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Vitamin D attenuates epithelial-mesenchymal transition of renal tubular epithelial cells in infant rats with hypothyroidism via the Traf6/TAK1 signalling pathway.

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Keywords: vitamin D; hypothyroidism; kidney injuries; epithelial-mesenchymal transition; TRAF6 protein; MAP3K7 protein (TAK1); signal transduction.

Abstract. In the hypothyroidism(HT) state, renal hemodynamics is disordered, oxidative stress is intensified, and inflammatory factors are activated. Vitamin D (VD) not only regulates calcium and phosphorus metabolism, but its active form (1,25-dihydroxyvitamin D) could also exert anti-inflammatory and anti-fibrotic effects by binding with the vitamin D receptor (VDR) widely distributed in the kidney. This study aimed to elucidate the impact of VD on the epithelial-mesenchymal transition (EMT) of renal tubular epithelial cells in HT-induced renal injury in young rats, as well as its regulatory mechanism involving the tumor necrosis factor receptor-associated factor 6 (Traf6)/transforming growth factor-β activated kinase 1 (TAK1) pathway. An HT model in young rats was established via propylthiouracil (PTU) gavage, and a functional rescue experiment was conducted by overexpressing TAK1 (pcDNA3.1-TAK1). The animals were divided into five groups: normal, HT, low-dose VD (HT+VD-L), high-dose VD (HT+VD-H), and HT+VD-H+pcDNA3.1-TAK1 (HT+VD-H+pc). Each group consisted of 10 rats. Serum creatinine (Scr) and blood urea nitrogen (BUN) levels were measured using an automatic biochemical analyzer. Renal apoptosis (TUNEL), TGF-β1/α-SMA/E-cadherin (immunohistochemistry), and Bcl-2/Bax/Traf6/TAK1/p-TAK1 (Western blot) expressions were assessed in renal tissue. VD significantly reduced Scr and BUN levels in the serum of HT young rats, downregulated renal tissue apoptosis, decreased TGF-β1 and α-SMA expressions, and upregulated E-cadherin expression. Additionally, VD inhibited Traf6, p-TAK1, and Bax expressions while increasing Bcl-2 expression. All differences were statistically significant.

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Vitamina D atenúa la transformación mesenquímica-epitelial de células epiteliales tubulares renales a través de la vía de señalización Traf6/TAK1 en ratas bebé con hipotiroidismo.

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Palabras clave: vitamina D; hipotiroidismo; daño renal; transición epitelio-mesénquima; proteína TRAF6; proteína MAP3K7 (TAK1); transducción de señales.

Resumen:El hipotiroidismo (HT) altera la hemodinámica renal, el estrés oxidativo y la inflamación. La vitamina D (VD) regula calcio/fósforo; su forma activa ejerce efectos antiinflamatorios/antifibróticos vía el receptor VD renal. Este estudio se centra en observar y aclarar el efecto de la VD sobre la transición epitelial-mesenquimal (EMT) de las células epiteliales tubulares renales en la lesión renal inducida por el HT en ratas jóvenes, así como su mecanismo regulador que involucra la vía del receptor del factor de necrosis del tumor 6 (traf 6) / y del factor de crecimiento transformante β activado por la quinasa 1(TAK1). El modelo de HT se estableció en ratas jóvenes mediante el método de alimentación por sonda de propil-tiouracilo (PTU) y se realizó un experimento de rescate funcional mediante la sobreexpresión de TAK1 (pcDNA3.1-TAK1). Los animales fueron divididos en cinco grupos: normal, HT, VD de baja dosis (HT+VD-L), VD de alta dosis (HT+VD-H), HT+VD-H + PC DNA 3.1-tak 1 (HT+VD-H + PC). Cada grupo estuvo compuesto por 10 ratas. Se determinaron los niveles de creatinina sérica (Scr) y nitrógeno de urea en sangre (BUN) con un analizador bioquímico automático. Se evaluó la expresión de la apoptosis renal (TUNEL), TGF-β1/α-SMA/E-Calcinina (immunohistoquímica) y Bcl-2/Bax/traf 6/tak 1/p-tak 1 (Western blot) en el tejido renal. VD redujo significativamente los niveles de SCR y BUN en suero de ratones de HT, redujo la apoptosis en tejido renal, redujo la expresión de TGF-β1 y α-SMA y aumentó la expresión de E-calciferina. Además, la VD inhibe la expresión de Traf6, p-TAK1 y Bax, mientras que aumenta la expresión de Bcl-2.

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INTRODUCTION

Hypothyroidism (HT) is a common disease of the endocrine system, which can occur at all ages. It occurs in fetuses, newborns and infants, which could directly affect the development of children's nervous system and skeletal system, directly lead to short stature or permanent mental retardation, and bring unpredictable harm to patients' families [1]. Studies have shown that HT directly causes changes in renal hemodynamics and glomerular filtration performance[2,3], which directly leads to certain renal interstitial fibrosis. Consequently HT patients are often accompanied by certain renal dysfunction, but the pathological mechanism of HT renal injury has not been fully clarified, and there is still a lack of specific drugs in clinic, especially for infants with HT-induced renal injury. Studies have shown that abnormal apoptosis in renal tissue induced by inflammatory stress plays an important role in the progression of the disease. Adan et al. [4]

showed that the expression of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) is increased in the serum of patients with insufficient thyroxine synthesis, and it was correlated with the expression of apoptosis-inhibiting protein B cell lymphoma-2 (Bcl-2). It was positively correlated with the expression of Bcl-2-related X protein (Bax). Narilla et al. [5], showed that the levels of translation and transcription of transforming growth factor-β 1 (TGF-β1) and α-smooth muscle actin (α-SMA) in the serum of patients with hypothyroidism were significantly increased. However, the transcription and translation levels of E-cadherin decreased significantly, and the patients exhibited specific pathological changes indicative of epithelial-mesenchymal transition (EMT) in renal tubules. Tumor necrosis factor receptor-associated factor 6 (Traf6)/ transforming growth factor β-activated kinase 1 (TAK1) signal is an endogenous pathway closely related to inflammatory stress and cell fibrosis. Wang et al. [6] demonstrated that inhibiting the TRAF6/TAK1 pathway and reducing the phosphorylated TAK1 (p-TAK1) expression in the HK-2 renal tubular fibrosis model induced by high glucose significantly suppressed myofibroblast marker α-SMA expression in HK-2 cells, thereby blocking cell fibrosis progression. Many clinical data and basic experimental data at home and abroad shows that vitamin D (VD) is not only a simple fat-soluble vitamin, but also a steroid hormone closely related to physiological processes such as immune regulation, cell stress and apoptosis. Therefore, in this study, the HT-model of young rats was established by intragastric administration of propylthiouracil (PTU). Based on the TRAF6/TAK1 signal, a function rescue experiment was conducted by overexpressing TAK1 (pc DNA3.1-TAK1). The effect of VD on the EMT of renal tubular epithelial cells in young rats with HT is discussed, aiming to provide a reliable direction for the clinical treatment of the disease.

MATERIALS AND METHODS

Test animals, main reagents and instruments

Fifty 3-week-old male SD rats with SPF cleanliness, weighing 45g～55g, were used in the experiment after being fed adaptively by the specialized personnel in our hospital at a temperature of 20℃～25℃ and a humidity of 55～60%)% for 12h. Experimental reagent vitamin D drops (Star Shark) (VD, 400U*36 pills, Star shark pharmaceutical (Xiamen) Co., Ltd., Sinopharm zhunzi H35021450); PTU (purity ≥99.9%) was purchased from sigma company in USA, and overexpression TAK1(pc DNA3.1-TAK1) and negative control pc DNA3.1-TAK1-NC were purchased from anbote genetic engineering technology Co., Ltd, Beijing. Other reagents and equipment were: Phosphate buffer solution (PBS), formaldehyde, xylene, neutral resin (Beijing Suolaibao Biotechnology Co., Ltd.), rabbit anti-rat Traf6, TAK1 and p-TAK1, Bcl-2, Bax, glyceraldehyde triphosphate dehydrogenase (GAPDH) monoclonal antibody (Shanghai Biyuntian Biotechnology Co., Ltd.), Immunofluorescence, immunohistochemistry and hematoxylin and eosin (HE) staining kit (Shanghai Wohong Biotechnology Co., Ltd.), Protein western blot kit, deoxynucleotide terminal-mediated nick end labeling (TUNEL) kit (Nanjing Senbeijia Biotechnology Co., Ltd.), enzyme-linked immunosorbent assay kit (Nanjing Jiancheng Biotechnology Co., Ltd.). Tissue scissors, hemostatic forceps and other surgical instruments (Jinhua Yidi Medical Equipment Co., Ltd., Jinhua City, Zhejiang Province), Image Quant LAS4010 gel imaging system (GE Company, USA), CFX96 real-time fluorescent quantitative polymerase chain reaction (quantitative real-time polymerase chain reaction, QRT-PCR) amplifier (Bio-Rad Company, USA), Leica DMI6000B microscope (Leica Company, Germany), Z-5000 spectrophotometer (Hitachi Company, Japan), CK-150 high-speed freezing centrifuge (Sigma Company, USA).

Replication of the HT rat model

According to the method introduced by Santos et al.[7], a propylthiouracil (PTU) solution was prepared with normal saline at a concentration of 0.5% (5 mg/mL). Rats were treated by gavage with 10 mL/kg PTU solution every day, and at the same time, their weigh was recorded, and the dosage of PTU was adjusted every week according to their weight. After four weeks of continuous gavage, 2mL of tail vein blood of rats in each group was taken to detect thyroid stimulating hormone (thyroid-stimulating hormone, TSH) and total thyroxine (TT4), compared with the normal group, the serum TSH level of HT rats increased significantly, while TT4 level decreased significantly, which indicated that the model was successful.

Packet processing

After adaptive feeding, rats were randomly divided into a normal group, HT group, VD low-dose (HT+VD-L) group, VD high-dose (HT+VD-H) group and HT+VD-H+PCDNA3.1-Tak1 (HT+VD-H+PC) group, with 10 rats in each group. Rats in the HT+VD-L group were injected with 5×10^9 pfu/mL of pc DNA3.1-TAK1-NC via tail vein every day, and treated with 0.25 mg/kg of VD by gavage. Rats in the HT+VD-H group were injected with 5×10^9 pfu/mL of pc DNA3.1-TAK1-NC via the tail vein every day, and perfused with 1 mg/kg of VD. Rats in the HT+VD-H+pc group were injected with 5×10^9 pfu/mL of pc DNA3.1-TAK1 by the tail vein every day, and treated with 1 mg/kg of VD by gavage. Rats in the normal group were injected with 5×10^9 pfu/mL of pc DNA3.1-TAK1-NC by the tail vein every day, and treated with the same dose of normal saline by gavage for four weeks. Rats in the HT, HT+VD-L, HT+VD-H and HT+VD-H+pc groups still need to be treated with PTU to maintain the decline of thyroid function in experimental animals. During the experiment, rats were raised in a single cage, with access to drinking water and food.

Sample collection

After the last drug administration, 24-hour urine samples were collected from rats in each group to detect the levels of 24-hour urinary albumin (UAlb) in the offspring rats. Subsequently, the rats were anesthetized with an intraperitoneal injection of 30 mg/kg pentobarbital sodium. Then, 5 mL of blood was collected from the heart and placed in a benchtop refrigerated centrifuge for 15 minutes to obtain serum for testing. The rats were euthanized by cervical dislocation. Under a microscope and on a sterile operating table, the kidney tissues of the offspring rats were dissected.

Thyroid and renal function of rats in each group

According to the requirements of the ELISA kit, corresponding solutions were added to the standard wells and sample wells, and blank wells were set up. After antibody coating, plate washing, and reaction termination, the OD450 values of each well were measured using a multifunctional microplate reader. A standard curve was plotted, and the concentrations of TSH and TT4 in animal serum were calculated. At the same time, the levels of UAlb, serum creatinine (Scr), and blood urea nitrogen (BUN) in rats from each group were detected using an automatic biochemical analyzer.

Apoptosis in renal tissue of rats in each group

Partial renal tissue from rats in each group was taken and fixed, sliced, and washed according to the preliminary requirements of the TUNEL kit. TUNEL working solution was added, and the samples were incubated at 37°C in the dark. After dehydration and mounting, microscopic examination was performed. The apoptosis rate in renal tissue was statistically analyzed using the Image-Pro 6.2 software.

PCR detection of gene expression in renal tissue of rats in each group

Partial renal tissue samples from rats were collected, and an appropriate amount of Trizol lysis solution was added. Total RNA was extracted according to the requirements of the Trizol kit. After determining its concentration using a Z-5000 spectrophotometer, cDNA was synthesized using the Prime Script™ kit. qRT-PCR amplification was performed under the following conditions: initial denaturation at 95°C for 5 seconds, denaturation at 95°C for 5 seconds, annealing at 60°C for 60 seconds, for 40 cycles. The primer sequences are shown in Table 1. The relative expression levels of each target gene were represented by $2^{-\Delta\Delta CT}$.

Immunofluorescence detection of TNF-α and IL-6 expression in renal tissue of rats in each group

Partial renal tissue samples from rats were collected and subjected to fixation, embedding, slicing, microwave repair, and incubation in 3% hydrogen peroxide. After washing with PBS, the samples were blocked with goat serum and then incubated with primary antibodies against TNF-α and IL-6 (diluted 1:500) at 4°C overnight in the dark. The next day, fluorescent secondary antibodies were added, and the samples were incubated in the dark. Observation was performed under a microscope.

Immunohistochemical experiment to detect the expression of E-cadherin, α-SMA, and TGF-β1 in renal tissue

Partial renal tissue samples from rats were fixed, embedded, and sliced, followed by immunohistochemical staining according to the kit instructions. Primary antibodies against E-cadherin, α-SMA, and TGF-β1 (all diluted 1:500) and secondary antibodies (diluted 1:1500) were added. Observation was performed under a microscope, and statistical analysis was conducted using the Image J image processing software.

Expression levels of Bcl-2, Bax, Traf6, TAK1, and p-TAK1 in renal tissue of rats in each group

renal tissue from rats in each group was placed in lysis buffer at 4°C for 30 minutes, followed by centrifugation and dilution of the supernatant. Total protein was extracted from the samples using RIPA lysate. Fifty micrograms of protein samples were loaded for electrophoresis, followed by membrane transfer and blocking. Primary antibodies against Bcl-2, Bax, Traf6, TAK1, and p-TAK1 (all diluted 1:1000) and secondary antibodies (diluted 1:5000) were added, and the samples were incubated at room temperature. After color development for 30 minutes, GAPDH was used as a reference to analyze the grayscale values of the target proteins.

Statistical analysis

The SPSS19.0 software (International Business Machines Corporation, New York, USA) and Graphpad5.01 software (GraphPad Software Inc., San Diego, CA, USA) were used for statistical analysis of data, and the data were expressed as mean±SD. The t-test was conducted to compare the two groups, and the comparison between multiple groups was conducted by one-way analysis of variance, with $p<0.05$ indicating statistical significance.

RESULTS

Comparison of thyroid function of rats in each group

The results of the enzyme-linked adsorption kit showed that compared with normal rats, the serum TT4 levels in the HT, HT+VD-L, HT+VD-H and HT+VD-H+pc groups were reduced significantly, while the TSH level increased significantly. Compared with the HT group, the serum TT4 levels in the HT+VD-L group, HT+VD-H group, and HT+VD-H+pc group increased significantly, while the TSH level decreased significantly.

Compared with the HT+VD-L group, the level of TT4 in the serum of the HT+VD-H group was significantly higher, while the level of TSH was significantly lower. Compared with the HT+VD-H group, the level of TT4 in the serum of the HT+VD-H+pc group decreased significantly, and the level of TSH increased significantly, with statistical significance (all $p<0.05$) (Fig. 1).

Changes in renal function in each group of rats

The results showed that, compared with the normal group, the contents of UAlb, Scr, and BUN in serum of rats in the HT, HT+VD-L, HT+VD-H, and HT+VD-H+pc groups were significantly higher. In the HT+VD-L group, HT+VD-H group and HT+VD-H+pc group, the contents of UAlb, Scr and BUN in serum decreased significantly. Compared with the HT+VD-L group, the contents of UAlb, Scr and BUN in the serum of the HT+VD-H group decreased significantly (Fig. 2).

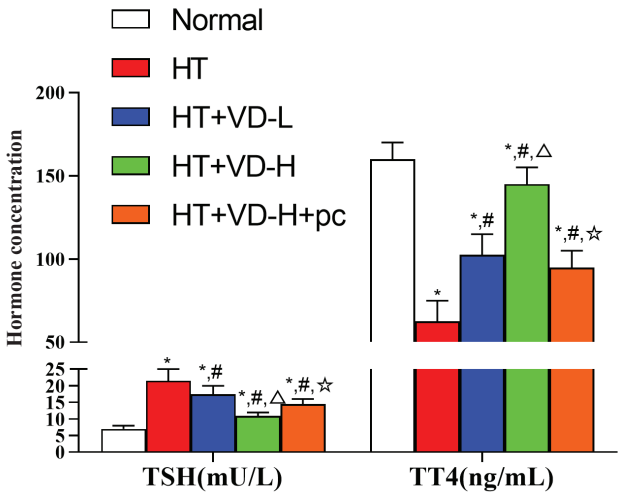

Fig. 1. Changes of Thyroid stimulating hormone (TSH) and Total thyroxine (TT4) in rats in each group. Note: One-way ANOVA. Compared with the Normal group, *: $p<0.05$ compared with HT group; #:$p<0.05$ compared with HT+VD-L group; Δ:$p<0.05$ compared with HT+VD-H group; ☆: $p<0.05$).

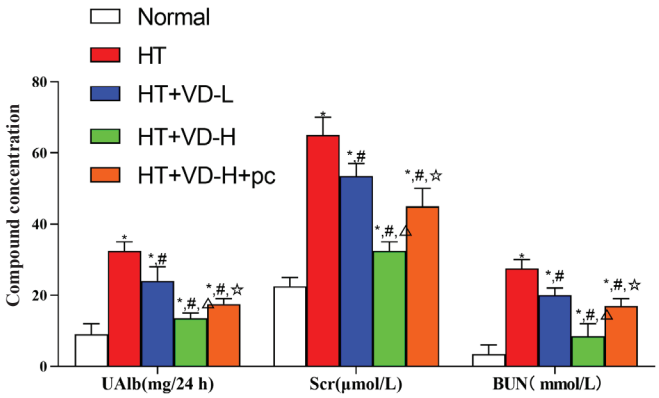

Fig. 2. Changes of 24-hour urinary albumin (UAlb), Serum creatinine (Scr) and Blood urea nitrogen (BUN) in rats of each group (Note: One-way ANOVA. Compared with the Normal group, *:$p<0.05$ compared with the HT group; #:$p<0.05$ compared with HT+VD-L group; Δ:$p<0.05$ compared with HT+VD-H group; ☆:$p<0.05$)

Apoptosis in the renal tissue of rats in each group

TUNEL staining results showed that compared with the normal group, the apoptosis rate in the kidney tissue of rats in the HT, HT+VD-L, HT+VD-H and HT+VD-H+pc groups was significantly higher. Compared with the HT group, the cells in the kidney tissue of rats in HT+VD-L, HT+VD-H and the HT+VD-H+pc groups were significantly higher. The apoptosis rate in the kidney tissue of the HT+VD-H group decreased significantly, and compared with the HT+VD-H group, the apoptosis rate in the kidney tissue of the HT+VD-H+pc group increased significantly, with statistical significance (all $p<0.05$) (Fig. 3).

Expression of related genes in the renal tissue of rats in each group

The results of PCR showed that compared with the normal group, the mRNA expressions of TNF-α, IL-6, α-SMA, TGF-β1, Traf6 and TAK1 in the renal tissues of the HT, HT+VD-L, HT+VD-H and HT+VD-H+pc groups were significantly increased, while the mRNA expression of E-cadherin was significantly decreased.

Compared with the HT group, the mRNA expressions of TNF-α, IL-6, α-SMA, TGF-β1, Traf6, and TAK1 in renal tissue of the HT+VD-L, HT+VD-H, and HT+VD-H+pc groups decreased significantly, while the mRNA expression of E-cadherin increased significantly.

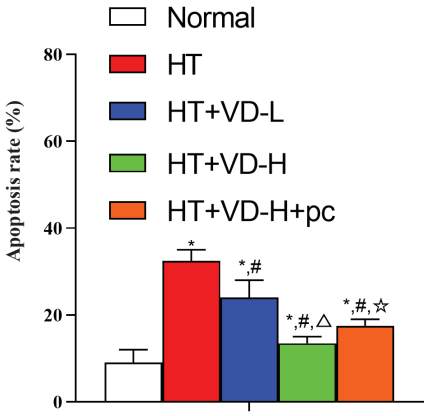

Fig. 3. The cell apoptosis of renal tissue and changes of renal function in rats of each group. Note: One-way ANOVA.Compared with the Normal group, *:p<0.05 compared with the HT group; #:p<0.05 compared with HT+VD-L group; △:p<0.05 compared with HT+VD-H group; ☆:p<0.05).

Compared with the HT+VD-L group, the mRNA expression of TNF-α, IL-6, α-SMA, TGF-β1, Traf6, and TAK1 in the HT+VD-H group decreased significantly, while the mRNA expression of E-cadherin increased significantly. Compared with the HT+VD-H group, the mRNA expressions of TNF-α, IL-6, α-SMA, TGF-β1, Traf6 and TAK1 in the kidney tissue of the HT+VD-H+pc group were significantly increased, while the mRNA expression of E-cadherin was significantly decreased, with statistical significance (all p<0.05), (Fig. 4).

Expression of TNF-α and IL-6 in the renal tissue of rats in each group

The results of immunofluorescence staining showed that compared with the normal group, the fluorescence intensity and expression of TNF-α and IL-6 in the renal tissue of the HT, HT+VD-L, HT+VD-H and HT+VD-H+pc groups increased significantly. Compared with HT+VD-L group, the fluorescence intensity of TNF-α and IL-6 in the HT+VD-H group decreased obviously. Compared with the HT+VD-H group, the fluorescence intensity of TNF-α and IL-6 in the HT+VD-H+pc group increased significantly (Fig. 5).

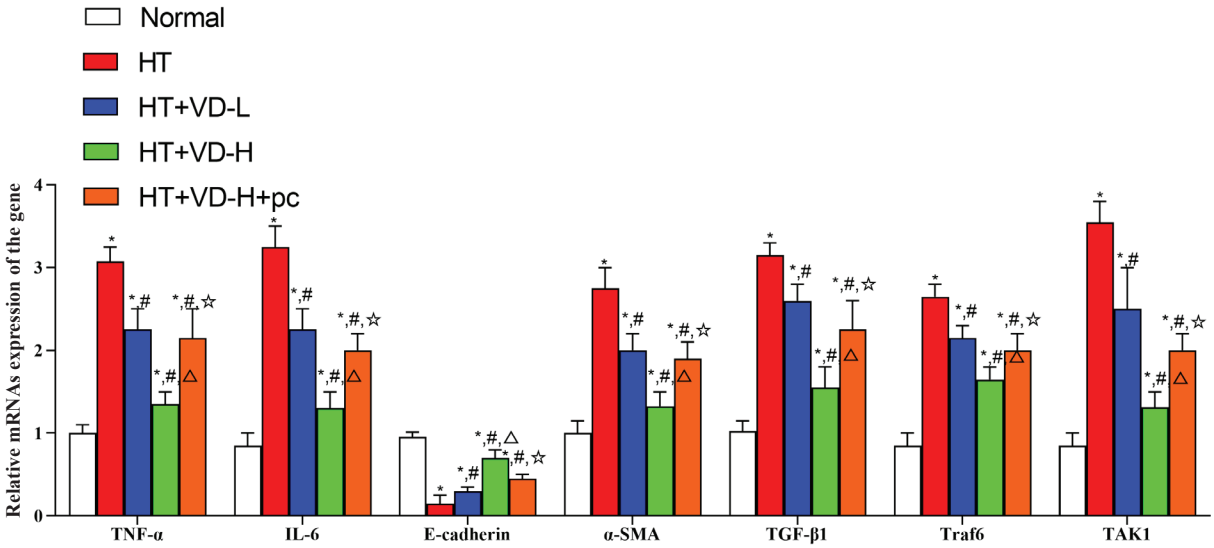

Fig. 4. The expression of related genes in renal tissue in rats of each group (Note: One-way ANOVA. Compared with the Normal group, *:p<0.05 compared with the HT group; #:p<0.05 compared with HT+VD-L group; △:p<0.05 compared with HT+VD-H group; ☆:p<0.05).

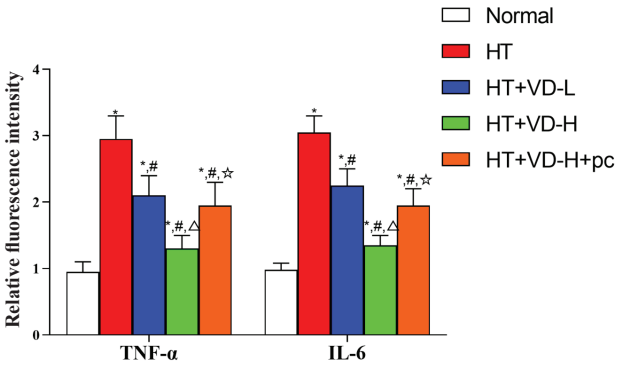

Fig. 5. The expression of TNF-α and IL-6 in renal tissue in rats of each group (Note: One-way ANOVA. Compared with the Normal group, *:p<0.05 compared with the HT group; #:p<0.05 compared with HT+VD-L group; △:p<0.05 compared with HT+VD-H group; ☆:p<0.05).

Expression of E-cadherin, α-SMA and TGF-β1 in the renal tissue of rats in each group

Immunohistochemical results showed that, compared with the normal group, the percentage of α-SMA and TGF-β1 positive cells in the renal tissue of rats in the HT, HT+VD-L, HT+VD-H and HT+VD-H+pc groups increased significantly. In contrast, the percentage of E-cadherin-positive cells decreased significantly, and the expressions of α-SMA, TGF-β1, and E-cadherin increased significantly. Compared with the HT group, the percentage of α-SMA and TGF-β1 positive cells in kidney tissue of the HT+VD-L, HT+VD-H and the HT+VD-H+pc groups decreased significantly, and the percentage of E-cadherin positive cells increased significantly. The expressions of α-SMA and TGF-β1 decreased significantly, and the expression of E-cadherin increased significantly, which was similar to that of HT. In the HT+VD-H group, the percentage of α-SMA and TGF-β1 positive cells in renal tissue decreased significantly, and the percentage of E-cadherin positive cells increased significantly. The expressions of α-SMA and TGF-β1 decreased significantly, and the expression of E-cadherin increased significantly. Compared with the HT+VD-H group, in the HT+VD-H+pc group, the percentage of α-SMA and TGF-β1 positive cells in rat kidney tissue increased significantly. In contrast, the percentage of E-cadherin-positive cells decreased significantly, and the expressions of α-SMA and TGF-β1 increased significantly, with statistical significance (all $p<0.05$)(Fig. 6).

Expression of Bcl-2, Bax, Traf6 and p-TAK1 in renal tissue of rats in each group

Western blot results showed that compared with the normal group, the expressions of Bax, Traf6, and p-TAK1 in renal tissue of rats in HT, HT+VD-L, HT+VD-Hand, and HT+VD-H+pc groups were significantly increased, while the expression of Bcl-2 was significantly decreased. The expressions of Bax, Traf6 and p-TAK1 in the renal tissue of rats in the HT+VD-L, HT+VD-H and HT+VD-H+pc groups decreased significantly, while the expression of Bcl-2 increased significantly. Compared with the HT+VD-L group, the expressions of Bax, Traf6 and p-TAK1 in renal tissue of rats in the HT+VD-H group decreased significantly. In the HT+VD-H+pc group, the expressions of Bax, Traf6 and p-TAK1 in the renal tissue of rats were significantly increased, while the expression of Bcl-2 was significantly decreased, with statistical significance (all $p < 0.05$), while the expression of TAK1 was not statistically significantly different ($p>0.05$), (Fig. 7).

Mechanism of VD alleviating renal injury in HT rats

When HT occurs in rats, Traf6/TAK1 signal in renal tissue is activated, the level of p-TAK1 is increased, the level of transcription and translation of related inflammatory and interstitial fibrosis genes is increased, the level of inflammatory stress in animal renal tissue is increased, and the apoptosis rate of cells is increased, which promote the EMT progress of renal tubular epithelial cells and further affects the renal function

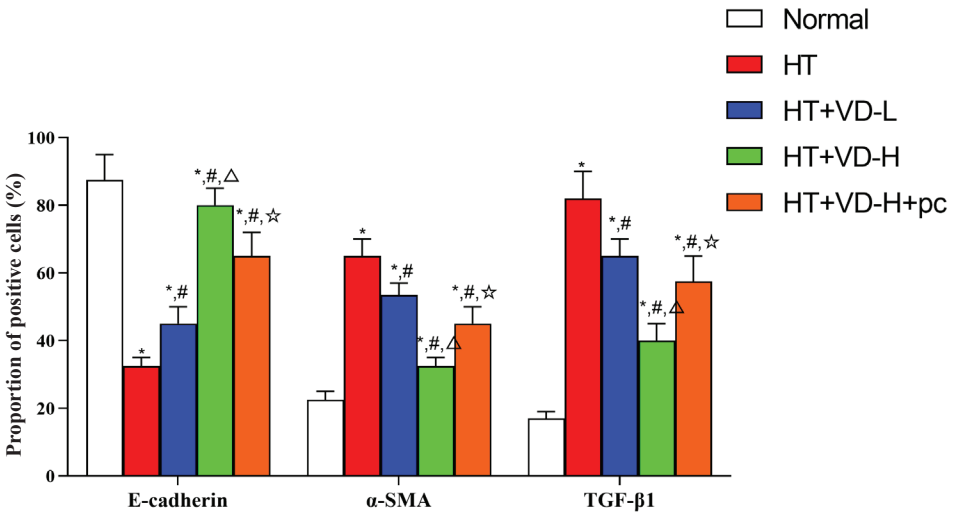

Fig. 6. Percentage of E-cadherin, α-SMA and TGF-β1 positive cells in renal tissue of rats in each group (Note: One-way ANOVA. Compared with the Normal group, *:p<0.05 compared with the HT group; #:p<0.05 compared with HT+VD-L group; △:p<0.05 compared with HT+VD-H group; ☆:p<0.05).

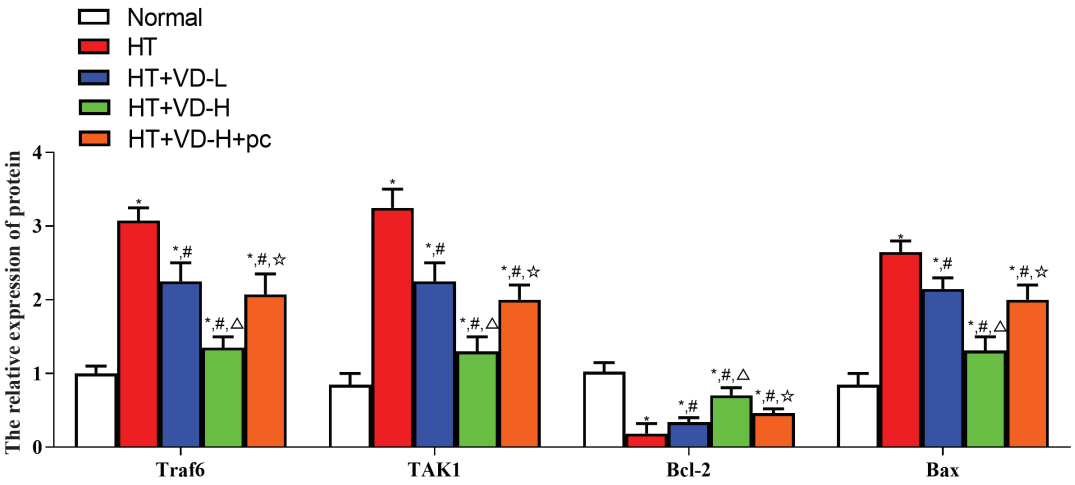

Fig. 7. The expression of Bcl-2, Bax,Traf6, TAK1 and p-TAK1 in renal tissue in rats of each group (Note: One-way ANOVA. Compared with the Normal group, *:p<0.05 compared with the HT group; #:p<0.05 compared with HT+VD-L group; △:p<0.05 compared with HT+VD-H group; ☆:p<0.05).

of animals. Following intervention with VD, the Traf6/TAK1 signal activation was significantly inhibited, leading to decreased levels of p-TAK1, reduced transcription and translation of related genes, lower apoptosis rates in cells within the animal kidney, and limited EMT progression. The rescue experiment was carried out using pCDNA3.1-TAK1, which overexpresses TAK1. The results showed that pcDNA3.1-TAK1 could partially reverse the protective effect of VD on renal tissue, and it was inferred that VD might play a protective role in renal tissue by inhibiting the Traf6/TAK1 signal (Fig. 8).

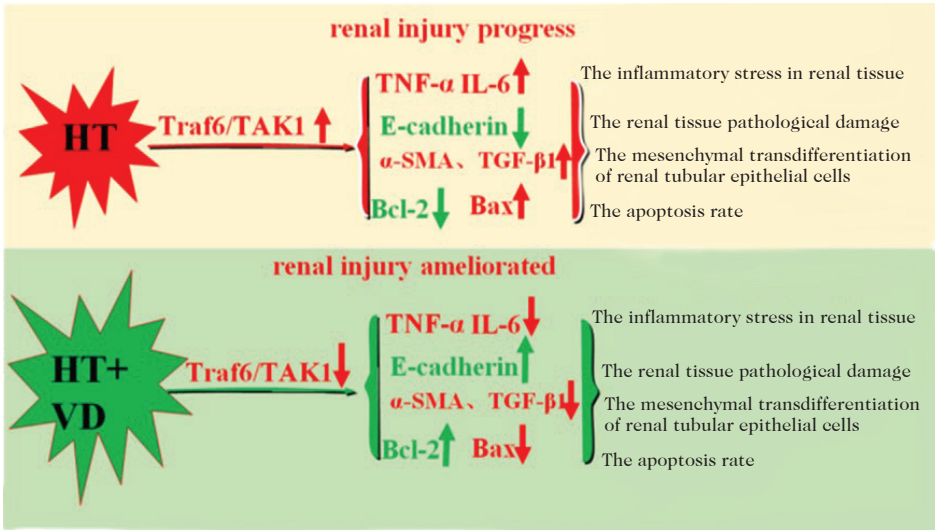

Fig. 8. The schematic diagram of the mechanism of VD attenuating renal injury in HT rats.

DISCUSSION

HT is an endocrine system disorder characterized by a series of metabolic impairments due to insufficient synthesis and secretion of thyroid hormones by the thyroid gland. The pathological mechanisms of this disease are highly complex, affecting multiple organs and tissues such as the liver, kidneys, and brain. Currently, patients could only manage the condition through lifelong oral administration of thyroid hormone supplements, and there is no definitive cure [8]. Renal injury induced by HT is one of the most prominent complications in the progression of the disease. Lathiya et al.[9] indicated that renal injury due to thyroid hormone deficiency was closely related to the progression of renal interstitial fibrosis in patients. Therefore, an in-depth exploration of the molecular mechanisms underlying epithelial-mesenchymal transition (EMT) in renal tubular epithelial cells in HT and the identification of reliable therapeutic targets are of great significance for the clinical treatment of the disease.

Vitamin D (VD), one of the essential vitamins for growth, development, and stress response, primarily participates in bone homeostasis by regulating calcium and phosphorus metabolism in cells. However, recent studies have shown [9] that VD exhibits multiple physiological effects, playing a role in pathophysiological processes such as inflammatory stress, immune regulation, neurotransmitter development, and antitumor activity. A clinical study by Durmuş et al.[10] revealed that supplementing COVID-19 patients with a certain amount of VD significantly reduced their serum levels of α-SMA and TGF-β1, decreased their urine albumin (UAlb) content and serum levels of creatinine (Scr) and blood urea nitrogen (BUN), thereby improving renal dysfunction. Wu et al. demonstrated that exogenous VD supplementation in a model of pituitary secretion deficiency significantly inhibited the expression of TNF-α and IL-6 in renal tissue, downregulated the apoptosis rate in animal renal tissue, and alleviated pathological damage to renal tissue. Li et al. [11] showed that in a model of HK-2 cell injury induced by high glucose, VD intervention significantly inhibited the expression of Bax in cells, increased the expression of E-cadherin, and inhibited the EMT process in cells. In this study, after the construction of an HT model in rats using propylthiouracil (PTU), results from

enzyme-linked immunosorbent assay kits and hematoxylin and eosin (HE) staining of thyroid tissue showed that compared with the normal group, HT rats had significantly elevated serum TSH levels, significantly decreased TT4 levels, and apparent thyroid pathological damage, indicating successful modeling. After VD intervention, renal function parameters (UAlb, Scr, BUN) in HT rats improved significantly, pathological damage to renal tissue was markedly reduced, the apoptosis rate of renal tissue cells decreased significantly, the expression of inflammatory mediators TNF-α and IL-6 in renal tissue was significantly lowered, and EMT in renal tubules was significantly restricted, confirming the protective effect of VD on the kidneys of HT rats.

To better evaluate the interventional effects of drugs and seek reliable therapeutic targets, it was necessary to delve deeper into the drug targets to promote their widespread use in clinical treatment. The activation of Traf6/TAK1 signalling was closely related to the progression of renal diseases such as acute kidney injury, chronic kidney disease, renal aging, and renal cell carcinoma, primarily exerting pro-inflammatory effects, promoting fibrosis, and regulating cell apoptosis and pyroptosis, thereby participating in the occurrence and development of related renal diseases. Wei et al.[12] showed that inhibiting the phosphorylation of TAK1 in a diabetic mouse model significantly reduced the expression of mesenchymal cell marker α-SMA in renal tissue and decreased the percentage of collagen deposition in renal tissue. The results of this study showed that the expression of Traf6 and p-TAK1 was significantly reduced in the renal tissue of rats in both high and low-dose VD groups. Functional rescue experiments using pcDNA3.1-TAK1 overexpressing TAK1 showed that pcDNA3.1-TAK1 could partially reverse the protective effect of VD on renal tissue, suggesting that VD may exert its protective effect on renal tissue by inhibiting Traf6/TAK1 signalling.

In summary, vitamin D can inhibit EMT in renal tubular epithelial cells of HT rats, reduce the apoptosis rate in renal tissue, alleviate pathological damage to renal tissue, and improve renal function. These effects are related to the inhibition of Traf6/TAK1 signalling activation. However, more detailed and systematic research is needed before vitamin D can be comprehensively used in the clinical treatment of the disease.

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Conflicts of interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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AC: conceived the study and drafted the manuscript. QH, HW: supervised the project and acquired funding, and critically revised the intellectual content. ZL, YL,JY, HH, RZ, JX, PL: performed the experiments and collected data.

REFERENCES

1. **Urgatz B, Razvi S.** Subclinical hypothyroidism, outcomes and management guidelines: a narrative review and update of recent literature. Curr Med Res Opin. 2023;39(3):351-365. *https://doi.org/10.1080/03007995.2023.2165811.*
2. **Yang H, Chen L, Liu Y.** Novel Causal Plasma Proteins for Hypothyroidism: A Large-scale Plasma Proteome Mendelian Randomization Analysis. J Clin Endocrinol Metab. 2023;108(2):433-442. *https://doi.org/10.1210/clinem/dgac575.*
3. **Rahimi-Bashar F, Vahedian-Azimi A, Dalvand S, Karimi L, Moshkani M, Alimohamadi Y, Johnston TP, Sahebkar A.** Prevalence of Amiodarone Induced Hypothyroidism and Hyperthyroidism in Patients with Heart Diseases: A Systematic Review and Meta-Analysis. Curr Med Chem. 2023;30(23):2690-2699. *https://doi.org/10.2174/0929867329666220831145651.*
4. **Adan AM, Özturk S, Adani AA, Jeele MOO.** Acute Renal Injury Due to Rhabdomyolysis Induced by Severe Hypothyroidism: First Case Report from Somalia. Int Med Case Rep J. 2022;15:575-578. *https://doi.org/10.2147/IMCRJ.S382350.*
5. **Narilla NSSH, Ramineni S, Yarlagadda S, Bandi KB.** Prevalence and outcomes of hypothyroidism--induced acute kidney injury. Clin Nephrol 2023; 99 (4): 187-190. *https://doi.org/10.5414/CN111013.*
6. **Wang Y, Zhao L, Qiu D, Shi Y, Duan H.** Effects of transforming growth factor beta-activated kinase 1 (TAK1) on apoptosis of HK-2 cells in the high glucose environment. Bioengineered. 2022;13(3):5880-5891. *https://doi.org/10.1080/21655979.2022.2040875.*
7. **Santos BR, Cordeiro JMDA, Santos LC, Santana LDS, Nascimento AEJ, Silva JF.** Kisspeptin Suppresses Inflammasome-NLRP3 Activation and Pyroptosis Caused by Hypothyroidism at the Maternal-Fetal Interface of Rats. Int J Mol Sci. 2023;24(7):6820. *https://doi.org/10.3390/ijms24076820.*
8. **Bajaj A, Shah RM, Kurapaty S, Patel AA, Divi SN.** Hypothyroidism and Spine Surgery: a Review of Current Findings. Curr Rev Musculoskelet Med. 2023;16(1):33-37. *https//doi.org/10.1007/s12178-022-09810-2.*
9. **Lathiya MK, Pepperl E, Schaefer D, Al-Sharif H, Zurob A, Cullinan SM, Charokopos A.** Vasopressin-induced hyponatremia in an adult normotensive trauma patient: A case report. World J Crit Care Med. 2023;12(1):35-40. https*://doi.org/10.5492/wjccm.v12.i1.35.*
10. **Durmuş ME, Kara Ö, Kara M, Kaya TC, Şener FE, Durmuş M, et al.** The relationship between vitamin D deficiency and mortality in older adults before and during COVID-19 pandemic. Heart Lung. 2023;57:117-123. https*://doi.org/10.1016/j.hrtlng.2022.09.007.*
11. **Li A, Yi B, Han H, Yang S, Hu Z, Zheng L, et al.** Vitamin D-VDR (vitamin D receptor) regulates defective autophagy in renal tubular epithelial cell in streptozotocin-induced diabetic mice via the AMPK pathway. Autophagy. 2022;18(4):877-890. https*://doi.org/10.1080/15548627.2021.1962681.*
12. **Wei TT, Yang LT, Guo F, Tao SB, Cheng L, Huang RS, et al.** Activation of GPR120 in podocytes ameliorates kidney fibrosis and inflammation in diabetic nephropathy. Acta Pharmacol Sin. 2021;42(2):252-263. *https://doi.org/10.1038/s41401-020-00520-4.*