1,25(OH)2D3 Attenuates IL-1β-Induced Epithelial-to-Mesenchymal Transition Through Inhibiting the Expression of lncTCF7

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The activated form of vitamin D3, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], regulates numerous cellular processes, including inhibition of cancer progression. IL-1β has been reported to facilitate cancer development, especially by inducing an epithelial-to-mesenchymal transition (EMT) in several malignant tumors. However, the underlying mechanism of 1,25(OH)2D3 and IL-1β in colorectal cancer (CRC) still remains largely unknown. To fill in this knowledge gap, we measured cell proliferation and invasion by CCK-8 and Transwell assays after stimulation with 1,25(OH)2D3 and IL-1β. E-cadherin and vimentin were chosen as markers of EMT measured by immunofluorescence, quantitative real-time PCR (qRT-PCR), and Western blot. The expression and function of the vitamin D receptor (VDR) was evaluated by Western blot and luciferase reporter assay. qRT-PCR and RNA-FISH were performed to detect the expression and location of lncTCF7 in vitro. The binding sites of VDR in the lncTCF7 promoter were confirmed by a chromatin immunoprecipitation assay. Based on the above experiments, we found that 1,25(OH)2D3 attenuates IL-1β-induced increased proliferation and invasion in colorectal cancer through enhancing VDR, which inhibits the expression of lncTCF7 by directly binding to its promoter region.

Key words: 1,25(OH)2D3; IL-1β; Vitamin D receptor; Epithelial–mesenchymal transition (EMT); lncTCF7

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the world. Several studies have reported that vitamin D3 exhibits antitumor activity in multiple cancers, especially CRC. Accumulating evidence shows that increasing vitamin D synthesis reduces the risk and progression of colorectal cancer, and vitamin D deficiency increases the risk of CRC. As the most active form of vitamin D in vivo, 1,25(OH)2D3 regulates up to 5% of genome transcription through the VDR signaling pathway. Long noncoding RNAs (lncRNAs) are defined as transcripts longer than 200 nucleotides that do not code for proteins. Some recent studies have shown that lncRNAs play an important role in cancer process. Jiang and Bikle reported that VDR regulated a variety of lncRNAs, including H19, HOTTIP, and Kcnq1ot1, in skin cancer, but the link between VDR and lncRNAs in CRC still largely remains unknown.

Inflammation has been reported as one of the hallmarks of cancer development. IL-1β is a member of the interleukin-1 family that has a complex function in inflammation, immunity, and hemopoiesis. IL-1β recently has been shown to induce the upregulation of BIRC3 and methylation of the estrogen receptor ERα gene, leading to EMT and chemoresistance in breast cancer. Searching on the website of GeneCards, we found that calcitriol, as one of the VDR agonists, is an IL-1β and TNF-α production inhibitor; thus, we hypothesized that vitamin D3 has the opposite effect of IL-1β, and its underlying mechanism in CRC remains unclear.

In this study, we examined the effect of 1,25(OH)2D3 and IL-1β in CRC cell lines, and we demonstrated that 1,25(OH)2D3 attenuates IL-1β-induced EMT through activating VDR signaling. In addition, we showed that 1,25(OH)2D3 inhibits cancer development partly through
the VDR/IncTCF7 axis. These results supplement our knowledge of the mechanism of 1,25(OH)2D3 in CRC.

MATERIALS AND METHODS

Cell Culture

The cell lines DLD1 and LoVo were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). DLD1 and LoVo were cultured in DMEM (Gibco, Carlsbad, CA, USA) and F-12K (Gibco) culture media, respectively, at 37°C with 5% CO2. The media were supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin (Keygen Biotech, Beijing, P.R. China), and 100 μg/ml of streptomycin (Keygen Biotech). The plasmid overexpressing IncTCF7 and its empty vector were obtained from GenePharma Co (Shanghai, P.R. China). Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) was used for the transfection.

RNA Extraction and qRT-PCR

Total RNA was extracted from cultured cells using TRIzol reagent (Takara, Dalian, P.R. China). RQ1 RNase-Free DNase (Promega, Madison, WI, USA) was used to remove DNA. RNA reverse transcription to cDNA was performed with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, Carlsbad, CA, USA), and quantitative real-time PCR (qRT-PCR) analysis was performed with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. The primer sequences are shown in Table 1. The results were normalized to the expression of β-actin. The delta Cq (ΔCq) value was used to represent the expression level of IncRNA in qRT-PCR. For each amplicon designed, the ΔCq value was normalized using the equation: ΔCq=Cq (target)−Cq (β-actin).

Western Blots

Lysate was extracted from cultured cells with a lysis buffer, and the protein content was assayed by a protein assay (BCA) kit (Thermo Fisher). Proteins were fractionated on 10% SDS-PAGE and transferred onto a PVDF membrane. Rabbit monoclonal antibodies against E-cadherin, vimentin, and VDR (Cell Signaling Technology, Danvers, MA, USA) were used as the primary antibodies. The antibody against GAPDH (Cell Signaling Technology) was used as a loading control. The secondary antibody was then added, and proteins of interest were visualized using Pierce ECL Western Blotting Substrate (Millipore, Boston, MA, USA).

Cell Proliferation

The effect of 1,25(OH)2D3 and IL-1β on proliferation of CRC cell lines was measured with Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan). DLD1 and LoVo

| Table 1. Primers |
|------------------|
| **E-cadherin**   |
| F: 5’-CGAGAGCTACACGTTCAAGG-3’ |
| R: 5’-GGGTGTCAGGGAAATAAGG-3’ |
| **Vimentin**     |
| F: 5’-TCCCGTGAAGCTGCTAACTA-3’ |
| R: 5’-CCAGAGGGAGTGATCCAGATAT-3’ |
| **VDR**          |
| F: 5’-GGTGACACCTGGCATGAGGACAG-3’ |
| R: 5’-GGTGAGGTCTTATGGTGG-3’ |
| **β-Actin**      |
| F: 5’-CCATGCAAGGTTTCAGAGGATGTA-3’ |
| R: 5’-CTCTTAAATGCAGGCAGCATAT-3’ |
| **GNAS-AS1**     |
| F: 5’-GCTGGCAGAGGAGCTAGATGG-3’ |
| R: 5’-CTGTGACAGTCGACTTCACCT-3’ |
| **lincRNA-p21**  |
| F: 5’-AGAGCTTCCACGAGGTGAC-3’ |
| R: 5’-GCTCCCACTCTACACCTCAG-3’ |
| **MEG3**         |
| F: 5’-CTCAGGCTGAGGGAGTGG-3’ |
| R: 5’-TGGTGGTCAGGACTTCCT-3’ |
| **HOTTIP**       |
| F: 5’-CCATATGGCAGCCTTATTAGG-3’ |
| R: 5’-TGGTGGTCAGGACTTCCT-3’ |
| **Kcnq1ot1**     |
| F: 5’-CGACATAGCCCTTATAGG-3’ |
| R: 5’-CAGGACCTTCACTGGAGAAT-3’ |
| **SRA1**         |
| F: 5’-CTGAGGCTGCTCTATGG-3’ |
| R: 5’-AGCCCTGATGTGTCTTCTT-3’ |
| **MALAT1**       |
| F: 5’-GGATGGAGTGGCTGCTAGTG-3’ |
| R: 5’-ATTGGCGCAATTCACCTAC-3’ |
| **SAMMSON**      |
| F: 5’-AGACAGACCTTTGGACCTGAG-3’ |
| R: 5’-AGTGGCTGCAATAACCA-3’ |
| **IncTCF7**      |
| F: 5’-AGGAGTCTGTTGACCTGAGC-3’ |
| R: 5’-AGTGGCTGCAATAACCA-3’ |
cells were resuspended, seeded at $1 \times 10^3$/100 μl/well in a 96-well plate, and incubated for different times with or without 100 nM 1,25(OH)2D3 in the absence or presence of 10 ng/ml of IL-1β. CCK-8 reagent was added to each well. The plate was incubated for another 2 h at 37°C, and the absorbance at 450 nm was measured.

Transwell Assays

The invasive ability of CRC cells was assayed by a Transwell plate with a filter of 8-μm pores (BD Biosciences, Franklin Lakes, NJ, USA), and the top chamber was precoated with 50 μl of Matrigel (BD Biosciences). A total of $5 \times 10^5$ cells in serum-free medium were added into the upper insert. In the lower compartment, 600 μl of medium containing 20% FBS was added. After incubation for 48 h, the cells on the upper surface of the filter were wiped off, and the cells adherent on the undersurface of the filter were fixed in methanol, stained with crystal violet, and counted under a microscope. Three visual fields were selected randomly for cell counting.

Immunofluorescence Analysis

After treatment with 100 nM 1,25(OH)2D3 for 48 h, DLD1 cells were washed with PBS, fixed with 3.7% formaldehyde for 10 min at room temperature, then permeabilized with 0.3% Triton X-100 in PBS for 10 min. Subsequently, cells were blocked with 5% bovine serum albumin in PBS for 1 h at room temperature, and then incubated with primary antibody for 1 h at room temperature. After being washed with PBS, the cells were incubated with FITC-conjugated secondary antibodies (Cell Signaling Technology) for 1 h at room temperature. After being washed with PBS, the nuclei were stained with DAPI. The fluorescence was visualized using Fluoview 1000 confocal microscope (Olympus, Tokyo, Japan). Antibody dilutions of 1:200 were used for E-cadherin (Cell Signaling Technology) and 1:100 for vimentin (Cell Signaling Technology).

RNA-FISH

A fragment of a 550-bp lncTCF7 cDNA was amplified from the lncTCF7 plasmid by using a high-fidelity DNA polymerase (Takara) as the DNA template. From this template, a fluorescein-labeled DNA as the lncTCF7 FISH probe was made with PCR Fluorescein Labeling Mix (Roche, Basel, Switzerland). DLD1 cells were cultured on slides treated with or without 100 nM 1,25(OH)2D3 for 48 h. The slide was soaked in proteinase K for 5 min and washed in 2× SSC/0.0001% NP-40 for another 2 min. The slide was then washed in 0.4× SSC/0.001% NP-40 at 560°C for 5 min followed by a second wash in 2× SSC/0.0001% NP-40 for another 2 min. The slide was covered with a drop of mounting medium containing DAPI and visualized using a Fluoview 1000 confocal microscope (Olympus).

Luciferase Reporter Assay

The promoter region of the human lncTCF7 gene, which contains putative binding sites, was amplified by PCR and then subcloned into the pGL3 vector. Luciferase reporter plasmids were constructed with a series deletion of the lncTCF7 promoter region (lncTCF7-luc) or mutated VDR binding sites (mutVIE). A pRL-TK plasmid expressing Renilla luciferase was used as an internal control. After cotransfection, DLD1 cells were cultured with 100 nM 1,25(OH)2D3 for 24 h. Luciferase activity was detected by a Dual-Luciferase Reporter Assay System (Promega). All of the experiments were performed in triplicate.

Chromatin Immunoprecipitation

DLD1 cells were treated with 100 nM 1,25(OH)2D3 for 24 h. ChIP assays were performed using SimpleChIP® Enzymatic Chromatin IP Kit (Cell Signaling Technology) following the manufacturer’s instructions. Antibody dilutions of 1:50 were used for VDR (Cell Signaling Technology). Primer sequences of the lncTCF7 promoter region were 5'-GTCGCTTAAGCCCAAGAGG-3' (forward) and 5'-CTATGTCTGGCCAGGGCTGA-3' (reverse).

Tumor Xenograft Model

Male BALB/c nude mice, 4 weeks of age, were purchased from Beijing Vital River Co. (Beijing, P.R. China) and maintained in the Experimental Animal Center of Peking University First Hospital. DLD1 cells ($1 \times 10^7$ cells) mixed with 50% Matrigel (BD Biosciences) were injected subcutaneously into the right flank of each nude mouse ($n=6$/group). The 1,25(OH)2D3 injection method was as described previously. Tumor volume was measured every 2 days and calculated as width$^2$ x length/2. After 2 weeks, the mice were sacrificed, the tumors were removed, and total mRNA and protein were collected. Animal study was approved by the Medical Ethics Committee of Peking University First Hospital.

Statistical Analysis

Data are presented as mean values ± SEM. Two-tailed Student’s t-test was used for data comparison. Values of $p<0.05$ were considered as statistically significant. Each experiment was repeated at least three times independently.

RESULTS

1,25(OH)2D3 Attenuates IL-1β-Induced Increased Proliferation and Invasion in CRC Cells

To detect whether 1,25(OH)2D3 and IL-1β affect CRC cell proliferation and invasion, CCK-8 and Transwell assays...
were performed. CCK-8 assays proved that 1,25(OH)2D3 inhibited the proliferation of DLD1 and LoVo cells, whereas IL-1β facilitated their proliferation (Fig. 1A). Furthermore, 1,25(OH)2D3 also inhibited cell proliferation that was promoted by IL-1β (Fig. 1A). Next, we detected their invasive ability after stimulation by 1,25(OH)2D3 and IL-1β. In a Transwell assay, for both DLD1 and LoVo cell lines, there were 30%–40% decreases in the 1,25(OH)2D3 group compared with the control (Fig. 1B and C). Cell numbers in the IL-1β group increased about twofold (Fig. 1B and C). However, cotreatment with 1,25(OH)2D3 and IL-1β showed that 1,25(OH)2D3 inhibited the effect of IL-1β on cell invasion.

1,25(OH)2D3 Inhibits CRC Cell EMT That Was Induced by IL-1β and Promotes the Expression of VDR

Given the effect of 1,25(OH)2D3 and IL-1β on CRC cell proliferation and invasion, we attempted to investigate the underlying molecular mechanisms. Epithelial-to-mesenchymal transition (EMT) plays an important role in cancer metastasis and invasion. VDR is pivotal in the 1,25(OH)2D3 signaling pathway. Thus, we set out to examine if 1,25(OH)2D3 and IL-1β affect EMT and VDR expression levels.

We measured the expression of epithelial and mesenchymal markers by immunofluorescence, qRT-PCR, and

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**Figure 1.** 1,25(OH)2D3 attenuates IL-1β-induced increased proliferation and invasion in colorectal cancer (CRC) cells. Two CRC cell lines, DLD1 and LoVo, were used for the following experiments. (A) Proliferation of CRC cells assessed by CCK-8 assays. 1,25(OH)2D3 (100 nM) inhibits CRC cell proliferation that was promoted by 10 ng/ml of IL-1β. Error bar: mean±SEM. *, #, ▲ p<0.05. (B) Cancer cell invasion was measured by Transwell assay. (C) Cells that had moved through the upper chamber were counted. The Ctrl group number was normalized to 1. The values represent the means±SEM. ***p<0.001.
Western blot. First, we performed immunofluorescence to detect the expression of E-cadherin and vimentin in DLD1 cells. The results suggested that 1,25(OH)2D3 promoted the expression of E-cadherin and inhibited the expression of vimentin (Fig. 2A and B). IL-1β showed an opposite effect, which could also be inhibited by 1,25(OH)2D3 (Fig. 2A and B).

qRT-PCR was performed to detect the mRNA levels of E-cadherin, vimentin, and VDR in DLD1 and LoVo cells after stimulating with 1,25(OH)2D3 and IL-1β. The results showed that 1,25(OH)2D3 increased the expression of E-cadherin but had a nonobvious effect on transcription of vimentin and VDR. On the other hand, IL-1β did not show any influence on E-cadherin, vimentin, or VDR at the transcriptional level (Fig. 3A). Figure 3B and C shows that 1,25(OH)2D3 enhanced the expression of E-cadherin and VDR, but inhibited the expression of vimentin while IL-1β demonstrated opposite effects in CRC cells, and 1,25(OH)2D3 attenuated the effect of IL-1β on EMT and VDR expression.

1,25(OH)2D3 Inhibits the Expression of lncTCF7 in CRC Cells

Previous studies have reported that many lncRNAs are involved in cancer progression through the Wnt/β-catenin pathway. It has been proven that vitamin D also inhibits colon cancer through the Wnt/β-catenin pathway. Therefore, we hypothesized that the inhibitory

**Figure 2.** 1,25(OH)2D3 inhibits CRC cell epithelial-to-mesenchymal transition (EMT) that was induced by IL-1β. (A) Effect of 1,25(OH)2D3 and IL-1β on E-cadherin synthesis in DLD1 cells. DLD1 cells were cultured on slides treated with or without 100 nM 1,25(OH)2D3 in the absence or presence of 10 ng/ml of IL-1β for 48 h before cells were fixed, and immunofluorescence of E-cadherin (green) and nucleus (blue) was performed. (B) Effect of 1,25(OH)2D3 and IL-1β on vimentin synthesis in DLD1 cells. DLD1 cells were cultured on slides treated with or without 100 nM 1,25(OH)2D3 in the absence or presence of 10 ng/ml of IL-1β for 48 h before cells were fixed, and immunofluorescence of vimentin (green) and nucleus (blue) was performed.
effect of 1,25(OH)2D3 on CRC may be partly through the action of those lncRNAs. We detected the expression level of some lncRNAs in DLD1 cells by qRT-PCR. As demonstrated in Figure 4A, several lncRNAs showed dysregulation after stimulation by 1,25(OH)2D3. Among them, the expression of lncTCF7 changed significantly (Fig. 4A). Moreover, 1,25(OH)2D3 decreased the expression of lncTCF7 in a dose- and time-dependent manner in both DLD1 and LoVo cells (Fig. 4B and C). Because 1,25(OH)2D3 showed the opposite effect with IL-1β, we wondered whether IL-1β could regulate lncTCF7. Figure 4D indicates that IL-1β increased the expression of lncTCF7, and 1,25(OH)2D3 attenuated this effect. To visualize the location of lncTCF7, RNA-FISH was employed. lncTCF7 was localized in both the nuclei and cytoplasm, and more abundant lncTCF7 RNA was seen in the control group compared with the 1,25(OH)2D3 group (Fig. 4E).

**lncTCF7 Facilitates EMT That Was Inhibited by 1,25(OH)2D3**

Considering the results that 1,25(OH)2D3 inhibits EMT in part through decreasing

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**Figure 3.** 1,25(OH)2D3 attenuates IL-1β-induced EMT and increases the expression of vitamin D receptor (VDR). (A) Effect of 1,25(OH)2D3 and IL-1β on mRNA expression of E-cadherin, vimentin, and VDR in DLD1, and LoVo cells were detected by qRT-PCR. β-Actin was used as an internal control. (B, C) The expressions of E-cadherin, vimentin, and VDR in DLD1 and LoVo cells were detected by Western blot after treatment with 1,25(OH)2D3 and IL-1β. GAPDH was used as an internal control. Data represent the mean ± SEM. *p < 0.05, **p < 0.01.
1,25(OH)2D3 INHIBITS THE EXPRESSION OF lncTCF7

Therefore, we overexpressed lncTCF7 by transfecting DLD1 and LoVo cells with lncTCF7 plasmid. Next, the expressions of E-cadherin and vimentin were assayed by Western blot with or without 1,25(OH)2D3 treatment. Compared with the empty plasmid group, E-cadherin expression was reduced and vimentin expression was enhanced in the lncTCF7 group (Fig. 5A and B). We also showed that lncTCF7 partially reversed the expression of E-cadherin and vimentin that was stimulated by 1,25(OH)2D3 (Fig. 5A and B). The results indicate that 1,25(OH)2D3-inhibited EMT was partially reversed by overexpressing lncTCF7.

**lncTCF7 Is Transcriptionally Inhibited by VDR in Response to 1,25(OH)2D3 Stimulation**

To further illuminate how 1,25(OH)2D3 influenced the expression of lncTCF7, we searched for predicted sites that may bind VDR in the lncTCF7 promoter region...
It showed that the promoter region of lncTCF7 harbored 10 potential VDR-inducible elements (VIEs) (Fig. 6A). Luciferase reporter plasmids were constructed with a serial deletion of the lncTCF7 promoter region or mutVIE (Fig. 6A). As shown in Figure 6B, the region between −1510 and −1070 in the lncTCF7 promoter was essential for VDR-mediated regulation. There are five potential VIEs in this region, and we chose two sites with high scores predicting that they are most likely to bind to VDR, VIE3 and VIE6. Mutations in VIE6 markedly increased the reporter activity inhibited by 1,25(OH)2D3 (Fig. 6C). To further prove this finding, a ChIP assay was performed to confirm the interaction between VDR and the promoter region of lncTCF7 in DLD1 cells. ChIP-PCR confirmed that VDR directly bound to the promoter region of lncTCF7 (Fig. 6D). Our study demonstrated that 1,25(OH)2D3 enhanced VDR, which inhibited transcription of lncTCF7 by binding directly to its promoter region.

**The Effect of the 1,25(OH)2D3–VDR–lncTCF7 Axis In Vivo**

Next, we detected whether 1,25(OH)2D3 influenced lncTCF7 in vivo. A total of 1 × 10⁷ DLD1 cells were inoculated into the nude mice. After a palpable tumor could be recognized, 0.5 μg/kg of 1,25(OH)2D3 was injected IP twice a day. As shown in Figure 7A and B, the 1,25(OH)2D3 group led to a significantly smaller tumor volume and slower tumor growth. Then we performed Western blotting to detect whether 1,25(OH)2D3 could regulate EMT-related proteins and VDR. Figure 7C indicates that 1,25(OH)2D3 increased the expression of E-cadherin and VDR and decreased the expression of vimentin. This result proves that 1,25(OH)2D3 inhibited EMT in vivo. Furthermore, the expression of lncTCF7 was detected by qRT-PCR, and there was a significant decrease in the 1,25(OH)2D3 group (Fig. 7D). These data demonstrate the 1,25(OH)2D3–VDR–lncTCF7 axis in vivo.

**DISCUSSION**

Research has shown that vitamin D3 and its analogs act as an efficient anticancer agent through several signaling pathways. Searching on the website of GeneCards, we found that calcitriol as one of the vitamin D3 analogs is an IL-1β and TNF-α production inhibitor. IL-1β has been found to promote stem cellness and invasiveness of CRC cells through Zeb1 activation. Soria et al. reported that coordinated expression of TNF-α and IL-1β had effects on EMT, leading to relapse of breast cancer. 1,25(OH)2D3 is one of the most activated forms.
1,25(OH)2D3 INHIBITS THE EXPRESSION OF lncTCF7

Our previous study demonstrated the protective effect of 1,25(OH)2D3 on intestinal epithelial barrier function that was injured by TNF-α. Therefore, we speculated as to whether 1,25(OH)2D3 will have any impact on IL-1β in CRC. We investigated the proliferation and invasion of CRC cell lines that were stimulated by 1,25(OH)2D3 and IL-1β and found that 1,25(OH)2D3 inhibited IL-1β-induced proliferation and invasion of CRC cells. EMT plays a critical role in tumor progression and invasion. E-cadherin and vimentin are the classical epithelial and mesenchymal markers, respectively. In previous studies, IL-1β has shown an influence on EMT. To reveal whether 1,25(OH)2D3 inhibits IL-1β-induced EMT in CRC cells, immunofluorescence analysis, qRT-PCR, and Western blots were performed. As expected, 1,25(OH)2D3 attenuated EMT that was induced by IL-1β.

Vitamin D3 inhibits cancer progression through the VDR pathway, and VDR dysregulation is related to CRC progression and recurrence. In our previous study, we have found that 1,25(OH)2D3 promoted the expression of VDR in CRC cell lines. We speculated that 1,25(OH)2D3 attenuates the IL-1β effect, leading to enhancement of the expression of VDR. Therefore, we investigated the expression of VDR after treatment with 1,25(OH)2D3 and IL-1β. Western blots showed that the expression of VDR is decreased after stimulation with IL-1β, but when cotreated with 1,25(OH)2D3 and IL-1β, the expression level of VDR is increased. These results show that 1,25(OH)2D3 inhibits IL-1β’s effect on the expression of VDR, but the mechanism of how 1,25(OH)2D3 attenuates the IL-1β effect on VDR expression needs further research.

It has been reported that lncTCF7 promotes aggressiveness and self-renewal of cancer stem cells in hepatocellular carcinoma. In our previous study, IncTCF7 has shown the ability to promote cell proliferation, migration, and invasion in vitro and tumorigenesis in vivo through activating the Wnt signaling pathway. Meanwhile, 1,25(OH)2D3 exerts an influence on cancer...
We hypothesized that there might be a link between 1,25(OH)2D3 and lncTCF7. We measured the expression of lncTCF7 in CRC cell lines and found that the lncTCF7 level was decreased after stimulation with 1,25(OH)2D3 in dose- and time-dependent manners. lncTCF7 was visualized by RNA-FISH in DLD1 cells, and this showed that 1,25(OH)2D3 downregulated the expression of lncTCF7. We also found that 1,25(OH)2D3 attenuates the effect of IL-1β on lncTCF7 expression.

To further reveal the upstream regulation of lncTCF7, we checked the web (http://genome.ufl.edu/mapper) and found that there are 10 potential sites that may allow VDR to bind to the lncTCF7 promoter region. A luciferase reporter assay and ChIP proved that VDR inhibited the expression of lncTCF7 through directly binding to its promoter region. Furthermore, overexpression of lncTCF7 reversed the inhibition of 1,25(OH)2D3 on EMT in both DLD1 and LoVo cells. In addition, in nude mice inoculation experiments, 1,25(OH)2D3 inhibited cell proliferation and EMT, upregulated VDR expression, and decreased the expression of lncTCF7 in vivo. These results illustrated that 1,25(OH)2D3 inhibits cancer progression partly through decreasing the expression of lncTCF7.

All of the above results prove the existence of a 1,25(OH)2D3–VDR–lncTCF7 signaling axis (Fig. 8) and may provide a novel therapeutic target for colorectal cancer in the future.
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