1α, 25-dihydroxyvitamin D_3 inhibits transforming growth factor β1-induced epithelial-mesenchymal transition via β-catenin pathway

Xin-Rong Xiong[1], Xin-Li Tian[2], Ru-Jie Huo[3], Yan-Ting Dong[3], Dai Liu[3], Jing-Cui Bai[3], Yun-Feng Qi[4], Xin-Rui Tian[3]

[1]Shanxi medical university, Taiyuan, Shanxi 030001, China;
[2]Cardiopulmonary Center, The Seventh Medical Center of People's Liberation Army General Hospital, Beijing 100730, China;
[3]Department of Respiratory and Critical Care Medicine, The Second Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001, China;
[4]Department of Respiratory Medicine, Jinhua Hospital of Traditional Chinese Medicine, Jinhua, Zhejiang 321017, China.

Abstract

Background: The transforming growth factor β1 (TGF-β1)-induced epithelial-mesenchymal transition (EMT) has been proven associated with the pathogenesis of asthmatic airway remodeling, in which the Wnt/β-catenin pathway plays an important role, notably with regard to TGF-β1. Recent studies have shown that 1α, 25-dihydroxyvitamin D_3(1α, 25$(OH)_2D_3$) inhibits TGF-β1-induced EMT, although the underlying mechanism have not yet been fully elucidated.

Methods: Alveolar epithelial cells were exposed to 1α, 25$(OH)_2D_3$, ICG-001, or a combination of both, followed by stimulation with TGF-β1. The protein expression of E-cadherin, α-smooth muscle actin, fibronectin, and β-catenin was analyzed by western blotting and immunofluorescence analysis. The mRNA transcript of Snail was analyzed using RT-qPCR, and matrix metalloproteinase 9 (MMP-9) activity was analyzed by gelatin zymogram. The activity of the Wnt/β-catenin signaling pathway was analyzed using the Top/Fop flash reporters.

Results: Both 1α, 25$(OH)_2D_3$ and ICG-001 blocked TGF-β1-induced EMT in alveolar epithelial cells. In addition, the Top/Fop Flash reporters showed that 1α, 25$(OH)_2D_3$ suppressed the activity of the Wnt/β-catenin pathway and reduced the expression of target genes, including MMP-9 and Snail, in synergy with ICG-001.

Conclusion: 1α, 25$(OH)_2D_3$ synergizes with ICG-001 and inhibits TGF-β1-induced EMT in alveolar epithelial cells by negatively regulating the Wnt/β-catenin signaling pathway.

Keywords: 1α, 25-Dihydroxyvitamin D_3; Vitamin D; β-Catenin; Epithelial-mesenchymal transition; Airway remodeling; Asthma

Introduction

The epithelial-mesenchymal transition (EMT) is a process by which epithelial cells are converted into a mesenchymal cell phenotype by losing their epithelial function and characteristics.[1] EMT is thought to be involved in the pathogenesis of airway remodeling in asthma.[2] Transforming growth factor β1 (TGF-β1) is a pleiotropic cytokine that exerts various effects in different cells, including cell proliferation, differentiation, immune function inhibition, and extracellular matrix formation.[3] TGF-β1 has been identified as a "master switch" in the induction of EMT and works through distinct signal transduction pathways, including Smad, non-Smad, and β-catenin. Among them, the β-catenin pathway is highly correlated with the pathogenesis of airway remodeling in asthma.[4] Therefore, the inhibition of the β-catenin signaling pathway represents a potential novel treatment for asthma via the attenuation of TGF-β1-induced EMT.

Vitamin D is a class of fat-soluble vitamins that are primarily synthesized in the skin and converted into the biologically active form in the liver and kidney via two hydroxylation steps. 1α, 25-dihydroxyvitamin D_3 (1α, 25$(OH)_2D_3$) is the most important active metabolite of vitamin D in the body.[5-9] Recent studies have shown that 1α, 25$(OH)_2D_3$ induces epithelial differentiation in normal cells and increases the expression of components of almost all types of cell adhesion structures, which are essential for obtaining and maintaining the epithelial phenotype. This indicates that 1α, 25$(OH)_2D_3$ may be a negative modulator of EMT.[1] Ramirez *et al* reported that 1α, 25$(OH)_2D_3$ reduces the expression of TGF-β1 and

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000830

Xin-Rong Xiong and Xin-Li Tian contributed equally to this work.

Correspondence to: Xin-Rui Tian, Department of Respiratory and Critical Care Medicine, The Second Hospital of Shanxi Medical University, 382 Wuyi Road, Xinghualing District, Taiyuan, China
E-Mail: tianxr@126.com

Chinese Medical Journal 2020;133(11)

Received: 10-10-2019 **Edited by:** Xiu-Yuan Hao

attenuates TGF-β1-induced EMT in rat lung epithelial cells.[10] However, the exact molecular mechanisms are unknown.

Vitamin D is involved in two pathways. Classically, vitamin D is mediated by membrane VDR to activate or repress the transcription of target genes. However, it also exerts a non-genomic effect via cross-talk with other signaling pathways.[11] One study previously showed that 1α, 25$(OH)_2D_3$ promotes the transport of β-catenin from the nucleus to the plasma membrane, competing with T-cell transcription factor 4 (TCF4) for β-catenin binding, thus inhibiting the Wnt/β-catenin signaling pathway.[12] Su *et al* found that 1α, 25$(OH)_2D_3$ promoted cardiac differentiation by inducing the expression of CK1α (a negative regulator of the Wnt signaling pathway).[13] However, the effects of 1α, 25$(OH)_2D_3$ on the β-catenin pathway in TGF-β1-induced EMT processes have not yet been reported. Our previous study showed that the inhibition of β-catenin by ICG-001 (a selective inhibitor of β-catenin transcriptional activity) suppressed TGF-β1-induced EMT in tubular epithelial cells.[14] In the present study, we investigated the mechanism by which 1α, 25$(OH)_2D_3$ induces EMT via TGF-β1 and its interaction with ICG-001 in alveolar epithelial cells.

Materials and Methods

Cell culture and treatment

Alveolar epithelial cells in rats were obtained from Baili (Shanghai, China). The cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C for 24 h in Dulbecco modified Eagle medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. Cells were seeded on six-well culture dishes at a density of 1×10^5 cells per well. When the cells reached approximately 80% confluency, the medium was substituted for serum-free DMEM overnight. Then, 1 μmol/L of 1α, 25$(OH)_2D_3$ and 5 μmol/L of ICG-001 were added either alone or in combination for 24 h, followed by stimulation with 10 ng/mL TGF-β1 (PeproTech, Rocky Hill, NJ, USA) for 48 h. The morphology of the cells was observed by inverted fluorescence microscopy.

Western blotting

After drug administration, the cells were incubated with radioimmunoprecipitation assay lysis buffer on ice for 30 min. The lysate was centrifuged at 12,000 r/min and 4°C for 30 min and the resulting supernatant was collected. The protein concentration was measured using a bicinchoninic acid protein assay kit (Beyotime Biotechnology, China). Equal amounts of total proteins (30 μg) were mixed with an equal volume of loading buffer and loaded onto 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The proteins were then transferred onto polyvinylidene fluoride membranes separately. After blocking with 5% powder skim milk for 1 h, the membranes were incubated with primary antibodies against E-cadherin (E-cad), α-smooth muscle actin (α-SMA), fibronectin (FN), and β-catenin (Abcam, Cambridge, MA, USA) overnight at 4°C, followed by incubation with anti-mouse or anti-rabbit horseradish peroxidase-conjugated antibodies (Abcam) at room temperature for 1 h. Protein expression was visualized using a chemiluminescence system (Olympus, Tokyo, Japan).

Immunofluorescence staining

Alveolar epithelial cells were plated in 24-well plates with coverslips. Once the cells grew to the appropriate density, they were stimulated with TGF-β1, ICG-001, or 1α, 25$(OH)_2D_3$ alone or a combination of these. After 48 h, the cells were washed with phosphate buffered saline (PBS) three times, fixed with 4% paraformaldehyde for 20 min at 4°C, incubated with 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) at 4°C for 15 min, and blocked with 10% bovine serum albumin in PBS for 20 min at room temperature. The treated cells were then exposed to antibodies against β-catenin, E-cad, α-SMA, and FN at 4°C overnight. The cells were then stained with the secondary antibodies IgG-Cy3 or IgG-fluorescein isothiocyanate in a dark room for 1.5 h and washed with PBS three times (3 min/wash). For nuclei labeling, the cells were incubated with 4',6-diamidino-2-phenylindole for 20 min in the dark. Fluorescence microscopy was used for image analysis.

Real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized using a Reverse Transcription System kit (Roche, Basel, Switzerland). The RT-qPCR reaction mixture consisted of cDNA (1 μL), forward and reverse primers (each 0.5 μL, Table 1), 2× RT-qPCR Master Mix (12.5 μL), and deionized water (for a total volume of 25 μL). The RT-qPCR reaction was run under certain conditions. The reaction parameters were 95°C for 2 min, followed by 40 cycles of 95°C for 35 s, 60°C for 1 min, and 72°C for 30 s. The grayscale ratio was calculated using the imaging software.

Table 1: Primer sequences for reverse transcription-quantitative polymerase chain reaction.

Gene name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
Snail	CTTGTGTCTGCACGACCTGT	CTTCACATCCGAGTGGGTTT
β-actin	GATTACTGCTCTGGCTCCTAGCA	GCCACCGATCCACACAGAGT

Gelatin zymogram

The cells were seeded at a suitable density on a six-well plate. The protein was extracted 3 days after drug administration. The protein sample was mixed with gelatin (Invitrogen) at 120 V for 2.5 h. After electrophoresis, the gel was eluted with 2.5% Triton X-100 and incubated with substrate buffer for 36 h at 37°C. The gel was then stained with Coomassie Brilliant Blue (BioRad, Hercules, CA, USA) and de-stained with a decolorizing solution. After bleaching, a white stripe was visible on the blue background.

Top flash assay

To assay the transcriptional activity of β-catenin, cells were transfected with Top/Fop Flash plasmids (Millipore Corp, Billeric, MA, USA) or a pRL-TK plasmid (control for transfection) using Lipofectamine 2000 (Invitrogen). After 48 h, a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to analyze the levels of Firefly and Renilla activity in the lysates. The luciferase assay was performed in triplicate for each experiment.

Statistical analysis

Statistical analysis was performed using SPSS (version 22.0) (SPSS, Inc., Chicago, IL, USA) for Windows. All data are presented as mean ± standard deviation. Differences between three or more groups were analyzed using one-way analysis of variance, followed by the Least Significant Difference-*t post-hoc* test. A P value <0.05 was considered statistically significant.

Results

1α, 25(OH)$_2$D$_3$ and ICG-001 inhibit TGF-β1-induced EMT

We first established a model of TGF-β1-induced EMT in alveolar epithelial cells. Under basal conditions, alveolar epithelial cells showed a cobble-toned-like morphology [Figure 1]. Cells were treated with 10 ng/mL TGF-β1 for 24 h, after which the cell morphology was found to change into a spindle-like morphology. The morphological changes were reversed by adding 1α, 25(OH)$_2$D$_3$ or ICG-001 or alone or in combination before TGF-β1 treatment. The morphology of the cells was not significantly changed by simultaneous treatment with 1α, 25(OH)$_2$D$_3$ and ICG-001 compared to the control. Subsequently, we investigated whether 1α, 25(OH)$_2$D$_3$ regulated the protein levels of key EMT markers in TGF-β1-treated cells by western blotting analysis. The western blotting results showed that the expression of epithelial cell marker E-cad was significantly reduced with increasing TGF-β1 stimulation. Moreover, the expression of the mesenchymal cell marker α-SMA and extracellular matrix FN was positively correlated with TGF-β1 treatment. Following treatment (eg, with 1α, 25(OH)$_2$D$_3$, ICG-001, or both), we found that the combined treatment of 1α, 25(OH)$_2$D$_3$ and ICG-001 significantly decreased the expression of α-SMA and FN, but increased the expression of E-cad compared to the 1α, 25(OH)$_2$D$_3$- and ICG-001-treated groups [Figure 1]. Immunofluorescence analysis showed similar results, demonstrating that 1α, 25(OH)$_2$D$_3$ or ICG-001 inhibited TGF-β1-induced EMT, while 1α, 25(OH)$_2$D$_3$ had a synergistic effect when combined with ICG-001.

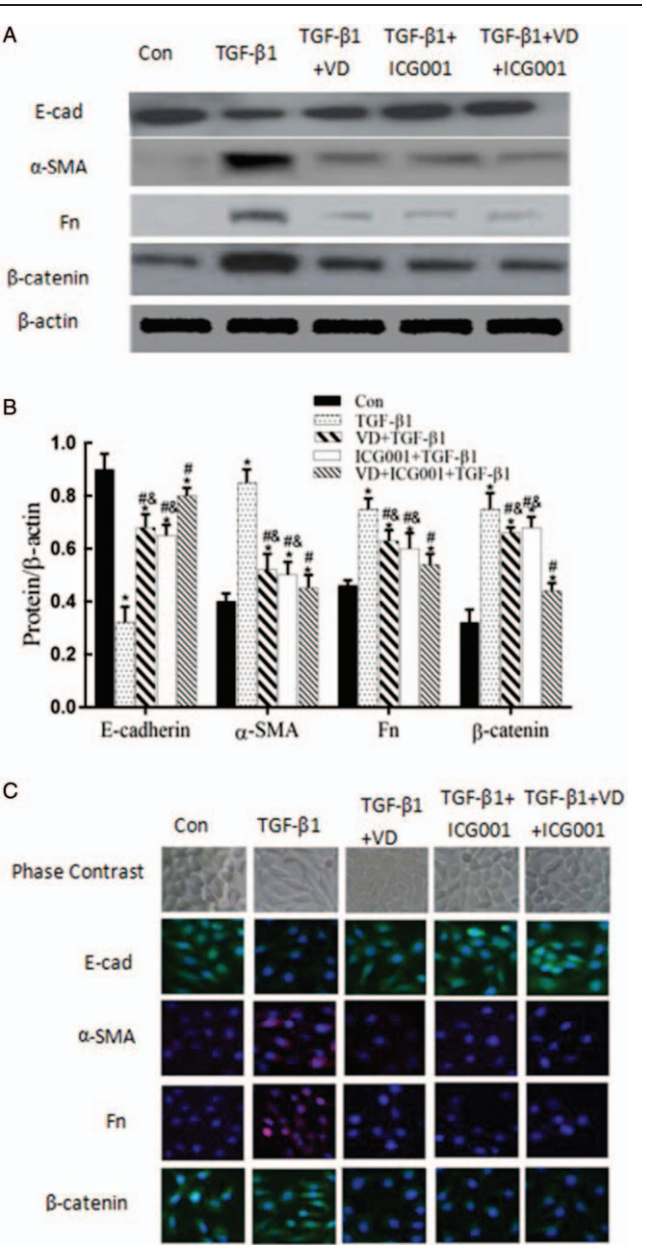

Figure 1: 1α, 25-dihydroxyvitamin D$_3$ and ICG-001 inhibit TGF-β1-induced epithelial-mesenchymal transition. (A) Representative western blots of E-cad, α-SMA, FN, and β-catenin compared with the β-actin control in the lysate of untreated and treated cells. (B) Quantitative analysis of (A) using relative densitometry intensity. Values are expressed as the mean ± SD, $^{*}P<0.01$ *vs.* Control group, $^{\#}P<0.01$ *vs.* TGF-β1 group, $^{\&}P<0.01$ *vs.* TGF-β1, 1α, 25-dihydroxyvitamin D$_3$ and ICG-001-treated group, $n=5$. (C) Phase-contrast images of alveolar epithelial cells untreated (Con) or treated with TGF-β1 (10 ng/mL) for 24 h, or treated with 1α, 25-dihydroxyvitamin D$_3$, ICG-001, or both in the presence of TGF-β1. Immunofluorescence images of E-cadherin, α-SMA, FN, and β-catenin staining in cells exposed to the respective treatments. α-SMA: α-Smooth muscle actin; Con: Control; E-cad: E-cadherin; FN: Fibronectin; TGF-β1: Transforming growth factor β1; VD: 1α, 25-Dihydroxyvitamin D$_3$.

1α, 25(OH)$_2$D$_3$ and ICG-001 act as negative regulators of the Wnt/β-catenin signaling pathway

To further explore the molecular mechanism by which 1α, 25(OH)$_2$D$_3$ suppressed TGF-β1-induced EMT, the transcriptional activity of the Wnt/β-catenin signaling

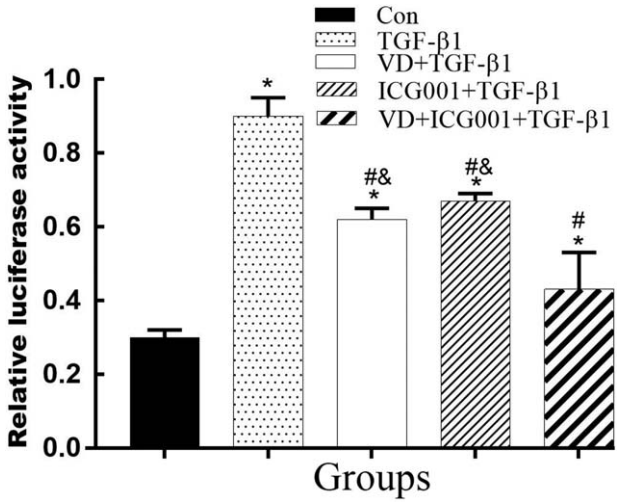

Figure 2: 1α, 25-dihydroxyvitamin D_3 and ICG-001 act as negative regulators of the Wnt/β-catenin signaling pathway. Interactions between β-catenin and LEF were shown using the Top-flash assay. The relative luciferase activity in cells transfected with the Fop-flash and Top-flash vectors are shown in the control, TGF-β1-treated, TGF-β1-1α, 25-dihydroxyvitamin D_3-treated, TGF-β1-ICG-001-treated, and TGF-β1-1α, 25-dihydroxyvitamin D_3-ICG-001-treated cells. Values are expressed as the mean ± SD. $^{*}P < 0.01$ *vs.* Control group, $^{\#}P < 0.01$ *vs.* TGF-β1 group, $^{\&}P < 0.01$ *vs.* TGF-β1, 1α, 25-dihydroxyvitamin D_3 and ICG001-treated group, $n = 5$. There was no statistical difference in TOP-flash activity between the control, TGF-β1-treated, TGF-β1-1α, 25-dihydroxyvitamin D_3-treated, TGF-β1-ICG-001-treated, and TGF-1α, 25-dihydroxyvitamin D_3- ICG-001-treated groups. Con: Control; TGF-β1: Transforming growth factor β1; VD: 1α, 25-Dihydroxyvitamin D_3.

pathway was investigated in-depth. The Wnt signaling pathway normally requires β-catenin to enter the nucleus, forming a complex with the transcription factor TCF/Lymphoid enhancer-binding factor(LEF) to initiate transcription of downstream regulatory genes. The transcriptional activity of endogenous β-catenin was measured using the Top/Fop Flash reporter assay, and the level of WNT pathway core protein β-catenin was determined using semi-quantitative analyses. We found that TGF-β1 markedly induced β-catenin-mediated transcriptional activity and increased β-catenin levels in alveolar epithelial cells. Following treatment with 1α, $25(OH)_2D_3$ or ICG-001 alone or in combination, the high levels of β-catenin-mediated TOP luciferase activity in the alveolar epithelial cells were downregulated [Figure 2]. This indicated that the transcription activity of TCF/LEF was decreased. Correspondingly, the expression level of β-catenin was reduced. A combination of 1α, $25(OH)_2D_3$ and ICG-001 inhibited the β-catenin signaling pathway more strongly than either one of the treatments alone, suggesting that 1α, 25 $(OH)_2D_3$ and ICG-001 negatively regulate the β-catenin pathway and the existence of a synergistic effect between 1α, $25(OH)_2D_3$ and ICG-001.

1α, 25(OH)$_2$D$_3$ and ICG-001 inhibit downstream transcription factors matrix metalloproteinase 9 (MMP-9) and Snail in the β-catenin signaling pathway

As a key transcription factor, Snail is also a downstream transcription factor in the Wnt/β-catenin pathway. As shown in the RT-qPCR results, we found that TGF-β1 increased the transcription level of Snail, which was inhibited after treatment with either 1α, $25(OH)_2D_3$ or

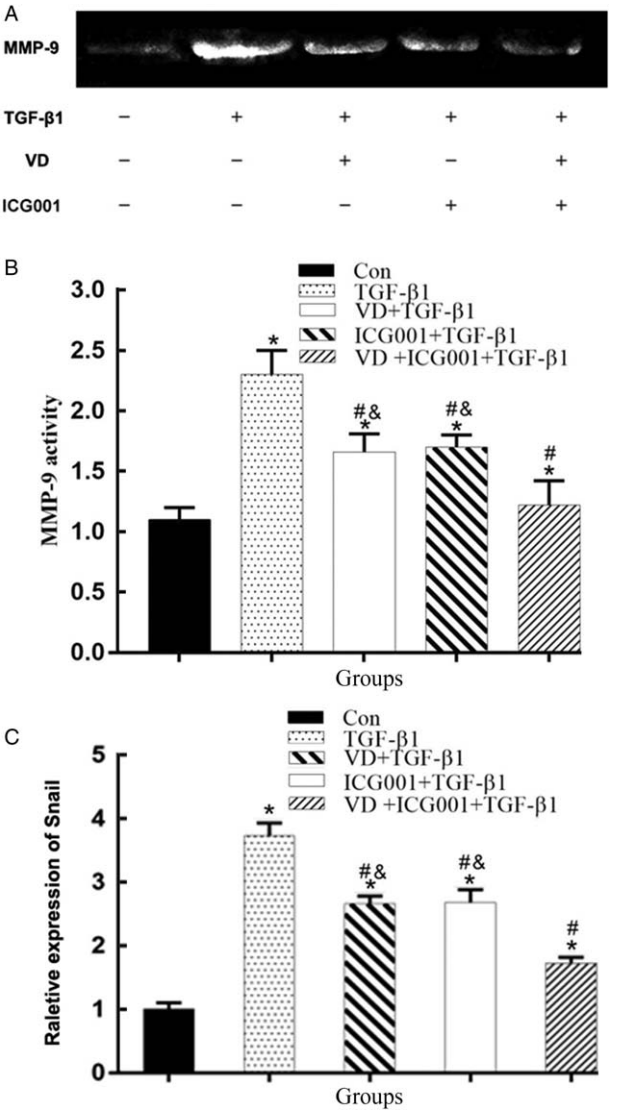

Figure 3: 1α, 25-dihydroxyvitamin D_3 and ICG-001 inhibit the downstream transcription factors MMP-9 and Snail in the β-catenin signaling pathway. (A) Representative MMP-9 gelatin zymography of supernatants from cells treated with the corresponding treatments. (B) Quantitative analysis of (A). Relative MMP-9 zymography intensity was calculated against the untreated medium. Values are expressed as the mean ± SD. $^{*}P < 0.01$ *vs.* Control group, $^{\#}P < 0.01$ *vs.* TGF-β1 group, $^{\&}P < 0.01$ *vs.* TGF-β1, 1α, 25-dihydroxyvitamin D_3 and ICG001-treated group, $n = 5$. (C) The expression of Snail in cells treated with the corresponding treatments was analyzed using real-time PCR. Values are expressed as the mean ± SD. $^{*}P < 0.01$ *vs.* Control group, $^{\#}P < 0.01$ *vs.* TGF-β1 group, $^{\&}P < 0.01$ *vs.* TGF-β1, 1α, 25-dihydroxyvitamin D_3 and ICG001-treated group, $n = 5$. Con: Control; MMP9: Matrix metalloproteinase 9; TGF-β1: Transforming growth factor β1; VD: 1α, 25-Dihydroxyvitamin D_3.

ICG001 alone or in combination [Figure 3C]. Due to the increased expression of MMP observed in EMT, we assessed the expression of MMP, specifically MMP-9, by gelatin zymography. Treatment of alveolar epithelial cells with TGF-β1 resulted in a significant increase in MMP-9 activity. After the administration of either 1α, $25(OH)_2D_3$ or ICG001 alone or in combination, TGF-β1-mediated MMP-9 activity was reduced [Figure 3A and 3B]. The RT-qPCR results indicated that Snail expression was significantly lower in the combination treatment group compared to the 1α, $25(OH)_2D_3$ and ICG-001 treatment groups. Meanwhile, the gelatin zymogram results showed

that the MMP-9 activity in the combination group was lower than that in either the 1α, $25(OH)_2D_3$ or ICG-001 groups. These results indicate that 1α, $25(OH)_2D_3$ and ICG-001 synergistically inhibit the downstream transcription factors MMP-9 and Snail in the β-catenin signaling pathway.

Discussion

Asthma affects more than 300 million people worldwide and is one of the most common chronic respiratory diseases.[15] Emerging evidence has suggested that the airway epithelium contributes to airway remodeling via the EMT.[16] Vitamin D is of particular concern in asthma due to its immunomodulatory functions.[17] Recent studies have shown that vitamin D deficiency is closely related to the development of asthma.[18] It has been clinically proven that vitamin D supplementation has a therapeutic effect on asthma.[19,20] However, the cellular and molecular mechanisms of vitamin D in airway remodeling in asthma have not yet been fully elucidated, and the use of vitamin D for the treatment of bronchial asthma remains controversial. In the present study, we investigated the effects of vitamin D on the EMT and its underlying mechanism to confirm whether vitamin D is able to effectively reduce airway remodeling, thereby providing therapeutic guidelines for the control of the pathogenesis of asthma using vitamin D.

The EMT plays an important role in the pathological process of airway remodeling in asthma. In EMT, TGF-β1 is an important cytokine.[21] Under the action of TGF-β1, epithelial cells lose their typical cell-cell junctions and cell polarity and acquire phenotypes that are more mesenchymal. This is characterized by the downregulation of epithelial markers E-cad and the upregulation of the mesenchymal markers α-SMA and FN.[22,23] Studies have shown that 1α, $25(OH)_2D_3$ regulates the EMT in epithelial cells, while 1α, $25(OH)_2D_3$ inhibits the EMT by inducing various target genes that encode cell adhesion and polarity proteins responsible for the epithelial phenotype by inhibiting key EMT inducers.[1] In our study using alveolar epithelial cells, we found that 1α, $25(OH)_2D_3$ increased the expression of E-cad and reduced the expression of α-SMA and FN in TGF-β1-treated alveolar epithelial cells. It was confirmed that 1α, $25(OH)_2D_3$ inhibits TGF-β1-induced EMT and may have a protective effect on airway remodeling.

TGF-β1 exerts its actions through different signal transduction pathways, including Smad, non-Smad, and β-catenin.[24] The classic Wnt/β-catenin pathway plays a key role in cell migration, cell proliferation, stem cell self-renewal, organogenesis, tissue homeostasis under physiological conditions, and tissue repair in injuries.[25] Under the stimulation of TGF-β1, β-catenin is activated and accumulated in the cytoplasm, enters the nucleus, and binds to the transcription factor TCF/LEF, forming a transcriptional activation complex, which promotes the transcription of EMT-inducing genes.[26,27] Thus, the β-catenin pathway regulates TGF-β1-induced EMT. Our previous study found that targeting β-catenin inhibited TGF-β1-induced EMT in renal epithelial cells by using ICG-001, a peptidomimetic small molecule that selectively blocks β-catenin-mediated transcriptional signaling.[14,28] In the present study, we showed that ICG-001 inhibited TGF-β1-induced EMT in alveolar epithelial cells and exerts a synergistic effect with 1α, $25(OH)_2D_3$.

To further investigate the effect of 1α, $25(OH)_2D_3$ on the β-catenin signaling pathway, we used the Top/Fop Flash luciferase reporter gene system, which is able to accurately detect the entry of β-catenin into the nucleus, and its combination with the transcription factor TCF/LEF.[29] Compared with the TGF-β1-treated group, the activity of the dual luciferase reporter plasmid decreased after treatment with 1α, $25(OH)_2D_3$ or ICG-001 alone. Therapy using a combination of 1α, $25(OH)_2D_3$ and ICG-001 further lowered the activity of the dual luciferase reporter plasmid. 1α, $25(OH)_2D_3$ was found to inhibit TGF-β1-induced EMT by negatively regulating the β-catenin/TCF signaling pathway in alveolar epithelial cells.

We also measured the expression of Snail and MMP-9, which are downstream target genes in Wnt/β-catenin pathway.[30,31] Snail is a zinc-finger transcription factor that represses epithelial genes and activates mesenchymal phenotype genes.[32] MMP-9 is a family of MMP whose primary function is to degrade and remodel the homeostasis of extracellular matrices.[33] Both Snail and MMP-9 are downstream products of the TGF-β1/β-catenin signaling pathway and participate in TGF-β1-induced EMT in epithelial cells. Treatment with 1α, $25(OH)_2D_3$ was found to significantly reduce the expression of Snail and MMP-9 upon stimulation with TGF-β1. It was further confirmed that 1α, $25(OH)_2D_3$ inhibited TGF-β1-induced EMT via the β-catenin signaling pathway.

In conclusion, the present study found that 1α, $25(OH)_2D_3$ inhibited TGF-β1-induced EMT in alveolar epithelial cells. More importantly, 1α, $25(OH)_2D_3$ was found to negatively

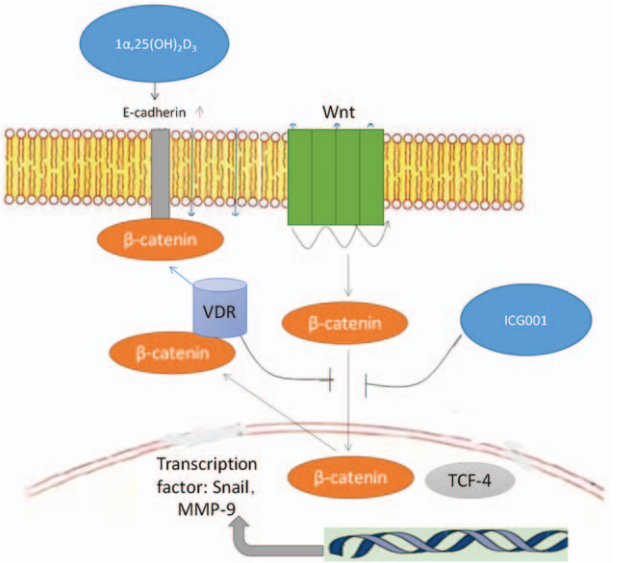

Figure 4: 1α, 25-dihydroxyvitamin D_3 promotes the translocation of β-catenin from the nucleus to the plasma membrane, and competes with T-cell transcription factor 4 (TCF4) for β-catenin binding. This results in the inhibition of the Wnt-β-catenin-TCF4 signaling pathway and a reduction in the expression of transcription factors (Snail and MMP-9) and synergy with ICG-001. MMP9: Matrix metalloproteinase 9; TGF-β1: Transforming growth factor β1.

regulates the Wnt/β-catenin/TCF signaling pathway [Figure 4]. The inhibitory effect of 1α, 25$(OH)_2D_3$ on EMT provides a basis for advances in airway remodeling in asthma. As such, our results provide an experimental basis for the prevention and treatment of asthma by 1α, 25$(OH)_2D_3$.

Acknowledgement

The authors would like to thank Editage (www.editage.cn) for English language editing.

This work was supported by the Natural Science Foundation of Shanxi Province of China [2013011055-1]; Fund Program for the Scientific Activities of Selected Returned Overseas Professionals in China {2016[366]}; Fund Program for the Scientific Activities of Selected Returned Overseas Professionals in Shanxi Province {[2017]144}; and Key Research and Development Program (International Scientific and Technological cooperation) of Shanxi Province.

Conflicts of interest

None.

References

1. Larriba MJ, García de Herreros A, Muñoz A. Vitamin D and the epithelial to mesenchymal transition. Stem Cells Int 2016;2016:6213872. doi: 10.1155/2016/6213872.
2. Zhu X, Li Q. Epithelial mesenchymal transition in airway remodeling of asthma and its molecular regulation. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2018;43:566–570. doi: 10.11817/j.issn.1672-7347.2018.05.016.
3. Chi L, Xiao Y, Zhu L, Zhang M, Xu B, Xia H, *et al*. microRNA-155 attenuates profibrotic effects of transforming growth factor-beta on human lung fibroblasts. J Biol Regul Homeost Agents 2019;33:1415–1424. doi: 10.23812/19-41A.
4. Kwak HJ, Park DW, Seo JY, Moon JY, Kim TH, Sohn JW, *et al*. The Wnt/beta-catenin signaling pathway regulates the development of airway remodeling in patients with asthma. Exp Mol Med 2015;47:e198. doi: 10.1038/emm.2015.91.
5. Shin MH, Lee Y, Kim MK, Lee DH, Chung JH. UV increases skin-derived 1alpha,25-dihydroxyvitamin D3 production, leading to MMP-1 expression by altering the balance of vitamin D and cholesterol synthesis from 7-dehydrocholesterol. J Steroid Biochem Mol Biol 2019;195:105449. doi: 10.1016/j.jsbmb.2019.105449.
6. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266–281. doi: 10.1056/NEJMra070553.
7. Plum LA, DeLuca HF. Vitamin D, disease and therapeutic opportunities. Nat Rev Drug Discov 2010;9:941–955. doi: 10.1038/nrd3318.
8. Campbell FC, Xu H, El-Tanani M, Crowe P, Bingham V. The yin and yang of vitamin D receptor (VDR) signaling in neoplastic progression: operational networks and tissue-specific growth control. Biochem Pharmacol 2010;79:1–9. doi: 10.1016/j.bcp.2009.09.005.
9. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. Nat Rev Cancer 2014;14:342–357. doi: 10.1038/nrc3691.
10. Ramirez AM, Wongtrakool C, Welch T, Steinmeyer A, Zügel U, Roman J. Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. J Steroid Biochem Mol Biol 2010;118:142–150. doi: 10.1016/j.jsbmb.2009.11.004.
11. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007;7:684–700. doi: 10.1038/nrc2196.
12. Huang Y, Wang L, Jia XX, Lin XX, Zhang WX. Vitamin D alleviates airway remodeling in asthma by down-regulating the activity of Wnt/beta-catenin signaling pathway. Int Immunopharmacol 2019;68:88–94. doi: 10.1016/j.intimp.2018.12.061.
13. Hlaing SM, Garcia LA, Contreras JR, Norris KC, Ferrini MG, Artaza JN. 1,25-Vitamin D3 promotes cardiac differentiation through modulation of the WNT signaling pathway. J Mol Endocrinol 2014;53:303–317. doi: 10.1530/JME-14-0168.
14. Hao S, He W, Li Y, Ding H, Hou Y, Nie J, *et al*. Targeted inhibition of beta-catenin/CBP signaling ameliorates renal interstitial fibrosis. J Am Soc Nephrol 2011;22:1642–1653. doi: 10.1681/ASN.2010101079.
15. Morrill MS. The effects of maternal employment on the health of school-age children. J Health Econ 2011;30:240–257. doi: 10.1016/j.jhealeco.2011.01.001.
16. Yang ZC, Yi MJ, Ran N, Wang C, Fu P, Feng XY, *et al*. Transforming growth factor-beta1 induces bronchial epithelial cells to mesenchymal transition by activating the Snail pathway and promotes airway remodeling in asthma. Mol Med Rep 2013;8:1663–1668. doi: 10.3892/mmr.2013.1728.
17. Ali NS, Nanji K. A review on the role of vitamin D in asthma. Cureus 2017;9:e1288. doi: 10.7759/cureus.1288.
18. Berraies A, Hamzaoui K, Hamzaoui A. Link between vitamin D and airway remodeling. J Asthma Allergy 2014;7:23–30. doi: 10.2147/JAA.S46944.
19. Agrawal T, Gupta GK, Agrawal DK. Vitamin D supplementation reduces airway hyperresponsiveness and allergic airway inflammation in a murine model. Clin Exp Allergy 2013;43:672–683. doi: 10.1111/cea.12102.
20. Korn S, Hübner M, Jung M, Blettner M, Buhl R. Severe and uncontrolled adult asthma is associated with vitamin D insufficiency and deficiency. Respir Res 2013;14:25. doi: 10.1186/1465-9921-14-25.
21. Hackett TL. Epithelial-mesenchymal transition in the pathophysiology of airway remodelling in asthma. Curr Opin Allergy Clin Immunol 2012;12:53–59. doi: 10.1097/ACI.0b013e32834ec6eb.
22. Li YZ, Peng X, Ma YH, Li FJ, Liao YH. Matrine suppresses lipopolysaccharide-induced fibrosis in human peritoneal mesothelial cells by inhibiting the epithelial-mesenchymal transition. Chin Med J (Engl) 2019;132:664–670. doi: 10.1097/CM9.0000000000000127.
23. Chen SY, Du Y, Song J1. MicroRNA-340 inhibits epithelial-mesenchymal transition by impairing ROCK-1-dependent Wnt/ β -catenin signaling pathway in epithelial cells from human benign prostatic hyperplasia. Chin Med J (Engl) 2018;131:2008–2012. doi: 10.4103/0366-6999.238145.
24. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. Cell Res 2009;19:156–172. doi: 10.1038/cr.2009.5.
25. Nusse R, Clevers H. Wnt/beta-catenin signaling, disease, and emerging therapeutic modalities. Cell 2017;169:985–999. doi: 10.1016/j.cell.2017.05.016.
26. Rao TP, Kuhl M. An updated overview on Wnt signaling pathways: a prelude for more. Circ Res 2010;106:1798–1806. doi: 10.1161/CIRCRESAHA.
27. Baarsma HA, Konigshoff M. 'WNT-er is coming': WNT signalling in chronic lung diseases. Thorax, 2017 2017;72:746–759. doi: 10.1136/thoraxjnl-2016-209753.
28. Henderson WR Jr, Chi EY, Ye X, Nguyen C, Tien YT, Zhou B, *et al*. Inhibition of Wnt/beta-catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. Proc Natl Acad Sci U S A 2010;107:14309–14314. doi: 10.1073/pnas.1001520107.
29. Su J, Zhang A, Shi Z, Ma F, Pu P, Wang T, *et al*. MicroRNA-200a suppresses the Wnt/beta-catenin signaling pathway by interacting with beta-catenin. Int J Oncol 2012;40:1162–1170. doi: 10.3892/ijo.2011.1322.
30. Liu Y. New insights into epithelial-mesenchymal transition in kidney fibrosis. J Am Soc Nephrol 2010;21:212–222. doi: 10.1681/ASN.2008121226.
31. Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. Am J Physiol Lung Cell Mol Physiol 2010;298:L715–L731. doi: 10.1152/ajplung.00361.2009.
32. Lamouille S, Xu J, Derynck R. Molecul1ar mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014;15:178–196. doi: 10.1038/nrm3758.
33. Qorri B, Kalaydina RV, Velickovic A, Kaplya Y, Decarlo A, Szewczuk MR. Agonist-biased signaling via matrix metalloproteinase-9 promotes extracellular matrix remodeling. Cells 2018;7:E117. doi: 10.3390/cells7090117.

How to cite this article: Xiong XR, Tian XL, Huo RJ, Dong YT, Liu D, Bai JC, Qi YF, Tian XR. 1α, 25-dihydroxyvitamin D_3 inhibits transforming growth factor β1-induced epithelial-mesenchymal transition via β-catenin pathway. Chin Med J 2020;133:1298–1303. doi: 10.1097/CM9.0000000000000830