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Circle RNA FOXP1 promotes cell proliferation in lung cancer by regulating miR-185-5p/Wnt1 signaling pathway

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Abstract. – OBJECTIVE: It is reported that circular RNA plays an important role in various cancers in recent years. However, there is less investigation reported in lung adenocarcinoma (LUAD) about circRNA. This study aims to explore the role and molecular mechanism of circle RNA FOXP1 in LUAD procession.

PATIENTS AND METHODS: The levels of circFOXP1 and miR-185-5p in LUAD cell lines and LUAD cancer samples were examined by RT-PCR. The functions of circFOXP1 and miR-185-5p at LUAD cells were detected by cell transfection of the overexpression or repression. The A549 and H1299 cell proliferation were detected by MTT assay and colony formation assay. And the cell apoptosis was detected by TUNEL assay. The expression levels WNT1 were measured by Western blot in A549 and H1299 cells. Furthermore, the luciferase assay detected the direct interaction between circFOXP1 and miR-185-5p or miR-185-5p and WNT1.

RESULTS: The circFOXP1 expression was increased in LUAD patients and LUAD cell lines. The downregulation of circFOXP1 significantly repressed LUAD cell proliferation and promoted cell apoptosis. Moreover, the luciferase assay results confirmed that circFOXP1 directly interacted with miR-185-5p. Overexpression of miR-185-5p could reverse the effect of circFOXP1 in LUAD cell. Besides, the luciferase results showed that miR-185-5p directly interacted with WNT1. miR-185-5p overexpression inhibited the WNT1 expression, while circFOXP1 repression decreased the WNT1 level in LUAD cell lines. The downregulating WNT1 could reverse the effects of miR-185-5p inhibition in LUAD cell lines. Furthermore, WNT1 was significantly upregulated in LUAD cancer tissues. In addition, circFOXP1 level was negatively correlated with miR-185-5p expression and positively correlated with WNT1 expression in LUAD cancer tissues.

CONCLUSIONS: These data suggested that circFOXP1 promoted cell proliferation and repressed cell apoptosis in LUAD by regulating miR-185-5p/WNT1 signaling pathway. It provides a novel potential therapeutic agent for the treatment of LUAD.

Key Words:
CircFOXP1, MiR-185-5p/WNT1 pathway, LUAD cancer, Cell proliferation.

Introduction

Lung cancer is one of the most common malignant tumors, which also has the highest death rate worldwide[1]. The risk factors of lung cancer include smoking, air pollution, occupational factors and chronic lung diseases[2,3]. Lung adenocarcinoma (LUAD) is a non-small cell carcinoma; its incidence accounts for 40% of primary lung tumors[4]. LUAD originates from the bronchial mucosal epithelium and is different from squamous cell lung cancer. LUAD is more likely to occur in women and non-smokers[5]. Currently, it is treated in the same way as lung cancer, including surgery, radiotherapy, chemotherapy and molecular targeted drug therapy (EGFR-TKI therapy)[6,7]. However, the molecular mechanism of LUAD is not fully understood.

Circular RNAs (circRNAs), a new type of long-stranded non-coding RNA, are characterized by covalently closed continuous rings with no polarity of 5' or 3' ends and are formed from exonic and intronic sequences[8,9]. It's widely distributed in thousands of species, including plants, animals and humans[10,11]. CircRNAs play import-

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ant roles in RNA alternative splicing, regulators of transcription and miRNA sponges[12]. Their functions are influenced by related miRNAs, and the circRNA-miRNA axis was found as potential functional modulators in a series of physiological and pathological processes including cell proliferation, differentiation, invasion and tumor progression[13,14]. Nevertheless, little is known about the mechanisms of action of circRNAs, including that in human LUAD cancer.

CircFOXP1, a newly identified circular RNA, was reported to promote gallbladder cancer progression by regulating PKLR expression[15]. And it was also reported that circFOXP1 induced by SOX9 to promote hepatocellular carcinoma progression by inhibiting miR-875-3p and miR-421 [16]. But the role of circFOXP1 in lung adenocarcinoma is unclear. Studies have shown that miR-185-5p has been identified as a tumor suppressor in some human cancers, including hepatocellular carcinoma[17], prostate cancer[18], melanoma[19] and so on. However, miR-185-5p is rarely reported in lung cancer.

Abnormal expression of the Wnt/β-catenin signaling pathway is common in human malignancies[20]. In non-small cell lung cancer (NSCLC), β-catenin and APC mutations are uncommon, but Wnt signal is very important in NSCLC cell lines. The inhibition of Wnt could reduce cell proliferation[21]. The recovery of WNT inhibitor function is associated with downregulated WNT signaling, decreased cell proliferation and increased apoptosis[22]. Wnt signaling may increase the resistance to cisplatin, docetaxel and radiotherapy, while Wnt inhibitors may restore the sensitivity[23]. The available data suggested that Wnt signaling has a substantial impact on the occurrence, prognosis and treatment tolerance of NSCLC.

This study aimed to investigate the function and mechanism of circFOXP1 (hsa_circ_0008234) which is significantly upregulated in LUAD tissues. CircFOXP1 sponged miR-185-5p, which promoted LUAD progression in GBC by targeting WNT1. Our results provide a new idea and a novel potential therapeutic target for the LUAD treatment.

Patients and Methods

Patients

LUAD cancer tissues and matched adjacent normal tissue samples were collected from 15 patients who underwent surgery in our hospital from 2016 to 2018, with an average age of 51.7 years. The patient, in clinical stage I-IV, was confirmed to have lesions that could be removed from pathological tissues before surgery. They all kept normal in cardiac, blood system, liver and kidney functions and did not receive any chemoradiotherapy before surgery. At the same time, they all were the primary lung cancer and did not have another type of cancer. The samples after surgery were stored in a -80°C ultra-low temperature refrigerator for extracting RNA and protein. Each patient signed a written informed consent before surgery. This research was approved by the Ethics Committee of our hospital.

Cell Culture

Human bronchial epithelial cell (BEAS-2B) and human LUAD cells (A549, H1299) were purchased from Cell Library of the Chinese Academy of Sciences (Shanghai, China). All cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) media (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen), 100 μg/mL penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and cultured at 37°C in a CO_2 incubator (5% CO_2 and 95% air, 95% humidity). The cells were cultured by passage after fusion of 70% to 80% and by transfection after fusion of 80% to 90%. BEAS-2B cell line served as normal control.

shRNA, Plasmid Construction and Cell Transfection

Cells were transfected using lipofectamine 3000 according to the manufacturer's instructions. shRNAs targeting mRNA to knockdown circFOXP1 and expression vector to overexpression circFOXP1 were synthesized by RiboBio (China). sh-circFOXP1#1 sense Oligonucleotide: CACCGAAAGGGAAAGGTTCCCGTGTCTTCAAGAGAGACACGGGAACCTTTCCCTTTTTTTTTG, sh-circFOXP1#2 sense Oligonucleotide: CACCGCTCCCAAAAGGGAAAGGTTCCTTCAAGAGAGGAACCTTTCCCTTTTTGGGAGTTTTTTG. sh-circFOXP1, miR-185-5p mimics or negative controls were transfected into A549 and H1299 cells (Qiagen, Hilden, Germany). And the infecting cells were collected at 72 h after transfection. Next, puromycin was used to screen cells for at least one week to obtain stable transfected cells.

MTT Assay

The transfected A549 and H1299 cells were plated into 96-well plates (5×10^3 cells/well) and cultured at 37°C in a CO_2 incubator for 24 h. The MTT (Solarbio) solution (dark) was added to each

well and incubated for 4 h. 150 µl dimethyl sulfoxide (DMSO) (Solarbio, Beijing, China) was added and centrifuged for 15 min after removing the medium. Plates were gently shaken to dissolve blue formazan crystals and absorbance was tested at 490 nm using a microplate reader (Thermo Scientific, Vario Skan Flash, Waltham, MA, USA). Cell survival rate (%) =OD (experimental group)/ OD (blank control group) ×100%. Wells containing only LUAD cells served as blanks. The experiment was repeated three times.

RT-PCR Assays

Real-Time Quantitative RT-PCR amplification was used to test the expression levels of circFOXP1 and miR-185-5p. The LUAD tissues and the cells in each treatment group were collected. The total RNAs were extracted with TRIzol and template cDNAs were synthesized with reverse transcription kit (TaKaRa, Otsu, Shiga, Japan). The PCR system was using the UItraSYBR Green qPCR Mixture reagents (TaKaRa, Otsu, Shiga, Japan). Each sample was repeated 3 times and amplified by the following protocol: 30 sec at 95°C for the initial denaturation, followed by 95°C for 5 sec and 60°C for 30 sec for 40 cycles. After the reaction, the amplification curve and dissolution curve of PCR were checked. Ct value was automatically output in the instrument software, and the $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels. The Q-PCR primers were designed by Ribobio (Guangzhou, China), circFOXP1 forward primer: 5'- CCACATGCCTCTACCAATGGA-3', circFOXP1 reverse primer: 5'- CAGCACTTGTTGCTGGAGGAT-3'; miR-185-5p reverse transcriptional primer: 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCAGGA-3', miR-185-5p Q-PCR forward primer: 5'-TGGAGAGAAAGGCAGT-3', miR-185-5p reverse primer: 5'- TGTCGTGGAGTCGGC-3'; GAPDH forward primer: 5'-CAGGAGGCATTGCTGATGAT-3', GAPDH reverse primer: 5'-GAAGGCTGGGGCTCATTT-3'. U6 forward primer: 5'- CTCGCTTCGGCAGCACA-3', U6 reverse primer: 5'- AACGCTTCACGAATTTGCGT-3'. GAPDH and U6 served as an endogenous control.

Western Blot Assays

Western blot analysis was used to assess the WNT1 protein level. The LUAD tissues and the cells transfected with sh-NC and shWNT1, sh-NC and sh-circFOXP1, miR-NC and miR-185-5p mimics were lysed by RIPA lysis buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The samples centrifuged at 12000 g for 10 min at 4°C and the concentrations were determined by the BCA Protein Assay Kit (Beyotime, China). Total proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Beyotime, Shangai, China) and transferred to 0.22 µm polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 4% FBS buffer for 2 h and incubated overnight with primary antibodies (WNT1 1:1000, β-actin 1:2500) (Cell Signaling Technology, Danvers, MA, USA) at 4°C after washed triple by TBST. The β-actin was served as an endogenous control. After TBST washing, the chemiluminescence (ECL) reagent (Cell Signaling Technology, Danvers, MA, USA) was exposed to detect the target protein straps and the ImageJ software was used to evaluate the signals of each protein band.

Luciferase Reporter Assay

Targetscan website was used to search for the potential target miRNAs of circFOXP1 or the potential target mRNAs of miR-185-5p. The partial sequences of circFOXP1 containing the putative binding sites of miR-185-5p were amplified by PCR and cloned to construct circFOXP1 wild-type (WT) reporter vector. The mutant miR-185-5p binding sites were constructed into circFOXP1 mutant-type (MUT) reporter vector by site-directed mutagenesis System (Thermo Fisher Scientific, Waltham, MA, USA). Then the constructed reporter vector was transfected into A549 and H1299 cells, respectively, together with miR-185-5p mimics or miR-NC. After transfected 48 h, luciferase activity of the cells was assayed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Statistical Analysis

The data were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) and reported as mean ± standard deviation of the number of experiments indicated. For all the data, one-way ANOVA analysis followed by a Student's *t*-test was used to assess the statistical significance of the difference between each group. LSD method was used as post-hoc test after validating ANOVA for pairwise comparisons. A statistically significant difference was considered at the level of $p < 0.05$.

Results

CircFOXP1 was Upregulated in LUAD Tissues and Cells

To examine whether circFOXP1 was associated with the tumorigenesis of LUAD, we detected circFOXP1 level in LUAD tissues firstly. Compared with adjacent normal tissues, circFOXP1 was significantly increased in LUAD tissues ($p<0.05$) (Figure 1A). In addition, the expression level of circFOXP1 in LUAD cell lines (H1299, H1975, A549) was strongly upregulated in normal BEAS-2B line ($p<0.05$) (Figure 1B). These data suggested that circFOXP1 was upregulated in LUAD tissues and cells.

CircFOXP1 Regulated Cell Proliferation and Apoptosis in Vitro

To explore the effect of circFOXP1 in LUAD, we transfected the circFOXP1 shRNAs (sh-circFOXP1#1, sh-circFOXP1#2) into A549 and H1299 cells for 24 h, 48 h, and 72 h. And the circFOXP1 levels were extremely lower than transfected sh-NC ($p<0.05$) (Figure 2A). Next, we used the MTT assay and colony formation assay experiment to test the cell proliferation after circFOXP1 downregulation. The results showed that the proliferative ability of A549 and H1299 cells was significantly repressed by circFOXP1 silence ($p<0.05$) (Figure 2B-C). Moreover, the TUNEL staining showed that the circFOXP1 repression extremely upregulated the cell apoptosis in lung cancer cell lines ($p<0.05$) (Figure 2D). In short, the circFOXP1 expression promoted cell proliferation and suppressed cell apoptosis in LUAD.

CircFOXP1 Acts as a Sponge for miR-185-5p

In order to investigate the molecular mechanism of circFOXP1 in LUAD, we detected the location of circFOXP1 in A549 and H1299 cells by using nuclear-cytoplasmic fractionation. And the results showed that circFOXP1 was mainly distributed in the cytoplasm of LUAD cells (Figure 3A), which means the regulatory role of circFOXP1 was in post-transcription. Thus, we speculated that circFOXP1 was a ceRNA to sponge certain miRNAs. Therefore, we used the starBase data to search the potential miRNAs for circFOXP1 and getting two miRNAs that could bind to circFOXP1. The luciferase assay showed that miR-185-5p was directly binding with circFOXP1 (Figure 3B-D). In addition, we also detected that circFOXP1 expression was downregulated in

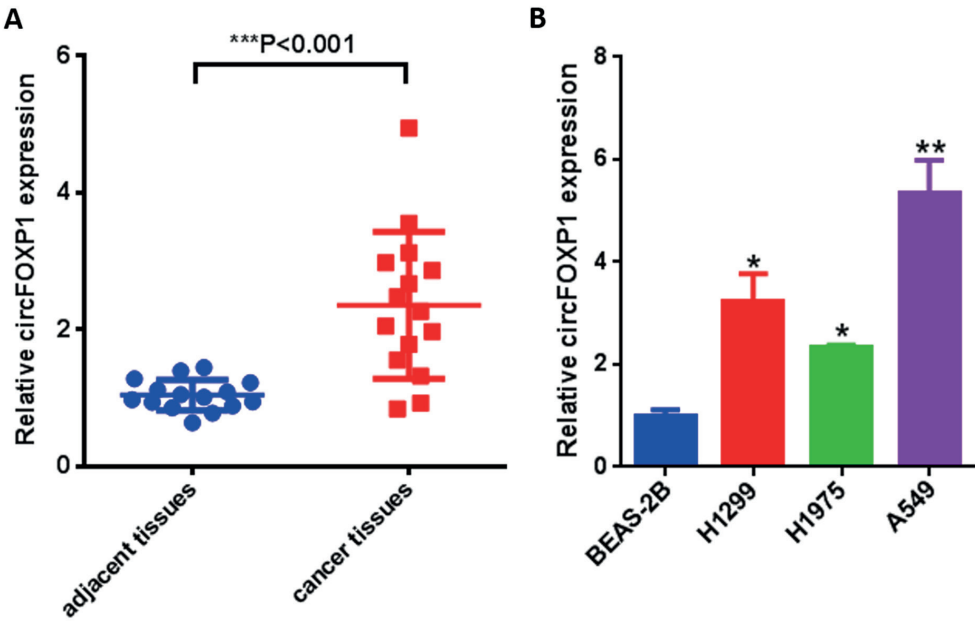

Figure 1. The expression of circFOXP1 in LUAD tissues and cell lines. **(A)** The circFOXP1 level in LUAD cancer tissues and adjacent normal tissues was detected by RT-PCR assay. **(B)** The circFOXP1 expression levels in three LUAD cell lines were detected by RT-PCR. Adjacent tissues or BEAS-2B served as the control group. Data are present the mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$(ANOVA).

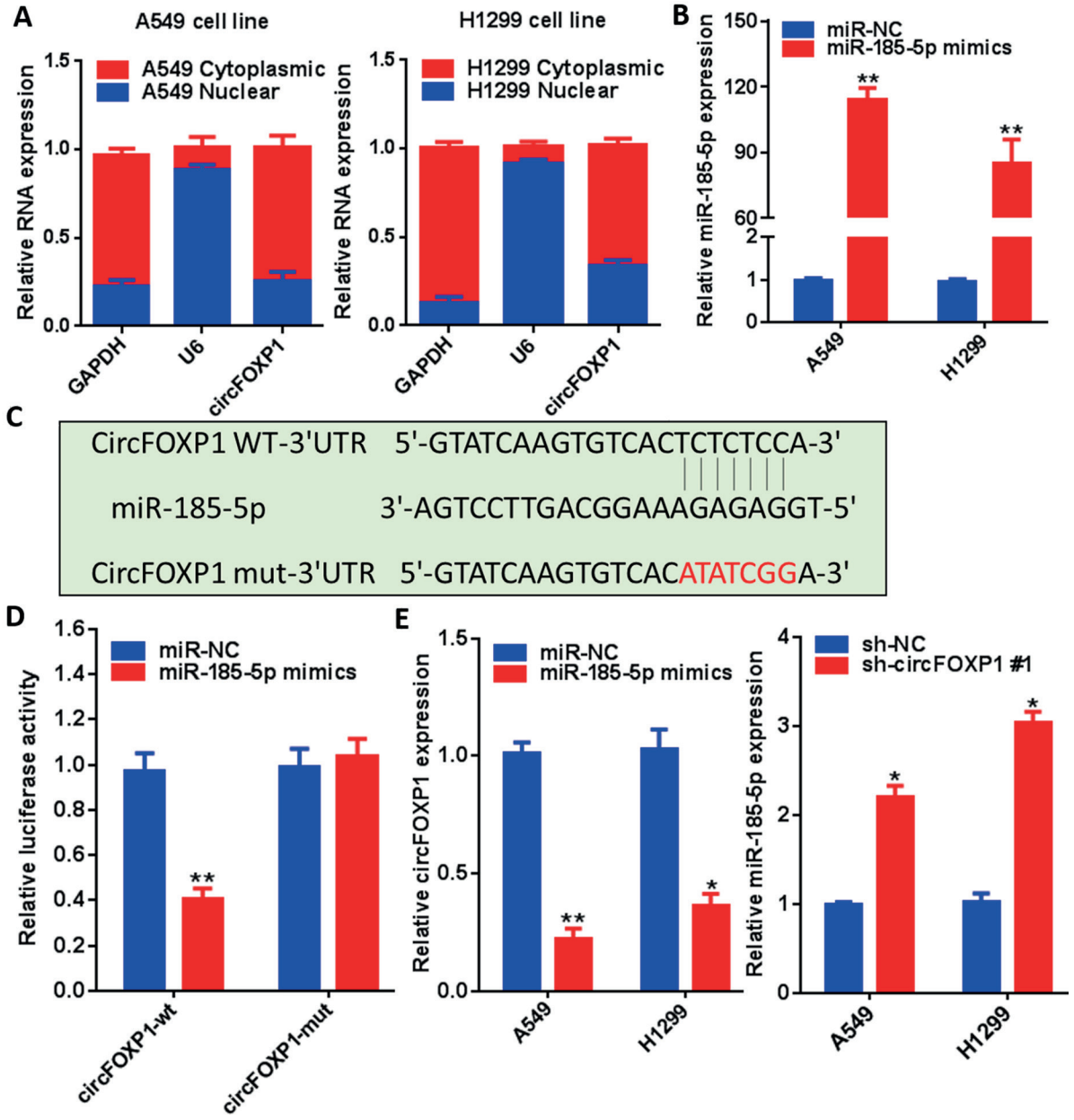

Figure 2. The circFOXP1 promotes cell survival in LUAD cell lines. **(A)** The circFOXP1 expression levels in A549 cells and H1299 cells were evaluated by RT-PCR assay. **(B)** The A549 and H1299 cells growth after circFOXP1 repression were indicated by MTT assay. **(C)** The cell proliferation ability after downregulation of circFOXP1 in A549 and H1299 cells was also detected by colony formation assay. **(D)** The cell apoptosis in A549 and H1299 cells after circFOXP1 repressed was determined by TUNEL assay. sh-NC served as the control group. Data are present the mean ± SD of three independent experiments. *p<0.05, **p<0.01(ANOVA).

miR-185-5p overexpression cells and the miR-185-5p expression was upregulated significantly in circFOXP1 repression cells (p<0.05) (Figure 3E). These results suggested that circFOXP1 acted as a sponge of miR-185-5p.

CircFOXP1 Function Is Partially Mediated by Repressing miR-185-5p

To explore the effect of miR-185-5p on cell viability after circFOXP1 overexpression, the cells were transfected with NC, circFOXP1, circFOX-

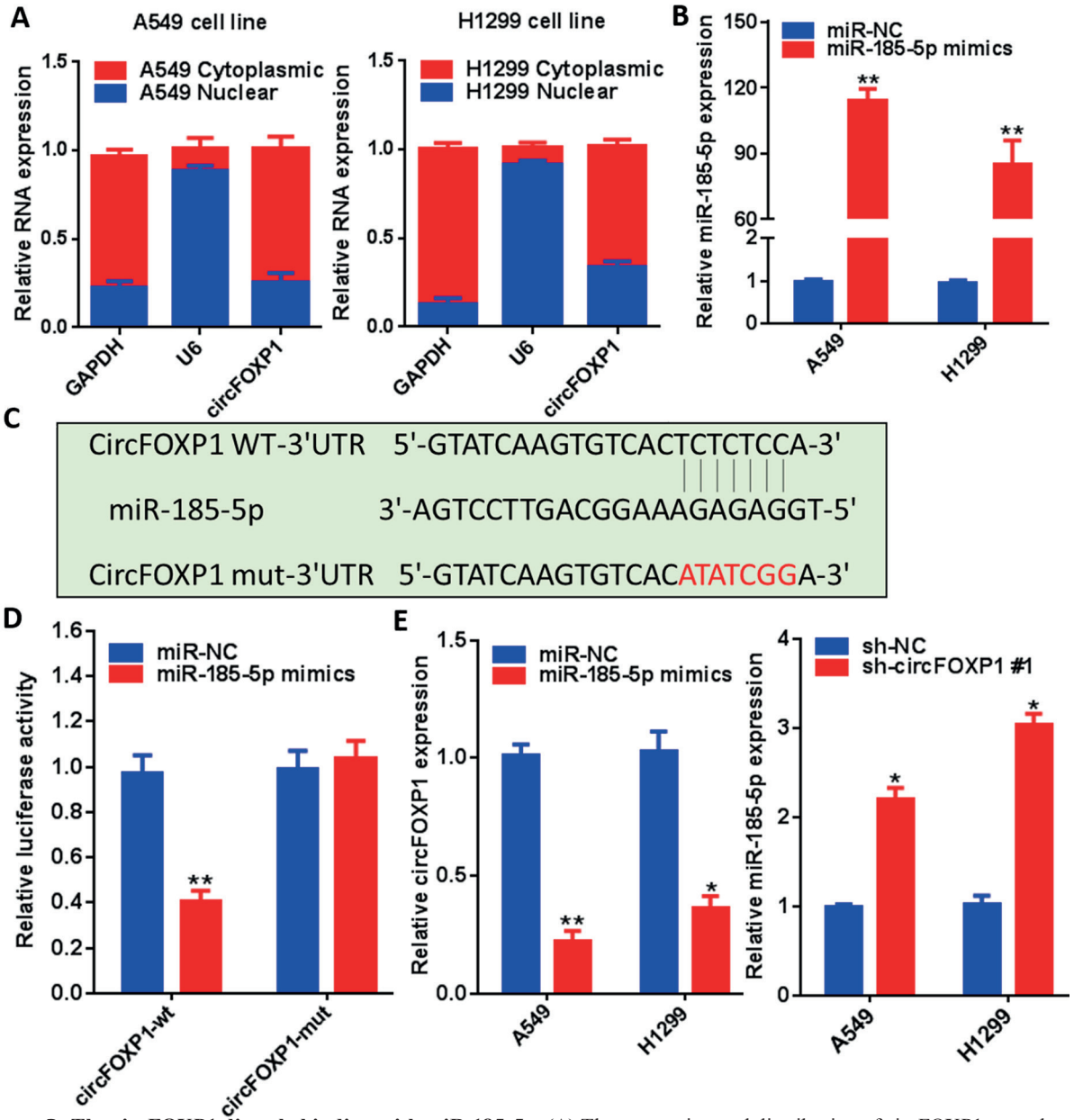

Figure 3. The circFOXP1 directly binding with miR-185-5p. (A) The expression and distribution of circFOXP1 at nuclear and cytoplasmic at A549 and H1299 cells were evaluated by RT-PCR. **(B)** The expression of miR-185-5p after transfected the miR-185-5p mimics in A549 and H1299 cells was detected by RT-PCR. **(C)**The binding sequence of circFOXP1 to miR-185-5p by starBase v2.0 database. **(D)** The circFOXP1 directly binding with miR-185-5p by luciferase assay. **(E)** The level of circFOXP1 in miR-185-5p overexpression cells and the level of miR-185-5p in circFOXP1 repression cells were assayed by RT-PCR. miR-NC or sh-NC as the control group. Data are present as the mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$.

P1+miR-NC, circFOXP1+ miR-185-5p mimics. The MTT assay and colony formation assay results showed that upregulation of circFOXP1 increased the LUAD cell lines survival rate, while the miR-185-5p expression could reversed this effect (Figure 4A-C). Then, we demonstrated the effect of miR-185-5p on cell apoptosis. The TUNEL staining results showed that overexpression of circFOXP1 decreased the cell apoptosis in A549 and H1299 cells, while the miR-185-5p expression increased cell apoptosis obviously ($p<0.05$) (Figure 4D). Those results indicated that the overexpression of circFOXP1 promoted LUAD cells survival by negatively regulating miR-185-5p.

WNT1 Is a Direct Target of miR-185-5p

To further support ceRNA hypothesis, we searched the downstream genes of miR-185-5p in StarBase database. Then we detected the interaction and expression of WNT1 in miR-185-5p overexpression cells. The luciferase assay result showed miR-185-5p binding with WNT1 (Figure 5A-B) ($p<0.05$). Besides, the WNT1 levels were significantly down-regulated in LUAD cell lines after overexpression of miR-185-5p or after repression circFOXP1 (Figure 5C-D) ($p<0.05$), indicating that WNT1 was regulated by miR-185-5p and circFOXP1. The overexpression of miR-185-5p inhibited the WNT1 expression and the circFOXP1 repression also inhibited the WNT1 expression. These results suggested that WNT1 were regulated by circFOXP1/miR-185-5p and may involve in LUAD.

circFOXP1/miR-185-5p/WNT1 Axis Drives LUAD Progression

To further verify whether circFOXP1 increased LUAD progression through miR-185-5p/WNT1 signal axis, we adopted some rescue experiments to confirm it. Firstly, we transfected sh-WNT1 in A549 and H1299 cells, and the Western blot

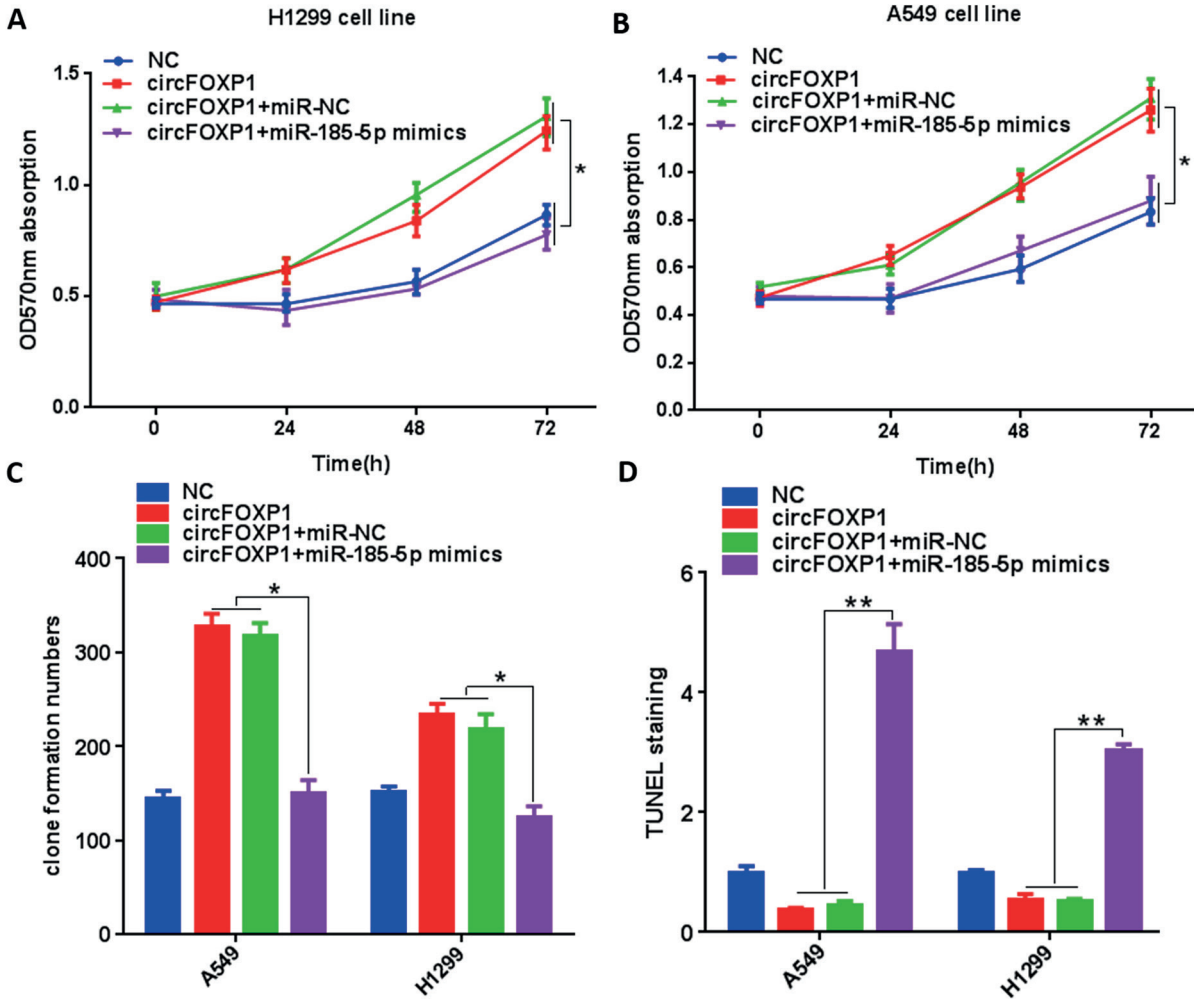

Figure 4. miR-185-5p reverses the circFOXP1-mediated cell survival in LUAD cell lines. **(A-B)** The A549 and H1299 cells survival were measured by MTT assay after transfected with the NC, circFOXP1, circFOXP1+ miR-NC, circFOXP1+ miR-185-5p. **(C)** The cell proliferation ability was examined by colony formation assay after transfected with the NC, circFOXP1, circFOXP1+ miR-NC, circFOXP1+ miR-185-5p in A549 and H1299 cells. **(D)** The cell apoptosis in A549 and H1299 cells was detected by TUNEL assay after transfected with the NC, circFOXP1, circFOXP1+ miR-NC, circFOXP1+ miR-185-5p. NC or circFOXP1+ miR-NC served as the control group. Data are present as the mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$ (ANOVA).

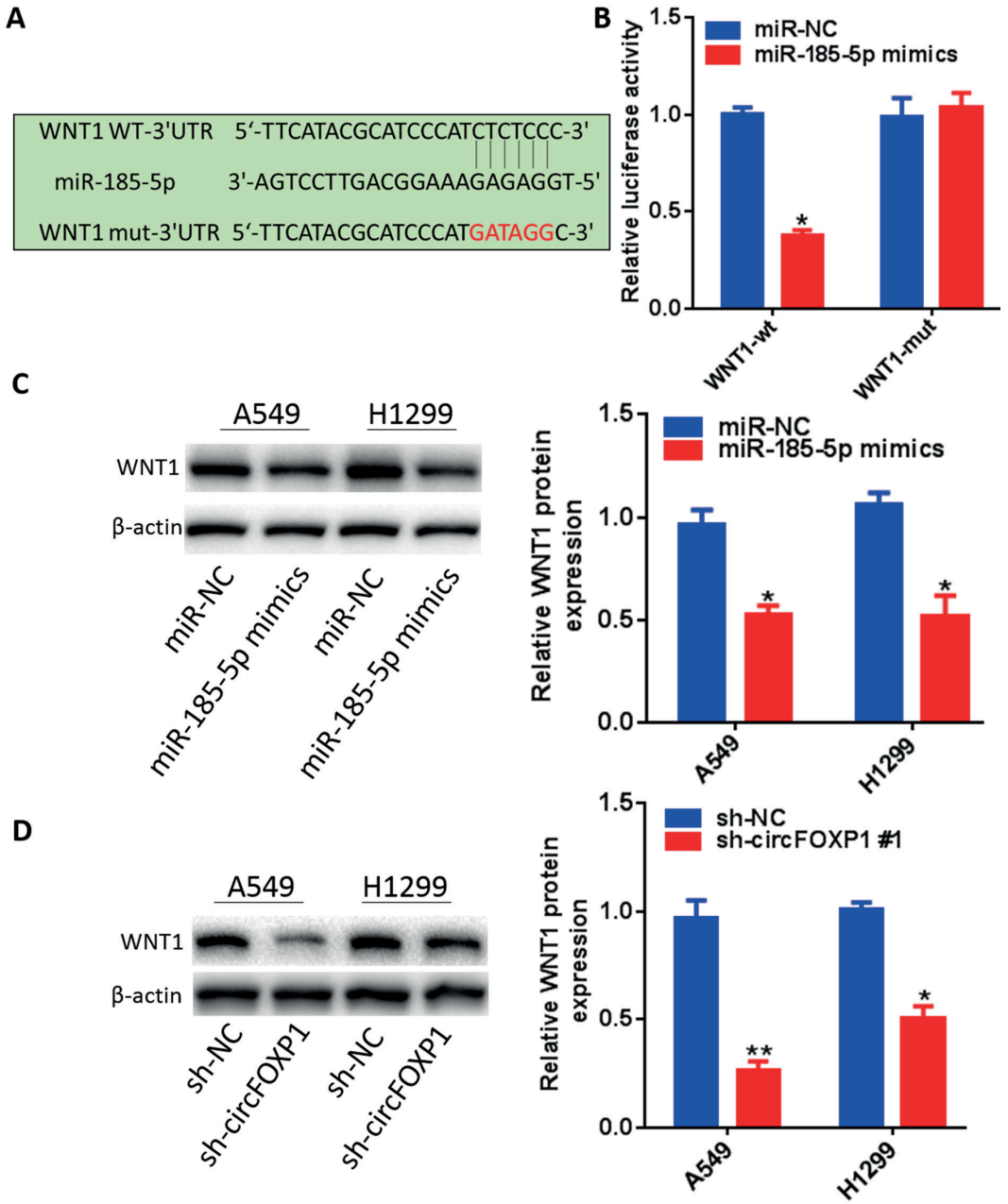

Figure 5. The WNT1 directly binding with miR-185-5p. **(A)** The binding sequence of WNT1 to miR-185-5p by starBase v2.0 database. **(B)** The WNT1 directly binding with miR-185-5p by luciferase assay. **(C-D)** The levels of WNT1 in miR-185-5p overexpression cells and in circFOXP1 repression cells were detected by Western Blot. miR-NC or sh-NC as the control group. Data are present as the mean ± SD of three independent experiments. *p<0.05, **p<0.01.

result showed that WNT1 expression levels were downregulated (Figure 6A). The MTT and colony formation assays results showed that WNT1 repression reversed the promotive effect of miR-185-5p inhibitor on the proliferation of circFOXP1 downregulated LUAD cells (Figure 6B-D). In addition, the TUNEL assay result showed that the cell apoptosis repressed by miR-185-5p inhibition was recovered by transfecting sh-WNT1 in circFOXP1 silenced LUAD cells (Figure 6E). In other words, circFOXP1/miR-185-5p/WNT1 axis could promote LUAD progression.

The Relationship Between circFOXP1 and WNT1 or miR-185-5p in LUAD Tissues

To confirm the relationship of circFOXP1 and WNT1 or miR-185-5p in LUAD cancer tissues,

the expression levels of WNT1 and miR-185-5p were performed. The western blot result showed that WNT1 protein level was significantly increased in LUAD cancer tissues compared with the adjacent tissues (Figure 7A). Besides, miR-185-5p was significantly downregulated in LUAD cancer tissues (Figure 7B). Furthermore, we calculated the correlation between miR-185-5p and

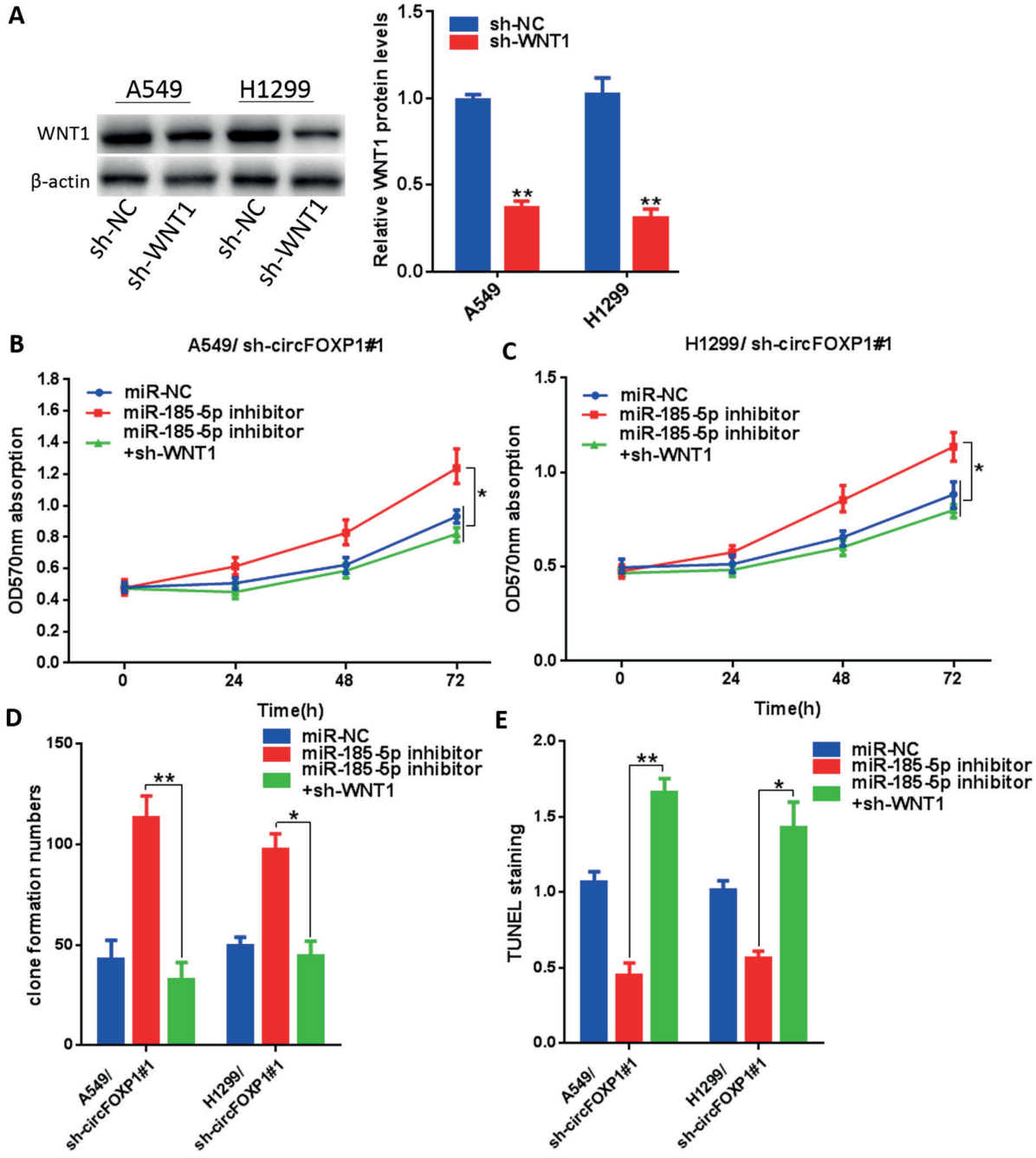

Figure 6. CircFOXP1 drives LUAD progression via miR-185-5p/WNT1 signaling. **(A)** WNT1 protein level in sh-WNT1 transfected A549 and H1299 cells was detected by Western blot assay. **(B-C)** The proliferation of circFOXP1 silenced in A549 and H1299 cells was tested after transfecting miR-NC, miR-185-5p inhibitor, miR-185-5p inhibitor + sh-WNT1 by MTT assay. **(D)** The cell proliferation of circFOXP1 silenced in A549 and H1299 cells after transfecting miR-NC, miR-185-5p inhibitor, miR-185-5p inhibitor + sh-WNT1 was also measured by colony formation assay. **(E)** The cell apoptosis of circFOXP1 silenced in A549 and H1299 cells was tested after transfecting miR-NC, miR-185-5p inhibitor, miR-185-5p inhibitor + sh-WNT1 by TUNEL assay. Data are present the mean ± SD of three independent experiments. *p<0.05, **p<0.01.

circFOXP1 expressions, between WNT1 and circFOXP1 expressions. The results showed that circFOXP1 level was negatively correlated with miR-185-5p expression and positively correlated with WNT1 expression in LUAD cancer tissues (Figure 7C-D).

Discussion

It is reported that circle RNA plays an important role in many cancer processes. Although the number of circRNAs which known functions are increasing, there are still thousands of circRNAs with functions that remain unknown. CircFOXP1, a new circle RNA, plays some crucial roles in the tumor progression in a series of cancers, such as in gallbladder cancer [15] and hepatocellular carcinoma [16]. In spite of some advanced findings, the expression and possible carcinogenic involvement of circFOXP1 in LUAD remains unknown. Therefore, making a better understanding of circFOXP1 may be useful as special markers or therapeutic targets in the diagnosis and treatment of

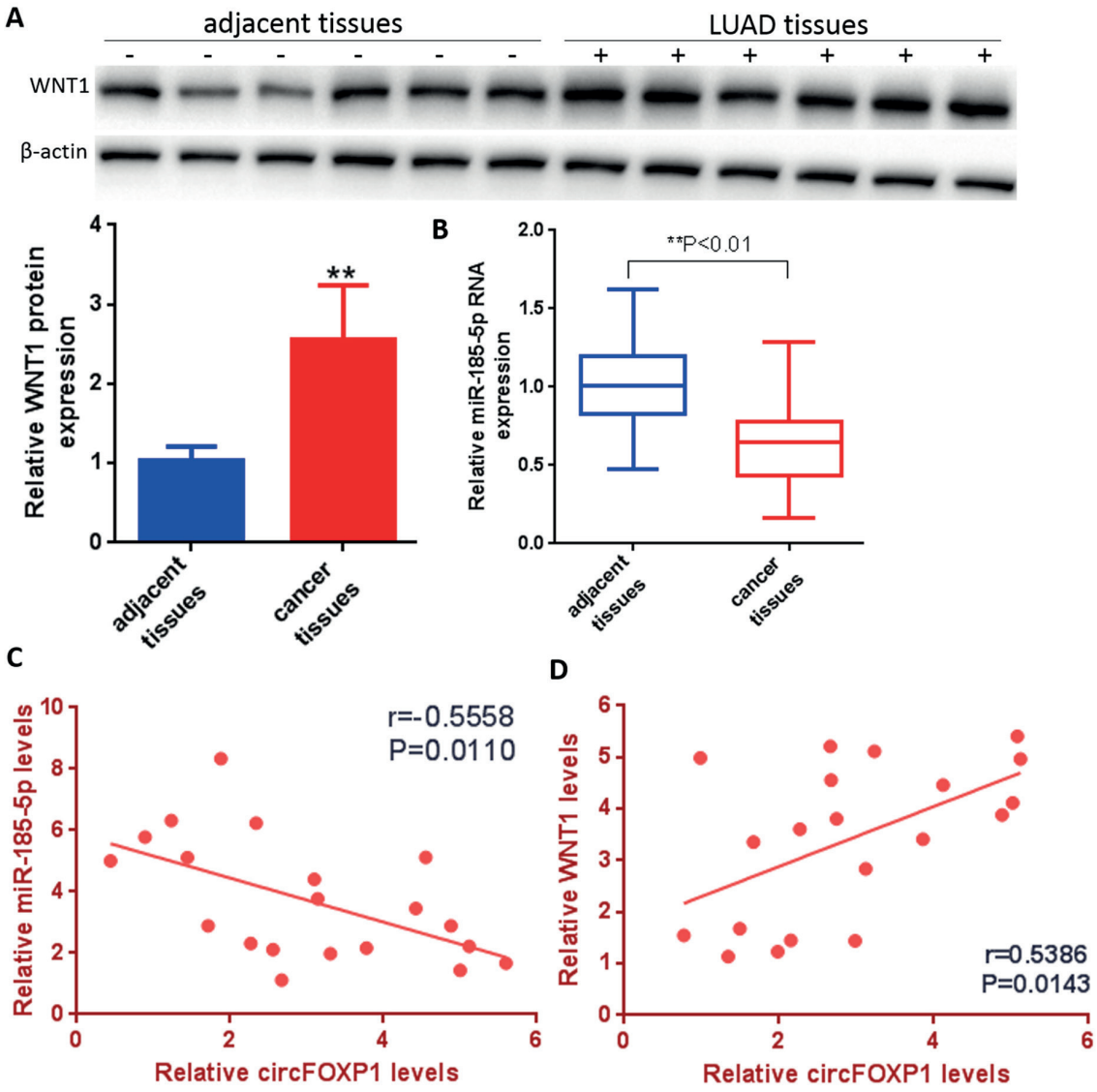

Figure 7. The relationship between circFOXP1 and WNT1/miR-185-5p in LUAD tissues. **(A)** WNT1 protein level in LUAD cancer tissues was detected by Western blot assay. **(B)** miR-185-5p expression in LUAD cancer tissues was assayed by RT-PC. **(C)** The relationship between circFOXP1 and miR-185-5p in LUAD tissues. **(D)** The relationship between circFOXP1 and WNT1 in LUAD tissues. Data are present as the mean ± SD of three independent experiments. *p<0.05, **p<0.01.

LUAD. In this report, the function and molecular mechanism of circFOXP1 in LUAD were detected and discussed. Our study is the first report that circFOXP1 was highly upregulated in LUAD tissues and cells. Furthermore, the downregulation of circFOXP1 repressed cell proliferation and increased cells apoptosis in A549 and H1299 cells. These results suggested that circFOXP1 serves as an oncogene and may act as a prognostic biological marker in LUAD.

Mechanistic studies revealed that circFOXP1 performed its tumor-promoting roles by sponging miR-185-5p to modulate WNT1 expression level. miR-185-5p was always reported as a tumor suppressor. Niu et al[24] showed that miR-185-5p inhibited cell migration and invasion of hepatocellular carcinoma by targeting ROCK2. Pei et al[25] showed that miR-185-5p modulated chemosensitivity of human non-small cell lung cancer by targeting ABCC1. Besides, miR-185-5p mimics could decrease cell proliferation, migration, invasion, stemness and EMT in glioma cells[26]. Herein, we detected the effects of circFOXP1 on miR-185-5p expression and found downregulated circFOXP1 significantly increased miR-185-5p level. Furthermore, miR-185-5p was downregulated in LUAD tissues and reversed the effects of circFOXP1 in LUAD cell lines, which is consistent with previous reports.

WNT1, a member of the proto-oncogene family, which is very conserved in evolution, has been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis. In NSCLC, Wnt1 overexpression affected the tumor proliferation and was a significant prognostic factor[27]. Xu et al[28] observed aberrant Wnt1/β-catenin expression was an independent poor prognostic marker in NSCLC after surgery. In our result, WNT1 was binding with miR-185-5p and regulated by miR-185-5p and circFOXP1. The WNT1 inhibition could reverse the effects of miR-185-5p, which decreased the LUAD cell proliferation and increased cell apoptosis. In addition, WNT1 expression was significantly upregulated in LUAD tissues. It is consistent with previous reports that miRNA-185 suppresses migration and invasion by regulating Wnt1 in colon cancer [29].

It is shown that endogenous circRNAs could act as miRNA sponges, which mean circRNAs can bind with miRNAs and inhibit their function, showing another mechanism that regulates miRNA activity [30]. Chen et al[31] reported circRNA_100290 sponging the miR-29 family in oral cancer. Han et al[32] showed circMTO1 acts as the sponge of miR-9 to suppress hepatocellular carcinoma progression. Moreover, Chen et al[33] showed has_circ_100395 inhibited lung cancer progression by regulated miR-1228/TCF21. In our study, circFOXP1 acted as miR-185-5p sponges to promote LUAD procession by regulating WNT1 expression. Our research indicated that circFOXP1 plays an oncogene role and may act as a prognostic biological marker in LUAD.

Conclusions

Circle RNA FOXP1 served as miR-185-5p sponges and regulated the LUAD procession through upregulating WNT1 expression level. Our study provides a new insight for understanding the molecular mechanisms and therapeutic target for LUAD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Skřičková J, Kadlec B, Venclíðek O, Merta Z. Lung cancer. Cas Lek Cesk 2018; 157: 226-236.
2) Malhotra J, Malvezzi M, Negri E, La Vecchia C, Boffetta P. Risk factors for lung cancer worldwide. Eur Respir J 2016; 48: 889-902.
3) de Groot P, Munden RF. Lung cancer epidemiology, risk factors, and prevention. Radiol Clin North Am 2012; 50: 863-876.
4) Mao Y, Yang D, He J, Krasna MJ. Epidemiology of lung cancer. Surg Oncol Clin N Am 2016; 25: 439-445.
5) Pascoe HM, Knipe HC, Pascoe D, Heinze SB. The many faces of lung adenocarcinoma: A pictorial essay. J Med Imaging Radiat Oncol 2018; 62: 654-661.
6) Denisenko TV, Budkevich IN, Zhivotovsky B. Cell death-based treatment of lung adenocarcinoma. Cell Death Dis 2018; 9: 117.
7) Lemjabbar-Alaoui H, Hassan OU, Yang YW, Buchanan P. Lung cancer: Biology and treatment options. Biochim Biophys Acta 2015; 1856: 189-210.
8) Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, Chen D, Gu J, He X, Huang S. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. Cell Res 2015; 25: 981-984.
9) Salzman J. Circular RNA expression: its potential regulation and function. Trends Genet 2016; 32: 309-316.

10) Meng X, Li X, Zhang P, Wang J, Zhou Y, Chen M. Circular RNA: an emerging key player in RNA world. Brief Bioinform 2017; 18: 547-557.

11) Chen I, Chen CY, Chuang TJ. Biogenesis, identification, and function of exonic circular RNAs. Wiley Interdiscip Rev RNA 2015; 6: 563-579.

12) Lasda E, Parker R. Circular RNAs: diversity of form and function. RNA 2014; 20: 1829-1842.

13) Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, Luo Y, Lyu D, Li Y, Shi G, Liang L, Gu J, He X, Huang S. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. Nat Commun 2016; 7: 11215.

14) Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Res 2016; 44: 2846-2858.

15) Wang S, Zhang Y, Cai Q, Ma M, Jin LY, Weng M, Zhou D, Tang Z, Wang JD, Quan Z. Circular RNA FOXP1 promotes tumor progression and Warburg effect in gallbladder cancer by regulating PKLR expression. Mol Cancer 2019; 18: 145.

16) Wang W, Li Y, Li X, Liu B, Han S, Li X, Zhang B, Li J, Sun S. Circular RNA circ-FOXP1 induced by SOX9 promotes hepatocellular carcinoma progression via sponging miR-875-3p and miR-421. Biomed Pharmacother 2020; 121: 109517.

17) Niu Y, Tang G. miR-185-5p targets ROCK2 and inhibits cell migration and invasion of hepatocellular carcinoma. Oncol Lett, 2019; 17: 5087-5093.

18) Tian C, Deng Y, Jin Y, Shi S, Bi H. Long non-coding RNA RNCR3 promotes prostate cancer progression through targeting miR-185-5p. Am J Transl Res 2018; 10: 1562-1570.

19) Chen X, Gao J, Yu Y, Zhao Z, Pan Y. Long non-coding RNA UCA1 targets miR-185-5p and regulates cell mobility by affecting epithelial-mesenchymal transition in melanoma via Wnt/β-catenin signaling pathway. Gene 2018; 676: 298-305.

20) Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. Oncogene 2017; 36: 1461-1473.

21) Stewart DJ. Wnt signaling pathway in non-small cell lung cancer. J Natl Cancer Inst 2014; 106: djt356.

22) Huang CL, Liu D, Ishikawa S, Nakashima T, Nakashima N, Yokomise H, Kadota K, Ueno M. Wnt1 overexpression promotes tumour progression in non-small cell lung cancer. Eur J Cancer 2008; 44: 2680-2688.

23) Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell 2017; 169: 985-999.

24) Niu Y, Tang G. MiR-185-5p targets ROCK2 and inhibits cell migration and invasion of hepatocellular carcinoma. Oncol Lett 2019; 17: 5087-5093.

25) Pei K, Zhu JJ, Wang CE, Xie QL, Guo JY. MicroRNA-185-5p modulates chemosensitivity of human non-small cell lung cancer to cisplatin via targeting ABCC1. Eur Rev Med Pharmacol Sci 2016; 20: 4697-4704.

26) Shen F, Chang H, Gao G, Zhang B, Li X, Jin B. Long noncoding RNA FOXD2-AS1 promotes glioma malignancy and tumorigenesis via targeting miR-185-5p/CCND2 axis. J Cell Biochem 2019; 120: 9324-9336.

27) Nakashima T, Liu D, Nakano J, Ishikawa S, Yokomise H, Ueno M, Kadota K, Huang CL. Wnt1 overexpression associated with tumor proliferation and a poor prognosis in non-small cell lung cancer patients. Oncol Rep 2008; 19: 203-209.

28) Xu X, Sun PL, Li JZ, Jheon S, Lee CT, Chung JH. Aberrant Wnt1/β-catenin expression is an independent poor prognostic marker of non-small cell lung cancer after surgery. J Thorac Oncol 2011; 6: 716-724.

29) Zhang W, Sun Z, Su L, Wang F, Jiang Y, Yu D, Zhang F, Sun Z, Liang W. miRNA-185 serves as a prognostic factor and suppresses migration and invasion through Wnt1 in colon cancer. Eur J Pharmacol 2018; 825: 75-84.

30) Panda AC. Circular RNAs act as miRNA sponges. Adv Exp Med Biol 2018; 1087: 67-79.

31) Chen L, Zhang S, Wu J, Cui J, Zhong L, Zeng L, Ge S. circRNA_100290 plays a role in oral cancer by functioning as a sponge of the miR-29 family. Oncogene 2017; 36: 4551-4561.

32) Han D, Li J, Wang H, Su X, Hou J, Gu Y, Qian C, Lin Y, Liu X, Huang M, Li N, Zhou W, Yu Y, Cao X. Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression. Hepatology 2017; 66: 1151-1164.

33) Chen D, Ma W, Ke Z, Xie F. CircRNA hsa_circ_100395 regulates miR-1228/TCF21 pathway to inhibit lung cancer progression. Cell Cycle 2018; 17: 2080-2090.