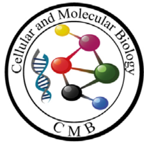
Cell. Mol. Biol. (ISSN: 1165-158X)



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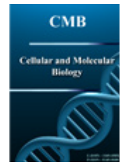

Original Article

Up-regulation of lncRNA WT1-AS ameliorates Aβ-stimulated neuronal injury through modulation of miR-186-5p/CCND2 axis in Alzheimer's disease

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Article Info

Article history:

Received: November 12, 2023
Accepted: January 12, 2024
Published: January 31, 2024

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Abstract

As a common neurodegenerative disorder, Alzheimer's disease (AD) seriously threatens human life. Long non-coding RNAs (lncRNAs) exhibit essential functions in AD development. Nevertheless, the detailed effects and possible mechanisms of lncRNA Wilms tumor 1 Antisense RNA (WT1-AS) in AD are largely unknown. In our studies, a total of 30 serum samples from AD patients were collected, and WT1-AS expressions were detected through qRT-PCR analysis. Additionally, an in vitro AD model was constructed by treating $A\beta_{1-42}$ in human neuroblastoma cells. Functional assays were implemented to assess the impacts of WT1-AS on $A\beta_{1-42}$-stimulated human neuroblastoma cell proliferation together with apoptosis. Moreover, relationship of WT1-AS, microRNA (miR)-186-5p as well as cyclin D2 (CCND2) could be predicted through bioinformatics tools as well as proved via dual-luciferase reporter experiments. Our results showed that WT1-AS together with CCND2 were low-expressed, while miR-186-5p presented high expression in AD serum samples together with $A\beta_{1-42}$-stimulated human neuroblastoma cells. WT1-AS over-expression or miR-186-5p depletion notably promoted the proliferation, reduced the apoptosis, and decreased the p-Tau protein expressions of human neuroblastoma cells induced with $A\beta_{1-42}$. Moreover, miR-186-5p combined with WT1-AS, and CCND2 was modulated by miR-186-5p. Furthermore, CCND2 elevation partially offsets the impacts of miR-186-5p elevation on $A\beta_{1-42}$-stimulated cell proliferation as well as apoptosis mediated with WT1-AS up-regulation. Our results indicated that up-regulation of lncRNA WT1-AS ameliorated Aβ-stimulated neuronal damage through modulating miR-186-5p/CCND2 axis, offering a novel direction for AD therapy.

Keywords: Alzheimer's disease; lncRNA WT1-AS; Neuronal damage; Apoptosis; CCND2

1. Introduction

As a kind of frequent neurodegenerative disease causing dementia, the clinical features of Alzheimer's disease (AD) are progressive memory impairment, cognitive dysfunction, personality alterations, as well as language barriers [1]. Along with the aging population, the occurrence rate of AD is increasing every year. It is estimated that by 2050, there will be one AD patient in every 85 people [2]. AD, as the fourth killer completing with stroke, cardiovascular and cerebrovascular diseases and cancer, seriously endangers the physical and mental health of the aged, as well as results in a huge strain on the family together with society [3]. The main pathological features of AD are senile plaque (the main component is β-amyloid (Aβ)) deposition of cerebral cortex and hippocampus, neurofibrillary tangles (the main component is phosphorylated tubulin tau protein), and partial loss of neural synapses [4]. The main mechanisms of AD include abnormal modification of some proteins, amyloid toxicity mechanisms, central cholinergic injury, microtubule-associated protein differentiation, immune function mutation and so on [5]. Aβ can cause damage to synaptic transmission function and memory loss. At the same time, it can also cause calcium overload, oxidative damage, neuronal loss, and mediate inflammatory response [6]. Aberrant secretion or immoderate production of Aβ will result in other pathological alterations of AD, thus Aβ is the core part of AD [7]. Due to the late onset of AD, there is no specific index to show the disease. The early stage of the disease is easy to ignore. When diagnosed, the patient has entered the late stage. Hence, it is essential to probe early diagnostic markers of AD.

Long non-coding RNAs (lncRNAs) are presented in the nucleus as well as cytoplasm, and exert critical effects on the physiological and pathological processes of AD through regulating the expression of related genes [8]. Evidences have suggested that lncRNAs acted as potential biomarkers in the therapy, diagnosis, as well as prevention of neurodegenerative diseases, AD included [9]. For instance, SOX21-AS1 silencing affected $A\beta_{1-42}$ mediated

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Doi: http://dx.doi.org/10.14715/cmb/2024.70.1.27

proliferation as well as apoptosis of human neuroblastoma cells [10]. Besides, EBF3-AS and EBF3 (early B cell factor 3) expressions were increased in APP/PS1 mice. EBF3-AS hindered the apoptosis of human neuroblastoma cells stimulated by $A\beta_{25-35}$ [11]. Furthermore, NEAT1 depletion attenuated Aβ-stimulated viability inhibition, as well as apoptosis and p-Tau promotion. These findings mirrored that lncRNAs might be a novel therapy targeting for AD treatment.

Wilms tumor 1 Antisense RNA (WT1-AS), a novel discovered lncRNA, is located on chromosome 11p13 [12]. As reported previously, WT1-AS acted as tumor repressors in many tumors [13, 14]. Besides, WT1-AS could reduce hypoxia/ischemia-stimulated neuronal damage in cerebral ischemic stroke [15]. Recent literature has revealed that WT1-AS was down-regulated in $A\beta_{25-35}$-treated human neuroblastoma cells, and WT1-AS up-regulation inhibited apoptosis. Moreover, *in vivo* experiments uncovered that WT1-AS over-expression improved learning as well as memory abilities of AD mice. Nevertheless, the potential and underlying mechanisms of WT1-AS in AD are rarely documented.

MicroRNAs (miRNAs) influence mRNA expression via binding to the 3'-untranslated region (3'-UTR) of the target mRNA. MiRNAs are related to physiological processes of many diseases, AD included [16]. Additionally, some miRNAs have been identified as candidate diagnostic biomarkers for AD, such as miR-34c [17] and miR-331-3p [18]. Literatures have found that miR-186-5p is highly expressed in AD [19], but its biological function in AD is still unclear.

Therefore, the current work focused on examining WT1-AS expression levels in AD serum samples and $A\beta_{1-42}$-induced human neuroblastoma cells, as well as probing the latent mechanisms of WT1-AS in AD.

2. Materials and methods

2.1. Clinical samples

Thirty AD serum samples and 30 healthy serum samples were acquired. The Ethics Committee had approved experimental procedures, and all patients signed written informed consents. The Chinese Clinical Trial Registry has approved this study (chiCTR2100047410).

2.2. Cell culture and transfection

Chinese Academy of Science (Shanghai, China) offered human neuroblastoma cell lines (SH-SY5Y together with SK-N-SH) and could be cultivated in MEM + F12 (1:1) medium and MEM medium containing ten percent fetal bovine serum (FBS) as well as 1% penicillin/streptomycin at 37°C with 5% CO_2.

For constructing *in vitro* AD model, human neuroblastoma cell cells were treated with Aβ (MedChemExpress, USA) with different doses (0, 5, 10, or 20 μM) in dimethyl sulfoxide (DMSO, Thermo Fisher, USA) for 24 h, or with 10 μM of Aβ for various times (0, 12, 24, or 48 h).

For cell transfection, pc-WT1-AS, pc-CCND2, negative empty vectors (pc-NCs), NC mimic/miR-186-5p mimic, and NC inhibitor/miR-186-5p inhibitor were acquired from Ambion (Austin, USA) and introduced into cells (5×10^5) by virtue of Lipofectamine 3000 (Invitrogen, USA).

2.3. Cell counting kit-8 (CCK-8)

Human neuroblastoma cells were cultured in 96-well plate (1×10^4 cells per well) and treated with 10 μM Aβ for 24 h. Afterwards, 10 μL CCK-8 reagent (Beyotime, China) could be supplemented into each well. The viability at 450 nm absorbance could be evaluated with the microplate reader.

2.4. Flow cytometry

An Annexin V-FITC/PI apoptosis kit (Keygen, China) was implemented for assessing cell apoptosis [20]. Cell suspension was centrifuged and then re-suspended, and then dyed with Annexin V-FITC as well as propidium (PI). A flow cytometer (BD Biosciences, USA) was adopted for analyzing cell apoptosis.

2.5. Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

TRIzol reagent (Thermo Fisher, USA) could be adopted for extracting total RNA from serum sample as well as cells. Briefly, total RNA could be transcribed to cDNA with be help of M-MLV reverse transcriptase (Promega, USA), followed by qRT-PCR with the help of a Taqman PCR kit (Thermo Fisher, USA). The primer sets for each gene are indicated as follows. WT1-AS forward, 5'-GCCTC TCTGTCCTCTTCTTTGT-3', and reverse 5'-GCTGTGAGTCCTGGTGCTTAG-3'. miR-186-5p forward, 5'-AAGAATTCTCCTTTTGGGCT-3', and reverse, 5'-GTGCGTGTCGTGGAGTCG-3'. CCND2 forward, 5'-CATCCTCACGGCCTCGGCT-3', and reverse, 5'-CGGCGTGTGTTTATCGGA ATCCA-3'. U6 forward, 5'-CTCGCTTCGGCAGCACA-3', and reverse, 5'-AACGCTTCACGAATT TGCGT-3', β-actin forward, 5'-ATCACTGCCACCCAGAAGAC-3', and reverse, 5'-TTTCTAGACG GCAGGTCAGG-3'. U6 and β-actin were adopted to be internal controls. Gene expression received calculation with the $2^{-\Delta\Delta CT}$ method.

2.6. Western blot

Extraction of total proteins was implemented with the help of RIPA Lysis and Extraction Buffer (Thermo Fisher, USA), followed by separating through SDS-PAGE. Afterwards, proteins were shifted into the PVDF membranes (Beyotime, China). The membranes received cultivation with the primary antibodies at 4°C overnight containing Cleaved-caspase-3 (1: 1, 000, ab32042, Abcam, USA), anti-p-Tau (1: 2, 000, 28866-1-AP, Proteictech, China), anti-Tau (1: 2, 000, 10274-1-AP, Proteictech, China), as well as β-actin (ab8226, 1: 1, 000) after blocking with skimmed milk. The membranes received treatment with the secondary horseradish peroxidase (HRP) antibodies (Sigma, Aldrich) for 60 min at 4°C, followed by observation with the Image Lab (Bio-Rad Laboratories, USA).

2.7. Dual-luciferase reporter gene assay

The sequence of wild type WT1-AS (WT1-AS-WT) and 3'-UTR of CCND2 (CCND2-WT), mutant type WT1-AS (WT1-AS-Mut) along with CCND2 (CCND2-Mut) were cloned into pmirGLO reporter vector (Genscript, China). Then, the vectors were treated in combination with miR-186-5p mimic or NC into cells with the help of Lipofectamine 3000 (Invitrogen, USA), followed by examining the luciferase activity with the help of dual-luciferase reporter assay system (Promega, USA).

2.8. Statistical analysis

All the above experiments were implemented three times. Data were analyzed with the help of GraphPad Prism 5.0 and presented as mean ± SD. Differences received comparison using one-way ANOVA and Turkey's post host. $P < 0.05$ represented statistical significance.

3. Results

3.1. WT1-AS presented low expression in serum samples from AD patients and in human neuroblastoma cells induced by Aβ$_{1-42}$

In order to evaluate the WT1-AS expressions in AD, a total of 30 serum samples were gathered. It was revealed that WT1-AS presented low expression in AD serum samples compared with that in healthy serum samples (Figure 1A). Next, WT1-AS expression was measured in Aβ$_{1-42}$-stimulated AD model. As displayed in Figure 1B, WT1-AS expressions were decreased in human neuroblastoma cells in a concentration-dependent way. Notably, the reduced fold change of WT1-AS expression was the most in human neuroblastoma cells when treating 10 μM of Aβ$_{1-42}$, thus we chose 10 μM for subsequent analyses. Moreover, WT1-AS levels were decreased in 10 μM Aβ$_{1-42}$-stimulated human neuroblastoma cells in a time-dependent way. Besides, the CCK-8 assay indicated that Aβ$_{1-42}$ (10 μM) notably depleted the proliferation of human neuroblastoma cells at 24 h (Figure 1C).

3.2. Up-regulated WT1-AS enhances human neuroblastoma cells proliferation while reduces cell apoptosis of induced by Aβ$_{1-42}$

To certify the impacts of WT1-AS on AD progression, pc-WT1-AS was transfected into Aβ1-42-induced human neuroblastoma cells to elevate WT1-AS expression (Figure 2A). Then CCK-8 assay uncovered that Aβ$_{1-42}$ obviously repressed human neuroblastoma cell proliferation, while WT1-AS elevation promoted human neuroblastoma cell proliferation induced with Aβ$_{1-42}$ (Figure 2B). In addition, based on flow cytometry assay, Aβ$_{1-42}$ obviously induced the apoptosis of human neuroblastoma cells, while WT1-AS over-expression reversed this phenomenon (Figure 2C). Moreover, western blot analysis displayed that Aβ$_{1-42}$ elevated p-Tau as well as Cleaved-caspase-3 levels, while WT1-AS over-expression decreased the p-Tau as well as Cleaved-caspase-3 levels in human neuroblastoma cells stimulated by Aβ$_{1-42}$ (Figure 2D).

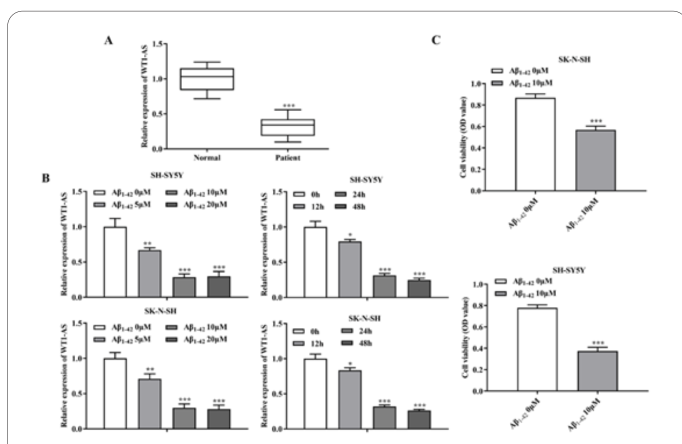

Fig. 1. WT1-AS is lowly expressed in Aβ$_{1-42}$-treated human neuroblastoma cells. (A) WT1-AS expression in AD serum samples from qRT-PCR assay; (B) WT1-AS expression in human neuroblastoma cells after treating different doses of Aβ$_{1-42}$ for 24 h, or Aβ$_{1-42}$ (10 μM) for indicated times. (C) CCK-8 assay evaluated the impact of Aβ$_{1-42}$ on human neuroblastoma cell proliferation. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. Control group.

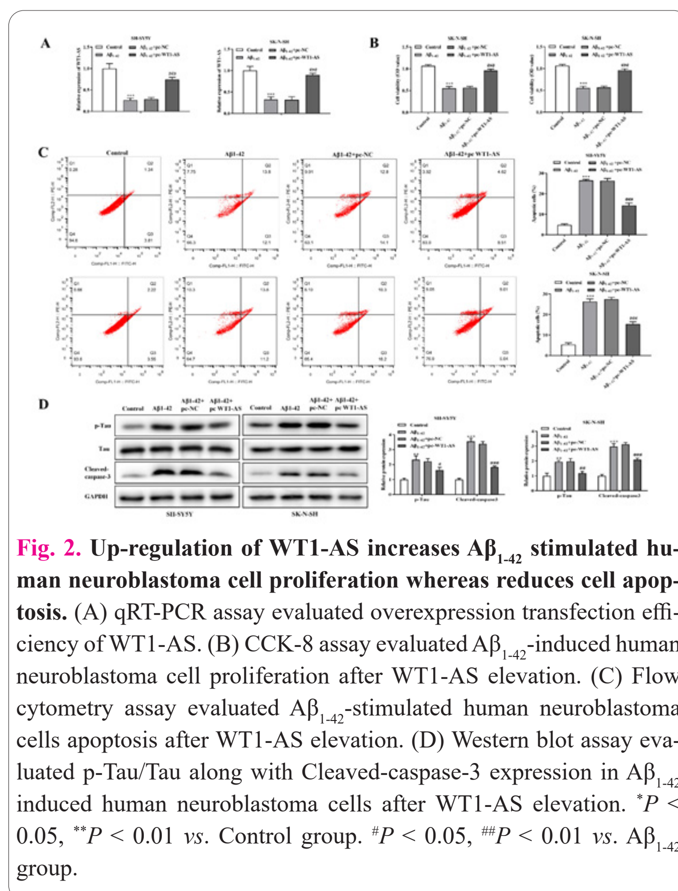

Fig. 2. Up-regulation of WT1-AS increases Aβ$_{1-42}$ stimulated human neuroblastoma cell proliferation whereas reduces cell apoptosis. (A) qRT-PCR assay evaluated overexpression transfection efficiency of WT1-AS. (B) CCK-8 assay evaluated Aβ$_{1-42}$-induced human neuroblastoma cell proliferation after WT1-AS elevation. (C) Flow cytometry assay evaluated Aβ$_{1-42}$-stimulated human neuroblastoma cells apoptosis after WT1-AS elevation. (D) Western blot assay evaluated p-Tau/Tau along with Cleaved-caspase-3 expression in Aβ$_{1-42}$ induced human neuroblastoma cells after WT1-AS elevation. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. Control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs*. Aβ$_{1-42}$ group.

3.3. MiR-186-5p binds to WT1-AS and exerts effects on proliferation and apoptosis of human neuroblastoma cells

Through bioinformatics tools, miR-186-5p could be speculated to possess the target domain of WT1-AS (Figure 3A). Then dual-luciferase reporter analysis unveiled elevated miR-186-5p distinctly lessened the luciferase intensity of WT1-AS-WT, whereas rarely affected WT1-AS-Mut activity (Figure 3B). Moreover, as uncovered in Figure 3C and 3D, miR-186-5p levels were obviously up-regulated. Furthermore, Figure 3E suggested that WT1-AS elevation declined miR-186-5p expression. Next, miR-186-5p inhibitor was introduced into Aβ1-42-induced human neuroblastoma cells, and the inhibition transfection efficiency could be verified through qRT-PCR analysis (Figure 3F). It was displayed that miR-186-5p inhibitor promoted Aβ1-42 stimulated human neuroblastoma cell proliferation (Figure 3G). In addition, flow cytometry assay implied that miR-186-5p inhibitor reduced Aβ1-42 stimulated human neuroblastoma cells apoptosis (Figure 3H). Moreover, western blot analysis displayed that miR-186-5p inhibitor down-regulation decreased the p-Tau along with Cleaved-caspase-3 levels in human neuroblastoma cells stimulated by Aβ$_{1-42}$ (Figure 3I).

3.4. CCND2 is targeted by miR-186-5p

Based on bioinformatics tools, it was shown that a complementary sequence of miR-186-5p and CCND2 3'-UTR (Figure 4A). Then, the dual-luciferase experiment confirmed that miR-186-5p elevation lessened the luciferase

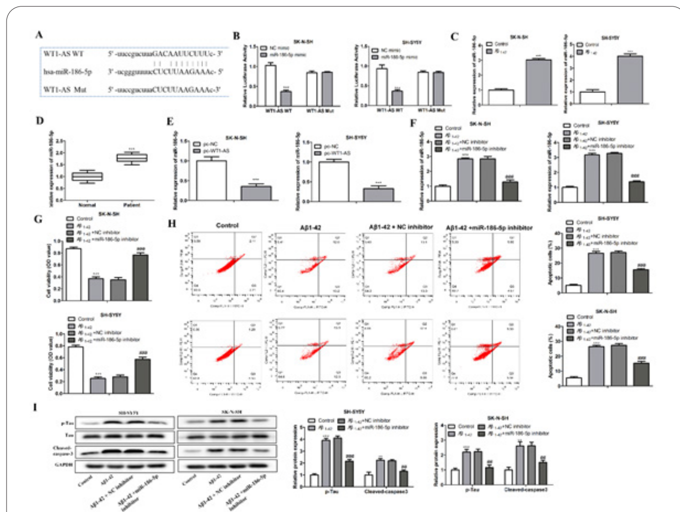

Fig. 3. Interaction between WT1-AS and miR-186-5p and effects of miR-186-5p on proliferation and apoptosis of human neuroblastoma cells. (A) Binding sites of WT1-AS and miR-186-5p. (B) Dual-luciferase reporter assay examined the luciferase activity of WT1-AS-WT/Mut after miR-186-5p elevation. $^{**}P < 0.01$ *vs*. NC mimic group. (C) MiR-186-5p expression in human neuroblastoma cells after treating $A\beta_{1\text{-}42}$ (10 μM) for 24 h through qRT-PCR assay. $^{**}P < 0.01$ *vs*. Control group. (D) MiR-186-5p expression in AD serum samples from qRT-PCR assay. $^{**}P < 0.01$ *vs*. Normal samples. (E) MiR-186-5p expression in human neuroblastoma cells after WT1-AS elevation from qRT-PCR assay. $^{**}P < 0.01$ *vs*. pc-NC group. (F) qRT-PCR assay evaluated the silence transfection efficiency of miR-186-5p. (G) CCK-8 assay evaluated $A\beta_{1\text{-}42}$-stimulated human neuroblastoma cell proliferation after miR-186-5p silence. (H) Flow cytometry assay evaluated $A\beta_{1\text{-}42}$-stimulated human neuroblastoma cells apoptosis after miR-186-5p silence. (I) Western blot assay evaluated p-Tau/Tau along with Cleaved-caspase-3 expression in $A\beta_{1\text{-}42}$-stimulated human neuroblastoma cells after miR-186-5p silence. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. Control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs*. $A\beta_{1\text{-}42}$ group.

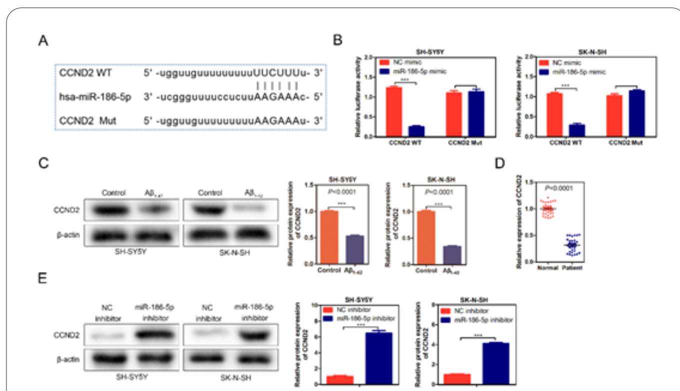

Fig. 4. CCND2 is targeted by miR-186-5p in AD. (A) Binding sites of miR-186-5p and CCND2. (B) Dual-luciferase reporter assay examined the luciferase activity of CCND2-WT/Mut after miR-186-5p elevation. $^{**}P < 0.01$ *vs*. NC mimic group. (C) CCND2 expression in human neuroblastoma cells after treating $A\beta_{1\text{-}42}$ (10 μM) for 24 h from western blot assay. $^{**}P < 0.01$ *vs*. Control group. (D) CCND2 expression in AD serum samples from qRT-PCR assay. $^{**}P < 0.01$ *vs*. Normal samples. (E) CCND2 expression in human neuroblastoma cells transfected with pc-WT1-AS from western blot assay. $^{**}P < 0.01$ *vs*. NC inhibitor group.

intensity of CCND2 WT, whereas barely affected CCND2 Mut activity (Figure 4B). Moreover, CCND2 levels were obviously downregulated in AD serum samples and cells shown in Figures 4C and 4D. Finally, as displayed in Figure 4E, CCND2 levels were over-expressed in human neuroblastoma cells after miR-186-5 suppression.

3.5. WT1-AS influences $A\beta_{1\text{-}42}$-stimulated human neuroblastoma cell proliferation along with apoptosis by miR-186-5p/CCND2 axis

To further verify whether WT1-AS exhibited its potential via modulating miR-186-5p/CCND2 axis, we implemented rescue assays. Firstly, human neuroblastoma cells were introduced with miR-186-5p mimic or pc-CCND2, respectively to elevate miR-186-5p or CCND2 expression (Figure 5A). As revealed in Figure 5B, miR-186-5p mimic partially reduced the promoting role of WT1-AS over-expression human neuroblastoma cells proliferation stimulated with $A\beta_{1\text{-}42}$, while CCND2 showed the opposite effects. MiR-186-5p mimic partially rescued the inhibitory role of WT1-AS over-expression on SH-SY5Y cells apoptosis induced with $A\beta_{1\text{-}42}$, while CCND2 showed the opposite effects (Figure 5C). Furthermore, miR-186-5p up-regulation partially restored the suppressive impacts of pc-WT1-AS on p-Tau as well as Cleaved caspase-3 expression, while CCND2 up-regulation exhibited the opposite effects (Figure 5D).

4. Discussion

With the in-depth study of lncRNAs, more and more researchers have shifted from the changes of lncRNA expressions in various diseases to the study of lncRNA

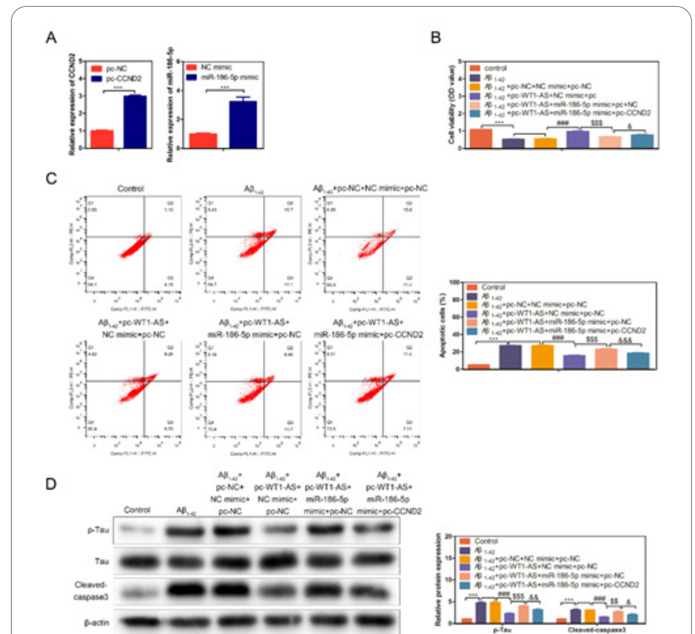

Fig. 5. WT1-AS regulates $A\beta_{1\text{-}42}$-induced SH-SY5Y cell proliferation and apoptosis via modulating miR-186-5p/CCND2 pathway. (A) Overexpression transfection efficiency of miR-186-5p/CCND2 from qRT-PCR assay. (B) CCK-8 assay assessed $A\beta_{1\text{-}42}$-stimulated human neuroblastoma cell proliferation after transfection. (C) Flow cytometry analysis measured $A\beta_{1\text{-}42}$-stimulated human neuroblastoma cells apoptosis after transfection. (D) p-Tau/Tau along with Cleaved-caspase-3 expression in $A\beta_{1\text{-}42}$-induced human neuroblastoma cells by western blot assay. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. Control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs*. $A\beta_{1\text{-}42}$ group, $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ *vs*. $A\beta_{1\text{-}42}$ + pc-WT1-AS + NC mimic + pc-NC group, $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$ *vs*. $A\beta_{1\text{-}42}$ + pc-WT1-AS + miR-186-5p mimic + pc-NC group.

functions and mechanisms [18]. In the study of blood and other tissues of AD patients, lncRNAs with different tables have been found continuously [21]. For example, lncRNA BACE1 levels presented up-regulation in AD patient's plasma. Accordantly, it was also validated that plasma BACE1 level presented elevation in AD patients [22]. Based on these, lncRNAs have great potential value as diagnostic markers in treating AD. Similarly, our research showed that WT1-AS was lowly expressed in AD serum samples, which was in consistent with previous study. These findings mirrored that lncRNAs might exert a vital role in progression of AD.

Aβ-treated human neuroblastoma cells have been reported for establishing AD models *in vitro* [23]. Consistently, an AD model in human neuroblastoma cells was also constructed through exposure of $Aβ_{1-42}$. As expected, $Aβ_{1-42}$ treatment inhibited the proliferation and induced apoptosis. Besides, Accumulating data has supported that Tau is a key element of AD pathophysiology [24]. In this research, we discovered that $Aβ_{1-42}$ treatment promoted p-Tau expression in human neuroblastoma cells. Functionally, former studies have reported that WT1-AS is implicated in tumor cell proliferation and apoptosis. Herein, WT1-AS over-expression notably elevated the proliferation, lessened the apoptosis, as well as decreased p-Tau protein expressions in $Aβ_{1-42}$ stimulated human neuroblastoma cells, which were in line with the reports about other lncRNAs on AD progression. For instance, MAGI2-AS3 levels were increased in $Aβ_{25-35}$ treated SH-SY5Y cells. MAGI2-AS3 depletion elevated neuronal viability while declined neuroinflammation in AD cell models [25]. Besides, BACE1-AS levels were up-regulated in human neuroblastoma cells upon $Aβ_{1-42}$ treatment. BACE1-AS silence relieved $Aβ_{1-42}$-stimulated cell injury [26]. In addition, $Aβ_{25-35}$ hindered human neuroblastoma cell viability whereas promoted cell apoptosis. SNHG1 depletion partially offsets the effects of $Aβ_{25-35}$ induction on cell viability and apoptosis. Report of Wang *et al*. reported that WT1-AS over-expression could repress OSI and apoptosis [27]. All above findings manifested that WT1-AS might work as a pathogenic factor in AD.

LncRNAs have been registered to serve as ceRNAs via regulating miRNA expression in AD progression. For example, BACE1-AS reduction rescued the suppressed proliferation and enhanced apoptosis of Aβ-treated human neuroblastoma cells stimulated by Aβ through regulating miR-214-3p. Besides, down-regulated NEAT1 reduced Aβ-stimulated inhibited viability as well as increased apoptosis and p-Tau levels via negatively regulating miR-107 [28]. More importantly, WT1-AS has also been proven to participate in the progression of diverse diseases via interacting with miRNAs [29, 30]. In line with the above literature, miR-186-5p was predicted to interact with WT1-AS in this research. MiR-186-5p has been documented to repress esophageal squamous cell carcinoma cell proliferation as well as gastric cancer cells aerobic glycolysis [31, 32]. Moreover, miR-186-5p has been confirmed to be decreased in the aged brain, which implies that miR-186-5p might be related to AD development [33]. As expected, miR-186-5p levels were confirmed highly expressed in AD serum samples and human neuroblastoma cells induced by $Aβ_{1-42}$. Functionally, miR-186-5p inhibitor notably enhanced the proliferation, lessened the apoptosis, as well as decreased the p-Tau protein expressions in $Aβ_{1-42}$ stimulated human neuroblastoma cells induced. Mechanistically, miR-186-5p over-expression partially restored the impacts of WT1-AS over-expression on biological activities of $Aβ_{1-42}$ stimulated human neuroblastoma cells. MiRNAs exhibit functional impacts usually through modulation of downstream targets in AD [34]. Consistently, our research predicted that miR-186-5p bond to CCND2 3'-UTR, which unveiled the potential of CCND2 to be the miR-186-5p target, and dual-luciferase reporter analysis further confirmed this prediction. CCND2, as a member of the cyclin proteins that regulate the progression of cells. CCND2 has been reported to modulate the progression of neurodegenerative diseases, AD included. For instance, tetrahydrocurcumin ameliorated Alzheimer's pathological phenotypes by regulating CCND2 [35]. In our research, CCND2 levels presented reduced in AD serum samples as well as human neuroblastoma cells induced with $Aβ_{1-42}$. Moreover, the modulatory mechanisms of WT1-AS/miR-186-5p/CCND2 in AD progression were investigated by rescue assays. As expected, CCND2 up-regulation partially restored miR-186-5p mimic functions in bioactivities of $Aβ_{1-42}$-induced human neuroblastoma cells transfected with pc-WT1-AS.

Additionally, our paper also presented some shortcomings. First of all, the samples of our study were relatively small. Besides, whether WT1-AS/miR-186-5p/CCND2 axis affected AD progression via certain signaling pathways was not investigated. Therefore, more studies will be performed in the future.

To sum up, WT1-AS and CCND2 levels were decreased, while miR-186-5p expression presented elevation in AD serum samples as well as human neuroblastoma cells induced by $Aβ_{1-42}$. Moreover, WT1-AS up-regulation notably promoted the proliferation, reduced the apoptosis, and decreased the p-Tau protein expressions in $Aβ_{1-42}$ stimulated human neuroblastoma cells, which might be achieved by regulating miR-186-5p/CCND2, indicating a novel avenue for AD therapy.

Informed Consent
The authors report no conflict of interest.

Availability of data and material
We declared that we embedded all data in the manuscript.

Authors' contributions
TY conducted the experiments and wrote the paper; ZX, WS, LL, WQ, LY and YY analyzed and organized the data; ZQ conceived, designed the study and revised the manuscript.

Funding
None.

Acknowledgement
We thanked The Affiliated Huai'an Hospital of Xuzhou Medical University approval our study.

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