P<0.001.

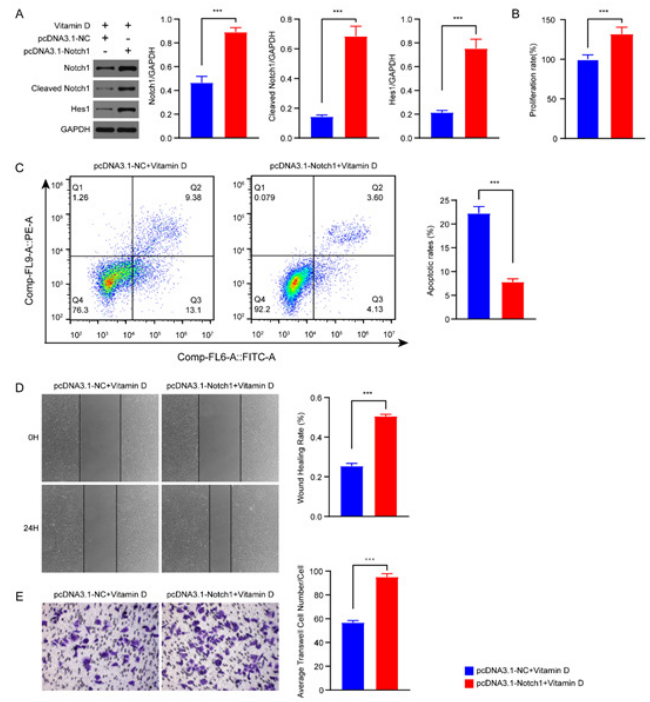

Figure 4. The inhibition ability of vitamin D on colorectal cancer progression was reversed by Notch1 overexpression. (A) The protein expression of Notch1, cleaved Notch1 and Hes1 was detected by western blotting, and quantitative analysis of the protein expression levels in SW480 cells treated with vitamin D in all groups was performed. (B) A CCK-8 assay was used to detect the effect of Notch1 overexpression on the viability of SW480 cells treated with vitamin D. (C) Cell apoptosis was analysed by flow cytometric analysis, and quantitative analysis of the cell apoptosis rate in SW480 cells in all groups. (D) A wound healing assay was used to detect the effect of Notch1 overexpression on the cell migration ability of SW480 cells treated with vitamin D. (E) Transwell assays were used to detect the effect of Notch1 overexpression on the invasion ability of SW480 cells treated with vitamin D. ***P<0.001.

The inhibitory effect of vitamin D on colorectal cancer progression was reversed by Notch1 overexpression

To investigate the effect of the Notch1 pathway on vitamin D inhibition of colorectal cancer growth, SW480 cells were transfected with pcDNA 3.1-Notch1. Western blot analysis showed that the NOTCH1 signaling pathway was activated in the NOTCH1 overexpression group compared to the control group (Figure 4A). In addition, overexpression of NOTCH1 reversed vitamin D's ability to inhibit SW480 cell proliferation (Figure 4B) and reduce apoptosis (Figure 4C). In addition, the results from cell migration (Figure 4D) and invasion (Figure 4E) analysis indicated that vitamin D suppression capabilities on cell migration and invasion were restored in PCDNA 3.1-Notch1 transfected cells. These results suggest that vitamin D inhibits CRC cell proliferation, invasion, and migration by inhibiting the NOTCH1 signaling pathway.

Discussion

Vitamin D has anticancer functions in a variety of cancers, but the relationship between vitamin D status and CRC risk and survival is inconclusive. In this study, we found that vitamin D may inhibit CRC progression in vitro. We investigated the impact of vitamin D on this pathway to clarify its antitumor mechanisms because Notch1 signaling pathways are crucial in the development of colorectal tumors and the progression of malignant disease. The outcomes demonstrated that vitamin D dramatically reduced NOTCH1 protein expression and hindered Notch1 pathways. Additionally, we discovered that overexpressing Notch1 may greatly counteract the vitamin D inhibitory effect on CRC cells. Together, our data demonstrated that NOTCH1 depletion caused by vitamin D in CRC cells slowed the growth of the disease.

Numerous epidemiological and observational studies published recently claim that vitamin D deficiency is selectively linked to CRC incidence and/or mortality (22, 23). However, there are controversies about data design, analysis, and interpretation in some epidemiologic and prospective interventional clinical studies (24, 25). Preclinical studies have suggested that vitamin D may inhibit proliferation and promote epithelial differentiation in CRC (9, 10). Similarly, we also found that vitamin D inhibits proliferation, sensitizes apoptosis, and promotes the migration and invasion of CRC cells. Overall, these results clearly support the idea that vitamin D may help prevent CRC and may even strengthen anticancer treatments. To further elucidate vitamin D's anticancer function and clarify the underlying mechanisms, further studies are needed.

Furthermore, we found that Notch1, cleaved Notch1, and Notch1 target Hes1 gene expression levels decreased after vitamin D treatment. It has been documented that Notch1 activation is a crucial initial event leading to CRC (16). More than 22% of CRCs show an increase in Notch1 receptor copy number, which causes tumor cell-autonomous signaling with poor prognostic value (26). Notch1 and its target HES1 were much more highly expressed in advanced cancers than in low-grade tumors, according to gene sequencing analyses (27). To investigate the effect of the Notch1 pathway on vitamin D inhibition of colorectal cancer growth, SW480 cells were transfected with pcDNA 3.1-Notch1. We found that overexpression of NOTCH1 reversed vitamin D's ability to inhibit SW480 cell progression, suggesting that vitamin D inhibits CRC cell proliferation, invasion, and migration by downregulating the Notch1 signaling pathway.

In conclusion, we found that the NOTCH1 pathway was frequently downregulated in CRC cells treated with vitamin D therapy. Vitamin D inhibited the metastasis and proliferation of CRC tumor cells in vitro, which may be caused by downregulating NOTCH1 expression levels. In addition, overexpressing NOTCH1 reversed vitamin D's ability to inhibit CRC cell progression. These results provide further insights into the mechanisms of vitamin D anticancer activity in CRC development and progression.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Consent for publications

The author read and approved the final manuscript for publication.

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