Sfrp2 regulates the WNT/β-catenin pathway to slow the development of aldosterone-producing adenoma

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Background: To explore a new drug therapy for aldosterone-producing adenoma (APA), and investigate whether *Sfrp2* (secreted frizzled-related protein 2) can influence the development of adrenal APA by regulating the WNT/β-catenin pathway.

Methods: Tissue samples from APA patients were collected to detect the expression of *Sfrp2* and *β-catenin* in APA. NCI-H295R cells were cultured with WNT/β-catenin pathway inhibitors to detect cell proliferation and aldosterone secretion. Then, the expression of *Sfrp2* was altered to determine the effect of *Sfrp2* expression on WNT/β-catenin pathway activity and aldosterone adenocarcinoma cells. Finally, a mouse APA model was established, and the mice were intravenously injected with WNT/β-catenin pathway inhibitors or transfected with the *Sfrp2* gene. The activity of the WNT/β-catenin pathway, blood pressure, aldosterone secretion, and cell growth in the mice were then observed.

Results: *β-catenin* was overexpressed in APA tissues, while *Sfrp2* was underexpressed. *Sfrp2* can negatively regulate *β-catenin* expression and control the activity of the WNT/β-catenin pathway. Increased *Sfrp2* expression inhibited the activity of the WNT/β-catenin pathway, which suppressed aldosterone secretion and APA cell proliferation. The *in vivo* experiments also demonstrated that inhibition of WNT/β-catenin pathway activity in mice reduced the arterial pressure and aldosterone concentration. The increased expression of *Sfrp2* can inhibit the WNT/β-catenin pathway in mice, and can also reduce arterial pressure and APA tissue growth.

Conclusions: *Sfrp2* can inhibit the WNT/β-catenin signaling pathway by suppressing the expression of *β-catenin*, thus controlling the concentration of aldosterone and hindering APA development. This study provides a novel therapeutic target for the treatment of APA and a new direction for future research.

Keywords: *Sfrp2*; WNT/β-catenin signaling pathway; aldosterone-producing adenoma; *CYP11B2*

Introduction

Primary aldosteronism (PA) is a clinical syndrome mainly characterized by water and sodium retention, hypertension, or hypokalemia due to the excessive secretion of aldosteronism by the adrenal cortex (1,2). PA is one of the most common causes of secondary hypertension, and the proportion of PA in hypertensive patients is about 10%, which can reach about 20% in patients with refractory hypertension (3,4). Aldosterone-producing adenoma (APA) is the most common subtype of PA. It usually occurs in the glomerulosa zone of the adrenal cortex and is a benign tumor that produces and secretes aldosterone. APA often causes severe hypertension, and commonly used antihypertensive drugs have poor efficacy (5). The commonly used APA treatment is APA resection, and after unilateral APA resection, the blood pressure of patients decreased to normal within 1 year, and more than 70% of other major symptoms disappeared (6). Before APA resection, drug therapy is often used to assist patients in adjusting their physical state. Meanwhile, when some patients cannot receive surgical treatment, drug therapy is also commonly used to reduce blood pressure or supplement potassium, so as to reduce the harm of APA (7).

Molecular study on the production of aldosterone have found that somatic mutations can contribute to the development of APA, and aldosterone synthase encoded by *CYP11B2* (cytochrome P450 11B2) gene is an essential enzyme in the synthesis of aldosterone and participates in the pathogenesis of APA (8). Studies have found that the oversecretion of aldosterone is related to the overexpression of *CYP11B2*, and various factors may affect the expression and function of *CYP11B2* in APA (9,10). Therefore, from the molecular perspective, targeted regulation of *CYP11B2* expression has a therapeutic effect in APA, and *CYP11B2* is an important therapeutic target of APA (11).

The Wnt/β-catenin signaling pathway, as a stem cell regulator, exists in numerous tissues and organs of the human body (12). In addition, due to the abnormal activation of the Wnt pathway in several tumors, there are many studies on the Wnt pathway gene as a therapeutic target, and it has been applied in the treatment of various tumors (13,14). Some researchers have tested the adrenocortical adenoma tissues of APA patients, and the results showed that *CYP11B2* and activated *β-catenin* were also highly expressed in areas where a large number of mast cells existed (15). Berthon *et al.* also showed that the WNT/β-catenin signaling pathway is abnormally activated in APA, and WNT pathway signaling may be related to APA development (16).

Secreted frizzled-related protein 2 (*Sfrp2*) blocks the activation of the WNT/β-catenin signaling pathway by competing with frizzled for WNT ligands. Therefore, *Sfrp2* is also known as an antagonist of WNT (17). Study have shown that *Sfrp2* can stimulate tumor angiogenesis and can be used as an effective target for anti-tumor angiogenesis therapy (18). *Sfrp2* may also be involved in regulating physiological activities such as heart development and tumorigenesis by blocking the WNT pathway (19). Moreover, *Sfrp2* participates in the physiological activities of tumor cells by regulating the activity of the WNT pathway. It has also been shown that low *Sfrp2* expression can lead to abnormal development of APA, and *Sfrp2* gene deletion can promote increased aldosterone secretion in mice (20).

The present study focused on the WNT pathway and explored the effect of *Sfrp2* expression on the WNT/β-catenin signaling pathway and APA through *in vitro* cell experiments and the establishment of a mouse APA model. We present this article in accordance with the ARRIVE and MDAR reporting checklists (available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-105/rc).

Highlight box

Key findings
- In this study, we learned that Sfrp2 can inhibit aldosterone secretion by suppressing β-catenin expression.

What is known and what is new?
- Previous studies have found that aldosterone oversecretion is related to overexpression of CYP11B2.
- In this study, we altered the expression of Sfrp2 to explore the secretion of aldosterone.

What is the implication, and what should change now?
- The study indicating that both the *Sfrp2* and WNT pathways can be used as therapeutic targets for inhibiting aldosterone secretion and for the treatment of other diseases caused by increased aldosterone concentration. However, the mechanism between *Sfrp2* and *β-catenin* still needs to be further explored.

Methods

Cell culture

Tissue samples from 16 APA patients and 11 normal adrenal tissue samples (NA) were collected from The Second Affiliated Hospital of Anhui Medical University. Normal

adrenal tissue samples were obtained from surgically removed tissue samples of patients with adrenal cysts, renal clear cell carcinoma, etc. Human adrenal cortical adenocarcinoma cells (NCI-H295R) were purchased from Ningbo Mingzhou Biotech Co., Ltd. (Zhejiang Province, China). All cells were cultured in dulbecco's modified eagle medium/Ham's F 12 nutrient medium (DMEM/F12 medium) containing 1% Insulin-Transferrin-Selenium (ITS), 1% penicillin/streptomycin, 0.01% gentamicin, and 10% fetal calf serum (FCS) at 37 ℃ and 5% carbon dioxide (CO_2). Cells in the Wnt-inhibited group were treated with 10 μM WNT pathway inhibitor KYA1797K (Selleck, Shanghai) and cultured in an incubator. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all the patients. The study was approved by the Ethics Committee of The Second Affiliated Hospital of Anhui Medical University (No. SL-YX 2023-SZR 090).

Reverse transcription-polymerase chain reaction (RT-PCR)

Patient tissue samples were collected and cells were lysed at room temperature with TRIzol reagent (Sigma-Aldrich, Shanghai). The extracted total RNA was reversely transcribed into complementary DNA (cDNA) using a reverse transcription kit (TAKARA, Beijing). PCR was performed on the extracted cDNA using a fluorescent quantitative PCR instrument (Jinan Olaibo Scientific Instrument Co., Ltd., Shandong, China). Using *GAPDH* as an internal reference, the $2^{-\Delta\Delta Ct}$ method was employed for data collection and analysis. The reaction system was as follows: pre-denaturation at 95 ℃ for 2 min; 95 ℃ reaction 15 s, 60 ℃ reaction 30 s, 72 ℃ reaction 30 s, for a total of 35 cycles. The primer sequence of *Sfrp2* was as follows: 5'-ATCGGTGCTGTGGCTCAA-3' (sense), 5'-CATCAGGATGCCGGGACT-3' (antisense). The primer sequence of *wnt5a* was as follows: 5'-TGCCACTTGTATCAGGACCA-3' (sense), 5'-CTGTGCTGCAGTTCCATCTC-3' (antisense). The primer sequence of *GAPDH* was as follows: 5'-GGGAAACTGTGGCGTGAT-3' (sense), 5'-AAAGGTGGAGGAGTGGGT-3' (antisense). The primer sequence of CYP11B2 was as follows: 5'-ACCATGGATGTCCAGCAA-3' (sense), 5'-GAGAGCTGCCGAGTCTGA-3' (antisense). The primer sequence of β-catenin was as follows: 5'-GATTACTGCTCTGGCTCCTAGC-3' (sense), 5'-GACTCATCGTACTCCTGCTTGC-3' (antisense).

Immunohistochemistry

The collected tissue samples were embedded in paraffin and then cut into thin slices and mounted on glass slides. The paraffin sections were dewaxed and then treated with 3% hydrogen peroxide for 15 min. Next, the sections were cleaned with a Phosphate Buffer Saline (PBS) solution and then treated with 10% goat serum and sealed at room temperature for 30 min. The paraffin sections were subsequently cleaned with a PBS solution, and a diluted primary antibody solution, such as rat anti-*β-catenin* antibody (1:200, FineTest®, Wuhan, China) or rabbit anti-*CYP11B2* antibody (1:2,000, Abcam, Cambridge, UK), was added to the sections and incubated at 4 ℃ overnight.

On the second day, horse radish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) secondary antibody solution (1:1,000, Solarbio, Beijing) or goat anti-rat IgG secondary antibody solution (1:1,000, Amyjet Scientific, Wuhan) was added to the slides and incubated at 37 ℃ for 30 min. The paraffin sections then were cleaned, and HRP avidin was added to the sections for 3 h. Diaminobenzidine (DAB) solution was added to the slices for 15 min to develop color, and the slices were sealed with neutral gum. Next, tissue staining was observed with a microscope (Precise Instrument Co., Ltd., Beijing).

Western blot analysis

NCI-H295R cells and APA tissues were collected, lysed with lysate (containing protease inhibitors), and seeded in culture plates for cell culture. The proteins were then collected and separated by electrophoresis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene fluoride (PVDF) membranes. After washing the membrane, 5% skim milk was added and it was sealed at room temperature for 1–2 h. The membrane was then placed in a primary antibody diluent and incubated overnight at 4 ℃. Next, the membranes were washed with a tris buffered saline tween (TBST) solution and added with HRP-labeled goat anti-rabbit IgG diluent (1:10,000, Solarbio) and incubated at room temperature for 2 h. After washing the membrane, the enhanced chemiluminescence (ECL) chemiluminescence method (Solarbio life science, Beijing) was utilized to detect the bands on the membrane. Image J software (National Institutes of Health, USA) was used to determine the absorbance value of the protein bands.

The primary antibodies used in the experiment included

the following: rabbit anti-*Sfrp2* antibody (1:500, Beyotime, Beijing), rabbit anti-*Wnt5a* (1:600, Beyotime, Shanghai), rabbit anti-*LEF* (1:1,000, Abcam), rabbit anti-TCF (1:1,000, Abcam), and rabbit anti-*CYP11B2* (1:2,000, Abcam).

Cell transfection

First, *Sfrp2*-targeting RNA interference plasmid vectors (*Sfrp2* siRNA) and *Sfrp2*-overexpressed lentivirus vectors (*Sfrp2* mimics) were constructed, and blank vectors were used as a negative control. NCI-H295R cells were then collected and inoculated into 24-well plates at a density of $2×10^5$ cells/well, and cultured until 95% cells grew adherent to the wall. 20 pmol of a gene carrier solution and 1 µL of Lipofectamine™ 2000 (Thermo Fisher Scientific, US) were respectively added to an Opti-MEM (Minimum Essential Medium) serum-free medium for dilution. The two diluents were then mixed together and let stand at room temperature for 5 min. When the cell density in the culture plate reached 90%, the culture medium in the culture plate was removed. An Opti-MEM serum-free medium containing transfection genes and transfection reagents was then added to the culture plate, and replaced with a serum-containing medium after 4–6 h of culturing.

Methyl thiazolyl tetrazolium (MTT) analysis

The NCI-H295R cells received drug induction or transfection culture as required, and were inoculated into 96-well plates after the cell growth became stable. The cells were cultured in the culture plate until the growth was stable, and then cell activity was determined by MTT. 200 µL of a 10% MTT solution (Procell, Wuhan) was added to each well of the culture plate, and the culture plate was cultured for 4 h in the dark. The culture medium in the culture plate was discarded, 100 µL of a dimethylsulfoxide (DMSO) solution was added to each well, and then the culture plate was placed on the shaking table for 10 min. The absorbance of each group of cell solutions at a wavelength of 450 nm was assessed using a microplate reader (Safe heart Technology Co. Ltd., Beijing), and the data were collected and processed. Each group had five multiple holes, and the blank control group was set. The experiment was repeated three times.

Establishment of a mouse APA model

The 20 C57BL/6 mice used in this study were purchased from cyagen Biotechnology, Inc (Guangzhou, China). The mice were fed in a single cage, fed and drank freely, and the room temperature was maintained between 20 and 25 ℃. Subcutaneous tumor formation was performed on mice using NCI-H295R cells, when the mice grew to the fourth week, all mice were anesthetized by intraperitoneal injection of 20% urethane (0.5 mL/100 g). Then, a mini-osmotic pump was inserted into their scapular region, through which aldosterone (1 µg/h) was pumped into the mice (21,22). The mice were continuously injected with aldosterone for 4 weeks, during which the tail artery systolic blood pressure was measured once a week. Experiments were performed under a project license granted by the Ethics Committee of Anhui Medical University (No. LLSC20231760), in compliance with guidelines of Institute of Cancer and Basic Medicine (IBMC) and the Chinese Academy of Sciences for the care and use of animals. A protocol was prepared before the study without registration.

10 mice implanted with a mini-osmotic pump were divided into two groups: an *Sfrp2* overexpression group and a control group. Mice in the *Sfrp2* overexpression group were intravenously injected with *Sfrp2* lentivirus vector solution every 3 days, 500 µL each time, a total of 6 injections; while mice in the negative control group were intravenously injected with lentivirus vector solution without the target gene. When the mice grew to 8 weeks of age, venous blood was collected and plasma aldosterone concentration (PAC) was determined by radioimmunoassay.

In addition, 10 mice implanted with a micro-osmotic pump were divided into two groups: a Wnt inhibition group and a control group. Mice in the Wnt-inhibition group were intraperitoneally injected with KYA1797K (25 mg/kg, Selleck), and mice in the control group were intraperitoneally injected with the same dose of normal saline. The mice were killed when they grew to 8 weeks old. The adrenal tissue of mice was resected, and the zona glomerulosa tissue was taken for follow-up detection.

Statistical analysis

SPSS 20.0 software (Chicago, USA) was used for statistical analysis. The mean value and standard deviation of a large amount of data from the same group were calculated. Data differences between the two groups were statistically analyzed using the *t*-test. P<0.05 was considered statistically significant, and GraphPad Prism 8.0 software (California, USA) was used for mapping. All experiments in this paper were repeated three times.
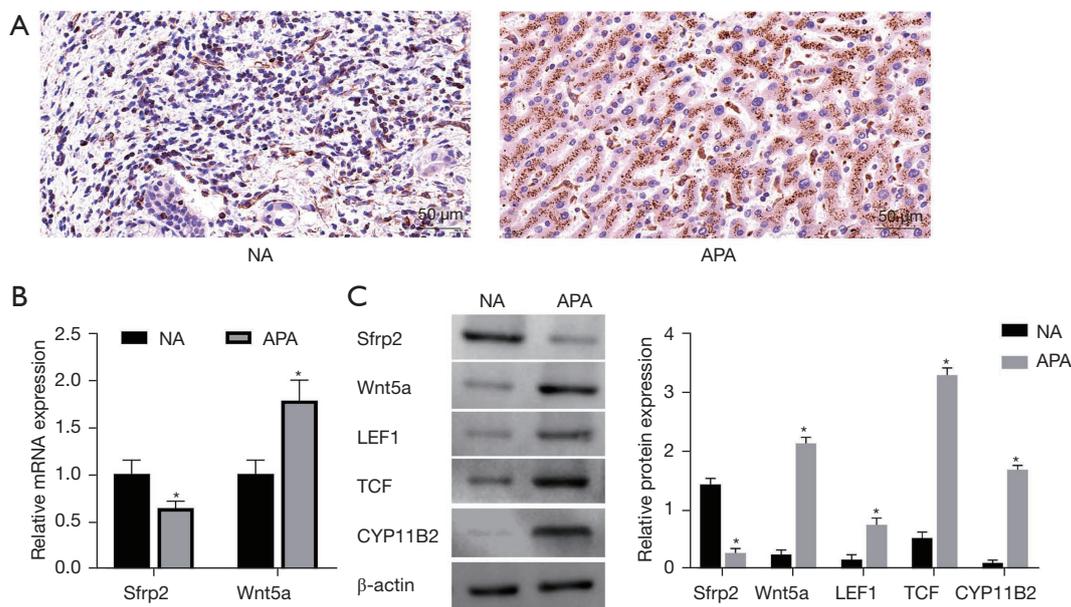
Figure 1 Expression of the WNT/β-catenin pathway in APA tissue. (A) The expression of *β-catenin* in APA tissues was assessed by immunohistochemistry. (B) The mRNA expression of *Sfrp2* and *Wnt5a* in APA tissues was assessed by RT-PCR. (C) Western blot analysis showed that Sfrp2 protein expression decreased in APA tissues, while *Wnt5a*, *LEF1*, *TCF*, and *CYP11B2* protein expression increased in APA group. *, $P<0.05$, compared with the NA group. NA, normal adrenal; APA, aldosterone-producing adenoma; Sfrp2, secreted frizzled-related protein 2; RT-PCR, reverse transcription-polymerase chain reaction; LEF1, lymphocyte enhancer factor-1; TCF, transcription factor; CYP11B2, cytochrome 11B2.

Results

Abnormal activation of the WNT/β-catenin pathway in adrenal aldosterone adenoma

Previous studies have reported abnormal activation of the WNT/β-catenin pathway in APA. Therefore, this study collected adrenal tissue samples from APA and non-APA patients and employed immunohistochemistry to detect the expression of *β-catenin* in the tissues. As shown in *Figure 1A*, *β-catenin* was expressed in large quantities in APA tissues. Of all the APA tissue samples, 12 cases (75%) showed high *β-catenin* expression, and the remaining 4 cases showed low expression or no significant difference. *β-catenin* exhibited low expression in NA tissue. Subsequently, the expression levels of *Sfrp2* and *Wnt5a* in the zona glomerulosa of the two groups of tissue samples were further assessed. Compared with NA group, *Sfrp2* expression in APA tissues decreased by about 30%, while *Wnt5a* was up-regulated in APA tissues (*Figure 1B*). Western Blot detection also showed that *Sfrp2* was highly expressed in NA tissues, while the

Wnt pathway proteins *Wnt5a*, *LEF1*, *TCF*, and aldosterone synthase *CYP11B2* were highly expressed in APA tissues (*Figure 1C*).

The WNT/β-catenin pathway regulates aldosterone secretion and influences the development of adrenal aldosterone adenoma

To verify the effect of WNT/β-catenin on aldosterone secretion, we used a WNT pathway inhibitor (KYA1797K) to inhibit *β-catenin* expression in NCI-H295R cells. Following induction culture with KYA1797K, the expression of *β-catenin* messenger RNA (mRNA) in H295R cells was decreased, and signal transduction of the WNT pathway was inhibited (*Figure 2A*). The expressions of the *LEF1*, *TCF*, and *CYP11B2* proteins decreased, as did the secretion of aldosterone (*Figure 2B*). Next, the proliferative ability of APA cells was assessed. The proliferative ability of cultured cells induced by KYA1797K was significantly decreased, and the activity of cells in the inhibition group
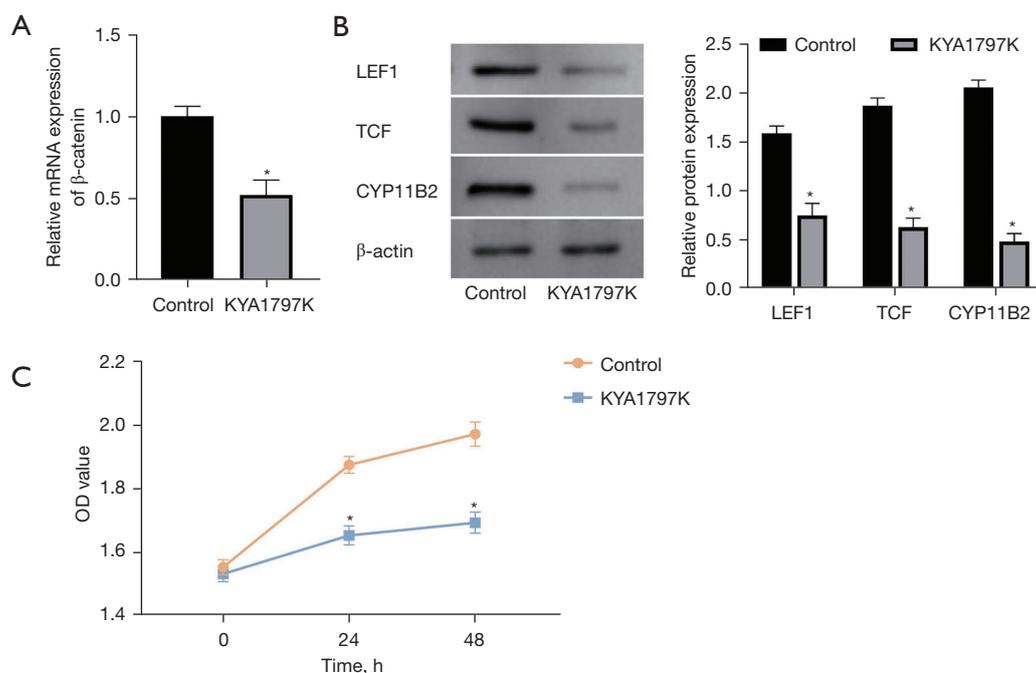
Figure 2 Effect of WNT/β-catenin pathway activity on aldosterone secretion. (A) The expression of β-catenin was assessed by RT-PCR. (B) Western blot analysis showed that the protein expressions of *LEF1*, *TCF* and *CYP11B2* decreased in KYA1797K group. (C) MTT was used to detect cell proliferation. *, P<0.05, compared with the control group. RT-PCR, reverse transcription-polymerase chain reaction; LEF1, lymphocyte enhancer factor-1; TCF, transcription factor; CYP11B2, cytochrome 11B2; MTT, methyl thiazolyl tetrazolium.

was lower than that in the control group (*Figure 2C*).

Change in the expression of Sfrp2, the regulation of Wnt pathway signaling, and the secretion of aldosterone

We further explored the regulatory role of *Sfrp2* expression on the Wnt pathway and aldosterone secretion. An *Sfrp2* silencing-RNA vector (*Sfrp2* siRNA) and overexpression vector (*Sfrp2* mimics) were transfected into NCI-H295R cells to detect the Wnt pathway activity in NCI-H295R cells. RT-PCR was used to test the transfection efficiency. The expression of *Sfrp2* mRNA was decreased and *β-catenin* mRNA was increased in the siRNA-transfected cells. Following transfection with the overexpression vector, the expression of *Sfrp2* mRNA was increased, while that of *β-catenin* mRNA was decreased.

Cell transfection was successful (*Figure 3A*). The expression of Wnt pathway-related proteins was assessed, and it was found that *β-catenin*, *LEF1*, *TCF*, and *CYP11B2* protein expression were increased in the *Sfrp2* siRNA group. The protein expression of *β-catenin*, *LEF1*, *TCF*, and *CYP11B2* decreased in the *Sfrp2* overexpression

group (*Figure 3B*). The cell proliferation tests showed that suppressing the expression of *Sfrp2* could promote the proliferative activity of APA cells, while increasing the expression of *Sfrp2* could inhibit this activity (*Figure 3C*). These results showed that *Sfrp2* acted as an inhibitor of the WNT pathway and negatively regulated the signal transduction of this pathway.

Increased expression of Sfrp2 in mice, inhibition of the activity of the WNT/β-catenin pathway, and secretion of aldosterone

We also established an APA mouse model. *Sfrp2* gene expression in mice was increased, and the venous blood of mice was collected to detect the expression changes of *β-catenin* and *CYP11B2* in plasma. Compared with the control mice, the plasma *β-catenin* and *CYP11B2* expressions of *Sfrp2*-overexpressed mice were decreased (*Figure 4A*). Subsequent tests showed that increased *Sfrp2* expression in APA mice could reduce the caudal arterial pressure and PAC (*Figure 4B,4C*). Also, increased *Sfrp2* gene expression in mice could inhibit WNT/β-catenin signaling, aldosterone
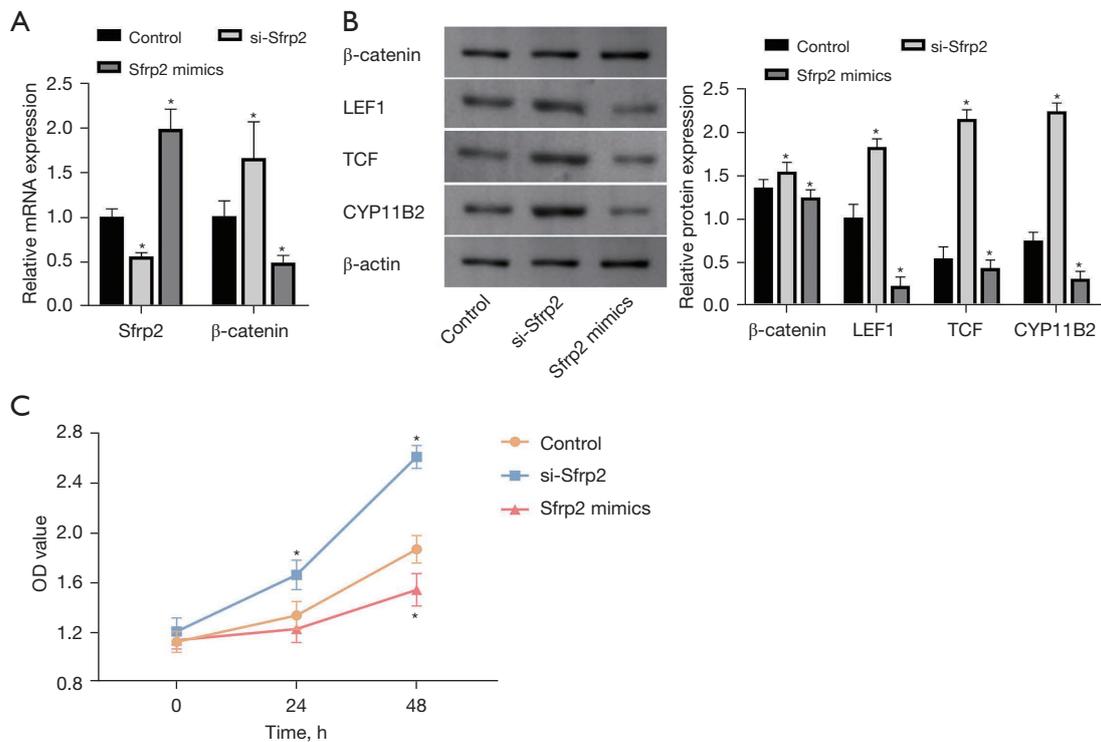
Figure 3 Effect of *Sfrp2* expression on the WNT pathway and aldosterone secretion. (A) RT-PCR was used to detect the expressions of *Sfrp2* and *β-catenin*. (B) The expression changes of *β-catenin*, *LEF1*, *TCF*, and *CYP11B2* protein were assessed by Western Blot. (C) MTT was used to detect cell proliferation. *, P<0.05, compared with the control group. RT-PCR, reverse transcription-polymerase chain reaction; Sfrp2, secreted frizzled-related protein 2; LEF1, lymphocyte enhancer factor-1; TCF, transcription factor; CYP11B2, cytochrome 11B2; MTT, methyl thiazolyl tetrazolium.
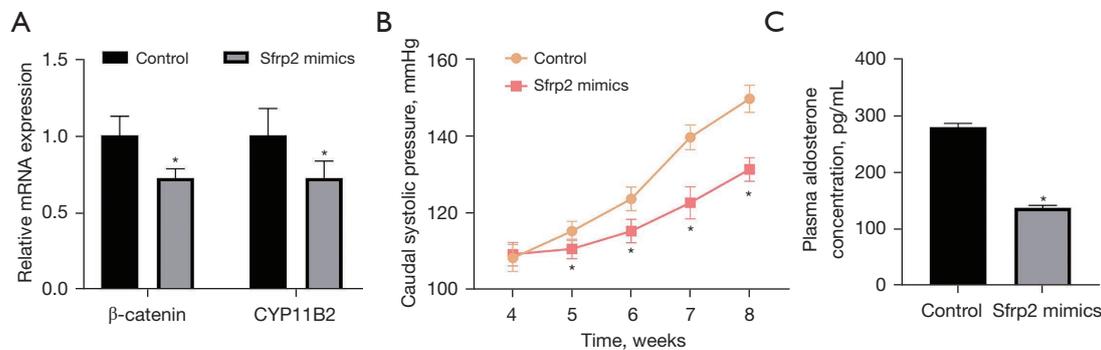
Figure 4 Effect of *Sfrp2* expression on WNT pathway and aldosterone secretion in mice. (A) RT-PCR was used to detect the expressions of *CYP11B2* and *β-catenin*. (B) A change in caudal artery systolic pressure was assessed. (C) The plasma aldosterone concentration was assessed after the mice grew to the 8 weeks of age. *, P<0.05, compared with the control group. Sfrp2, secreted frizzled-related protein 2; CYP11B2, cytochrome 11B2; RT-PCR, reverse transcription-polymerase chain reaction.
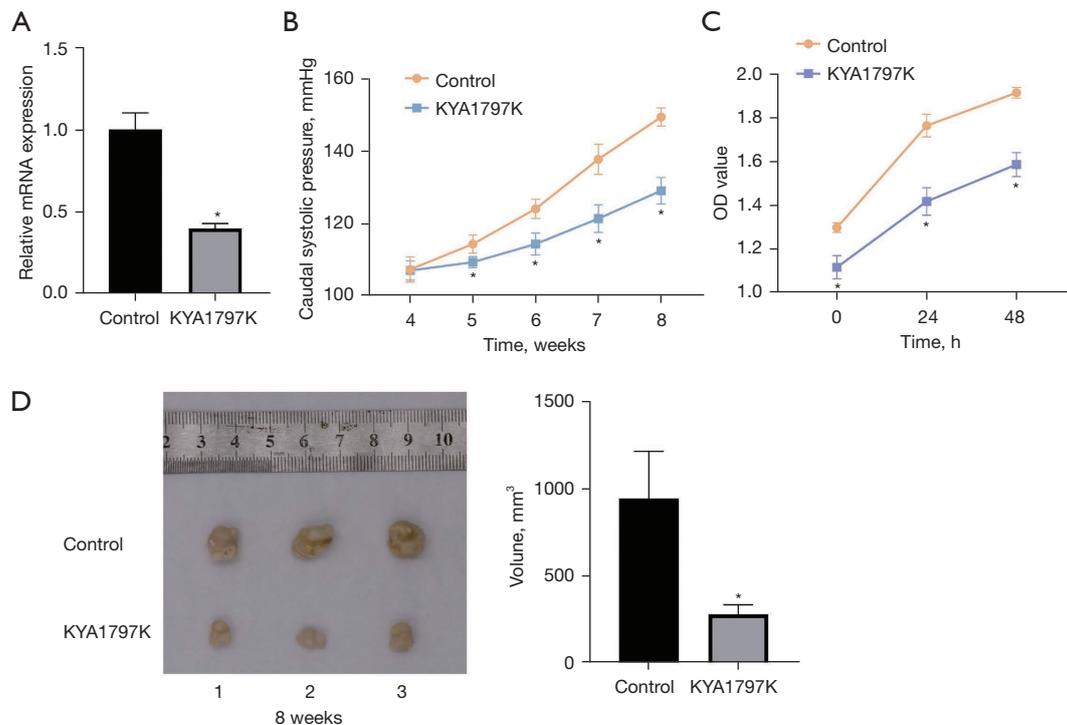
Figure 5 Effect of WNT/β-catenin pathway activity on the growth of aldosterone producing adenoma (APA). (A) The expression of *β-catenin* was assessed by RT-PCR. (B) A change in caudal artery systolic pressure was assessed. (C) Proliferative activity of aldosterone adenoma cells was assessed by MTT. (D) Tumor growth volume was observed after the mice grew to 8 weeks old. *, P<0.05, compared with the control group. RT-PCR, reverse transcription-polymerase chain reaction; MTT, methyl thiazolyl tetrazolium.

secretion, and APA development.

Inhibition of WNT/β-catenin pathway activity in mice and slowing the growth of adrenal aldosterone adenoma

The APA mice were intraperitoneally injected with a WNT/β-catenin inhibitor, KYA1797K. Compared with the inhibitor control group, the mRNA expression of *β-catenin* and arterial pressure in mice in the Wnt-inhibited group were decreased (*Figure 5A,5B*). The mice were killed and tumor tissues were collected for further detection. In the KYA1797K inhibition group, cell proliferation activity decreased, the tumor tissue was small, the growth rate was slow, and tumor growth was inhibited (*Figure 5C,5D*). Therefore, inhibiting the activity of the WNT/β-catenin pathway may help to slow the growth rate of adrenal aldosterone adenoma.

Discussion

PA is considered to be the leading cause of secondary

endocrine hypertension. Compared with essential hypertension, PA-induced hypertension causes more serious organ damage and is more likely to result in cardiovascular diseases (23,24). Therefore, early treatment of PA can effectively reduce the morbidity and mortality of cardiovascular and cerebrovascular diseases. For some patients with special conditions, such as PA during pregnancy and elderly patients, drug therapy is more appropriate (25,26). Study have shown that in tumor cells, the WNT pathway is overactivated often due to gene mutations, and a large number of *β-catenin* proteins enter the nucleus for *TCF/LEF* transcriptional regulation (27). Study have shown that mutations in *β-catenin* genes in APA lead to abnormal activation of the WNT/β-catenin signaling pathway (28). Therefore, studying the WNT/β-catenin signaling pathway as a therapeutic target is a novel approach.

In this study, we assessed high expression of *β-catenin* in tissue samples from APA patients. Meanwhile, WNT pathway-related proteins (*wnt5a*, *LEF1*, and *TCF*) were highly expressed in APA samples. The WNT/β-catenin

signaling pathway is abnormally activated in APA. As an antagonist of the WNT gene, *Sfrp2* plays a role in various tumors by inhibiting the signal transduction of the WNT pathway (29,30). This experiment also found that in APA, *Sfrp2* expression was low, while *CYP11B2* expression and aldosterone secretion were high. It is speculated that abnormal activation of the WNT/β-catenin signaling pathway may be related to the secretion of aldosterone, and *Sfrp2* can regulate aldosterone secretion by inhibiting the WNT/β-catenin signaling pathway.

KYA1797K is a WNT/β-catenin inhibitor that can promote the degradation of *β-catenin* by binding Axin and has been widely used in cancer stem cell studies (31,32). In this study, KYA1797K was applied to culture cells and inhibit the activity of the WNT/β-catenin signaling pathway. The results showed that inhibition of WNT/β-catenin signal transduction reduced the expression of *CYP11B2* and the proliferative activity of APA cells. Thus, inhibition of the WNT/β-catenin signaling pathway can reduce aldosterone secretion and slow down APA development.

After regulating the expression of *Sfrp2*, we found that *Sfrp2* could inhibit the expression of WNT/β-catenin pathway proteins and inhibit the secretion of aldosterone. In addition, the higher the expression of *Sfrp2*, the stronger the inhibition, and *Sfrp2* acts as a WNT/β-catenin inhibitor. A mouse APA model was then established for *in vivo* experimentation. According to the literature, the dose of aldosterone used in this study was 1 μg/h, which was consistent with the dose used in common mouse APA models (33). The up-regulation of *Sfrp2* gene expression in APA mice significantly reduced *β-catenin* expression and aldosterone concentration, and reduced arterial pressure in mice. After intravenous injection of KYA1797K in APA mice, the expression of *β-catenin* was inhibited, the arterial pressure of mice decreased, and the growth rate of adenoma slowed.

In this study, *in vivo* and *in vitro* experiments demonstrated that *Sfrp2* inhibits the WNT/β-catenin signaling pathway by inhibiting the expression of *β-catenin*, thereby suppressing the secretion of aldosterone in mice. Increasing the expression of *Sfrp2* in APA patients can effectively reduce the concentration of aldosterone and control patients' blood pressure, while simultaneously slowing the growth of APA tissues and delaying disease progression. Gene therapy with *Sfrp2* is an effective treatment for patients undergoing conservative treatment. Increased aldosterone concentration in the human body will lead to a series of secondary diseases such as hypertension, hypokalemia, and hyperaldosteronemia; therefore, reducing aldosterone secretion has a significant effect on a variety of diseases.

Conclusions

In this study, we learned that *Sfrp2* can inhibit aldosterone secretion by suppressing *β-catenin* expression, indicating that both the *Sfrp2* and WNT pathways can be used as therapeutic targets for inhibiting aldosterone secretion and for the treatment of other diseases caused by increased aldosterone concentration. However, the mechanism between *Sfrp2* and *β-catenin* still needs to be further explored.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the ARRIVE and MDAR reporting checklists. Available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-105/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-105/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all the patients. The study was approved by the Ethics Committee of The Second Affiliated Hospital of Anhui Medical University (No. SL-YX 2023-SZR 090). This experiment was performed under a project license granted

by the Ethics Committee of Anhui Medical University (No. LLSC20231760), in compliance with guidelines of Institute of Cancer and Basic Medicine (IBMC) and the Chinese Academy of Sciences for the care and use of animals.

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