Original Article

miR-140-5p inhibits proliferation, invasion, and promote apoptosis of non-small cell lung cancer cells through targeted inhibition of Wnt signaling pathway

Xiaobo Zhu1*, Hongyan Deng2*, Yueqiu Zhang3, Shijie Liu4

1Department of Oncology, The Second People Hospital of Dezhou, Dezhou, Shandong Province, China; 2Department of Oncology, Dezhou Hospital of Traditional Chinese Medicine, Dezhou, Shandong Province, China; 3Department of Respiration, Laoling People’s Hospital, Dezhou, Shandong Province, China; 4Department of Emergency, Yantai Municipal Laiyang Central Hospital, Yantai, Shandong Province, China. *Equal contributors and co-first authors.

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Abstract: Objective: To investigate the regulation mechanism of miR-140-5p on proliferation, invasion and apoptosis of non-small cell lung cancer (NSCLC) cells through Wnt signaling pathway. Methods: NSCLC cell line H1975 was subjected to cell culture, grouping and transfection. The cells were divided into five groups: Blank group, NC group, miR-140-5p mimic group, pcDNA3.1-Wnt1 group and miR-140-5p mimic + pcDNA3.1-Wnt1 group. The targeting relationship between miR-140-5p and Wnt1 was confirmed using dual luciferase reporter assay. The mRNA and protein expression levels of Wnt1, β-catenin, TCF-4, PCNA, E-cadherin, N-cadherin, Bcl-2 and Bax in each group were detected using quantitative real-time PCR (qRT-PCR) and Western blot. Cell viability of each group was determined using MTT assay. Transwell assay was used to detect the invasive ability and flow cytometry was used to detect cell cycle and apoptosis in each group. Results: Compared with the normal human lung epithelial cell line Beas-2B, the expression of Wnt1 was significantly up-regulated in lung cancer cell lines (P<0.05), with the highest expression in H1975 cells. miR-140-5p specifically inhibited Wnt1 expression. There were no significant differences between the blank group and the NC group regarding all measured indices (all P>0.05). Compared with the blank group, the mRNA and protein expression levels of Wnt1, β-catenin, TCF-4, PCNA, E-cadherin, N-cadherin, Bcl-2 and Bax in the miR-140-5p mimic group significantly decreased (all P<0.05), and the expression of E-cadherin and Bax significantly increased in the miR-140-5p mimics group (both P<0.05); meanwhile, the cell viability and invasion ability decreased, and the cell apoptosis increased in the miR-140-5p mimic group compared with the blank group (all P<0.05); moreover, the proportion of cells in G1 phase increased and the proportion of cells in S phase decreased significantly in the miR-140-5p mimic group compared with the blank group (both P<0.05). Compared with the blank group, the mRNA and protein expression levels of Wnt1, β-catenin, TCF-4, PCNA, N-cadherin and Bcl-2 in the pcDNA3.1-Wnt1 group significantly increased (all P<0.05), and the expression of E-cadherin and Bax significantly decreased in the pcDNA3.1-Wnt1 group (both P<0.05); meanwhile, the cell viability and invasion ability increased, and the cell apoptosis decreased in the pcDNA3.1-Wnt1 group compared with the blank group (all P<0.05); moreover, the proportion of cells in G1 phase decreased and the proportion of cells in S phase increased significantly in the pcDNA3.1-Wnt1 group compared with the blank group (both P<0.05). There are no differences between the miR-140-5p mimic + pcDNA3.1-Wnt1 group and blank group regarding cell invasion ability and cell apoptosis rate (both P>0.05). Conclusion: Overexpression of miR-140-5p inhibits the activation of Wnt signaling pathway by downregulating its target gene Wnt1, thereby inhibiting the proliferation, invasion, and promoting the apoptosis of NSCLC cells.

Keywords: miR-140-5p, Wnt1 gene, Wnt signaling pathway, non-small cell lung cancer, proliferation, apoptosis, invasion

Introduction

Lung cancer is one of the most common and life-threatening cancers in the world. Among all types of lung cancer, non-small cell lung cancer (NSCLC) is the most common one, accounting for more than 80% of lung cancer patients [1-3]. At present, the clinical treatment of NSCLC mainly relies on radiotherapy, chemotherapy and targeted therapy; however, none of those
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Current research indicates that Wnt signaling pathway regulates multiple life activities. In the field of oncology, Wnt signaling pathway is considered to be mainly involved in tumor occurrence, development, invasion, apoptosis, etc. [7-10]. The overexpression of Wnt signaling pathway significantly increases the occurrence of NSCLC; moreover, the expression of Wnt signaling pathway is found to be up-regulated in NSCLC. Previous studies have confirmed that the overexpression of Wnt1 could promote tumor growth, invasion, and inhibit tumor apoptosis [11-13]. In the classic Wnt signaling pathway theory, the activation of Wnt signaling pathway promotes the recruitment of β-catenin, which is transferred to intracellular signals and in turn affects tumor proliferation, invasion, and apoptosis [14-16]. MicroRNA is a research hotspot in recent years, especially in the field of oncology. In an organism, the same microRNA can act on multiple target genes, and multiple microRNAs can also act on the same target gene [17, 18]. According to the literature, multiple microRNAs, such as miR-148a-3p, miR-513b, miR-142-3p, etc. can affect tumor cell activities including proliferation, migration and invasion. The upstream microRNAs of the Wnt signaling pathway have not been fully defined [19-21]. Through bioinformatics analysis we found that there is a putative binding site between miR-140-5p and Wnt1. In addition, multiple previous studies demonstrated that miR-140-5p could inhibit the proliferation and epithelial-mesenchymal transition of NSCLC [22, 23]. However, there is no literature to confirm that miR-140-5p regulates the Wnt signaling pathway. Therefore, we hypothesized that miR-140-5p can inhibit the proliferation, invasion, and apoptosis of NSCLC cells by inhibiting the Wnt signaling pathway.

In this study, we used NSCLC cell line H1975 to explore the relationship between miR-140-5p and Wnt signaling, in an effort to reveal the effects of miR-140-5p and the Wnt signaling pathway on the proliferation, invasion, and apoptosis of NSCLC cells.

Materials and methods

Dual luciferase reporter assay

The bioinformatics analysis was performed using online software http://www.targetscan.org, and dual luciferase reporter assay was performed to verify whether Wnt1 is a target gene of miR-140-5p. The 3'UTR region of the Wnt1 gene was cloned and inserted downstream of the firefly gene of pmirGLO vector (Promega, USA), which was labeled as Wt-Wnt1. The mutated 3'UTR of Wnt1 gene was inserted downstream of the firefly gene of pmirGLO vector and labeled as Mut-Wnt1. miR-140-5p mimic and miR-140-5p mimic negative control (NC) were co-transfected into 293T cells with luciferase reporter vector, and the luciferase activity was detected 48 hours after transfection.

Cell culture

The normal human lung epithelial cell line Beas-2B, and 4 lung cancer cell lines NCI-H292, A549, H1299 and H1975 (ATCC, China) were purchased and cultured. Cells were cultured in RPMI1640 medium (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA), 50 U/mL penicillin (Gibco, USA), and 100 μg/mL streptomycin (Gibco, USA). Cells were placed in an incubator (Besun Medical Devices Co., Ltd., China) at 37°C and 5% CO₂. The medium was changed every 2 days and cells were passaged every 3-4 days. Cells in good condition and logarithmic growth phase were selected for transfection.

Cell grouping and transfection

The H1975 cells in logarithmic growth phase were seeded in a 6-well culture plate at a density of 1*10⁵ cells/well. DMEM medium containing no serum or antibiotic was used for cell culture one day before the transfection. The cells were divided into 5 groups: Blank group (no treatment), NC group (transfected with 50 nM miR-140-5p mimic NC), miR-140-5p mimics group (transfected with 50 nM miR-140-5p mimic), pcDNA3.1-Wnt1 group (transfected with 4 μg pcDNA3.1-Wnt1), and miR-140-5p mimic + pcDNA3.1-Wnt1 group (co-transfected with 50 nM miR-140-5p mimic and 4 μg pcDNA3.1-Wnt1). Transfection was performed in accordance with Lipofectamine 2000 instruc-
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At 6 h after transfection, culture medium was switched to RPMI1640 medium (Gibco, USA) containing 10% fetal bovine serum. At 48 h after transfection, cells were harvested for subsequent experiments.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from all groups of cells using Trizol (Thermo Fisher Scientific, USA). RNA was reverse transcribed into cDNA using PrimeScript™ RT reagent kit with gDNA Eraser kit (TaKaRa, Japan). The reaction mixture was prepared using SYBR® Premix Ex Taq™ II kit (Xingzhi Biotechnology Co., Ltd., China). The reaction mixture contained the following components: 25 μL SYBR® Premix Ex Taq™ II (2×), 2 μL upstream primer, 2 μL downstream primer, 1 μL ROX Reference Dye (50×), 4 μL DNA template, and 16 μL ddH₂O. qRT-PCR was performed using ABI PRISM® 7300 system (Kunke Instrument Equipment Co., Ltd., China). The reaction conditions were as follows: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 60°C for 30 s; the cycle was repeated 32 times followed by extension at 72°C for 1 min. Relative expression level was calculated using the formula: ΔCt = CT (target gene) - CT (GAPDH or U6). ΔΔCt = ΔCt (experimental group) - ΔCt (control group), with U6 as internal reference for miR-140-5p and GAPDH as internal reference for other genes. All primers are listed in Table 1.

Western blot

At 48 h after transfection, cells were harvested and total protein was extracted from all groups using RIPA lysate (Solarbio, China) containing PMSF. Protein concentration was determined using BCA kit (Thermo Fisher Scientific, USA), and the concentration was adjusted with deionized water. Samples mixed with loading buffer were boiled for 10 min. A total of 30 μg of each sample was loaded into the gel and subject to electrophoresis for 2 h at 80 V. Protein was then wet transferred onto PVDF membrane (Millipore, USA) at 110 V for 2 h. The PVDF membrane was blocked in 5% skim milk at 4°C for 2 h. After washing with TBST, the membrane was incubated with primary rabbit anti-human antibody Wnt1 (1:1,000, Abcam, UK), β-catenin (1:5,000, Abcam, UK), TCF-4 (1:1,000, Abcam, UK), PCNA (1:1,000, Abcam, UK), E-cadherin (1:500, Abcam, UK), N-cadherin (1:1,000, Abcam, UK), Bax (1:1,000, Abcam, UK), Bcl-2 (1:1,000, Abcam, UK), GAPDH (1:2,000, Abcam, UK), respectively, at 4°C overnight. The membrane was rinsed with TBST 3 times 10 min each time. Then the membrane was incubated with HRP-conjugated goat anti-rabbit IgG (1:5,000, Beijing Zhongshan Biotechnology Co., Ltd., China). After rinsing with TBST, the membrane was placed on a glass plate. A medium amount of solution A and solution B from Fluorescence Detection Kit (Ameshame, UK) were mixed in a dark room and applied onto the membrane. The membrane was exposed and photographed using Bio-Rad image analysis system (BIO-RAD, USA). Relative protein expression was calculated by comparing the grey value of each sample with the grey value of GAPDH using Image J software.

MTT assay

At 48 h after transfection, cells were harvested and seeded in a 96-well culture plate at a density of 3-6×10³ cells/well. The volume of each well is 100 μL, and each group has 6 replicate wells. At 24 h, 48 h, and 72 h, a volume of 20 μL MTT (Gibco, USA) solution at a concentration of 5 mg/mL was added into each well and the plate was incubated for 4 h in dark room. Then

### Table 1. qRT-PCR primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Wnt1</td>
<td>F: 5'-ATTGGTTCGGTTCTTCTCCG-3'</td>
</tr>
<tr>
<td>miR-140-5p</td>
<td>F: 5'-AGAGTTGCACCTTCTCAGG-3'</td>
</tr>
<tr>
<td>β-catenin</td>
<td>F: 5'-TCTGTGCTTGTACGGTCG-3'</td>
</tr>
<tr>
<td>TCF-4</td>
<td>F: 5'-CTAGCCTCTCTAAGTGAGATGC-3'</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>F: 5'-CCAGTGACCCGGCAAGA-3'</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>F: 5'-GCAGGCGCAGCAAAACATAC-3'</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>F: 5'-GGCTCTGAGTGAGATGAC-3'</td>
</tr>
<tr>
<td>Bax</td>
<td>F: 5'-CATATAACCCCCGTCACAGCAG-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5'-GGCTCTGAATCTCCTTTG-3'</td>
</tr>
<tr>
<td>U6</td>
<td>F: 5'-TCGGGACTACGGCTCGCA-3'</td>
</tr>
</tbody>
</table>
100 μL of DMSO was added to each well, and the absorbance value (OD) of each well at 570 nm was obtained using a microplate reader (Beijing Noah Instrument Co., Ltd., China). The cell viability curve was plotted with the time points as the abscissa and the OD values as the ordinate.

**Flow cytometry**

**Cell cycle:** Cells were harvested 48 h after transfection. Cells were washed with PBS 3 times and centrifuged at 3,000 r/min for 20 min. The supernatant was discarded and the cell density was adjusted to 1*10⁸/mL with PBS. Then 1 mL 75% ethanol pre-cooled at -20°C was added and the cells were fixed at 4°C for 1 h. Cells were centrifuged at 1,500 r/min for 5 min and washed with PBS twice. Then 100 μL Rnase A (Thermo Fisher, USA) was added and the cells were placed in 37°C water bath for 30 min. A volume of 400 μL PI (Sigma, USA) was added and the cells were placed in dark room at 4°C for 30 min. Cell cycle was measured with a flow cytometer (Beckman-Coulter, USA) by detecting the red fluorescence at a wavelength of 488 nm.

**Cell apoptosis:** At 48 h after transfection, cells were digested with trypsin containing no EDTA (Thermo Fisher, USA) and collected into a flow cytometry tube. The tube was centrifuged at 3,000 r/min for 30 min and the supernatant was discarded. Cells were washed with cold PBS 3 times then centrifuged at 3,000 r/min for 15 min. The supernatant was discarded. The Annexin-V-FITC/PI staining solution was made by mixing HEPES buffer (Thermo Fisher, USA), Annexin-V-FITC and PI in a ratio of 50:1:2. Cells were mixed with 100 μL Annexin-V-FITC/PI staining solution and let sit in room temperature for 15 min. After adding 1 mL HEPES buffer, cell apoptosis was measured in a flow cytometer at a wavelength of 488 nm. Cell apoptosis rate was determined by analyzing 10,000 cells using CELLquest software.

**Transwell invasion assay**

A Transwell insert (Shanghai Kelton Bio, China) was placed in a 24-well plate. The upper surface of the bottom membrane was coated with 1:8 Matrigel (Shanghai Qianchen Biotechnology, China) and air-dried at room temperature. Cells were digested and rinsed with PBS twice, then re-suspended with RPMI1640 medium and adjusted to a density of 1*10⁶ cells/mL. A volume of 200 μL cell suspension was added to the upper chamber and 600 μL RPMI1640 medium containing 20% fetal bovine serum (Gibco, USA) was added to the lower chamber. After 24 h the Transwell insert was removed and cells on the side surface of the chamber was wiped out with cotton swab. Cells were fixed with 4% paraformaldehyde (Beijing Leagen Bio, China) for 15 min, then stained with 0.5% crystal violet solution (Beijing Solarbio, China) for 15 min. The chamber was rinsed with PBS 3 times and observed with an inverted microscope. Five random fields (200×) were selected for photographing and counting cells that have migrated through the membrane.

**Statistical analysis**

All data were analyzed with SPSS21.0 software. Quantitative values were expressed as mean ± standard deviation. One-way ANOVA follow by Tukey post-hoc test was used to compare the differences between groups. A P value less than 0.05 was considered statistically significant.

**Results**

**Wnt1 mRNA expression was different between normal lung epithelial cell line and lung cancer cell lines**

The expression of Wnt1 gene in normal human lung epithelial cell line and lung cancer cell lines was detected by qRT-PCR. The results showed that Wnt1 expression was significantly up-regulated in lung cancer cell lines compared with normal human lung epithelial cell line Beas-2B (all P<0.05), with the highest expression in H1975 cells. See Figure 1.

**miR-140-5p specifically inhibited the expression of Wnt1**

Bioinformatics analysis via online software microrna.org (http://www.targetscan.org) predicted a putative binding site between miR-140-5p and Wnt1 (Figure 2A). Dual luciferase reporter assay showed luciferase activity significantly decreased in 293T cells co-transfected with miR-140-5p mimic and Wt-Wnt1 (all P<0.05), indicating that miR-140-5p specifically inhibited the expression of Wnt1 (Figure 2B).
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The expression level of Wnt pathway related genes Wnt1, β-catenin, and TCF-4 were detected by Western blot (Figure 3). There were no significant differences between the blank group and the NC group (P>0.05). The miR-140-5p group and the miR-140-5p mimic + pcDNA3.1-Wnt1 group had significantly higher miR-140-5p expression than other groups (both P<0.05). Compared with the blank group, the expression of Wnt1, β-catenin, and TCF-4 decreased in miR-140-5p mimic, but increased in pcDNA3.1-Wnt1 group (both P<0.05). There were no significant differences between the blank group and the miR-140-5p mimic + pcDNA3.1-Wnt1 group regarding β-catenin and TCF-4 expression (both P>0.05). The results indicated that miR-140-5p inhibited the activation of Wnt signaling pathway by targeting Wnt1.

The expression of cell proliferation, cell invasion and apoptosis related genes were different among all groups

The expression of cell proliferation related gene PCNA, cell invasion related gene N-cadherin and E-cadherin, and apoptosis related gene Bcl-2 and Bax were detected with qRT-PCR and Western blot (Figure 4). The NC group and the miR-140-5p mimic + pcDNA3.1-Wnt1 group showed no differences than the blank group (both P>0.05). Compared with the blank group, the expression of PCNA, N-cadherin and Bcl-2 decreased, but the expression of E-cadherin and Bax increased both in mRNA and protein level in the miR-140-5p mimic group (all P<0.05). In contrast, the expression of PCNA, N-cadherin and Bcl-2 increased, but the expression of E-cadherin and Bax decreased in the pcDNA3.1-Wnt group (all P<0.05).

miR-140-5p overexpression inhibited the viability of H1975 cells; Wnt1 overexpression reversed the effects of miR-140-5p

The NC group and the miR-140-5p mimic + pcDNA3.1-Wnt1 group showed no differences than the blank group in cell viability at all time points by MTT assay (all P>0.05). However, at 48 h and 72 h, cell viability decreased in the miR-140-5p mimic group and increased in the pcDNA3.1-Wnt1 group compared with the blank group (all P<0.05). The miR-140-5p mimic + pcDNA3.1-Wnt1 group had increased cell viability over the miR-140-5p mimic group (P<0.05). See Figure 5.

miR-140-5p overexpression promoted apoptosis of H1975 cells; Wnt1 overexpression reversed the effects of miR-140-5p

The NC group and the miR-140-5p mimic + pcDNA3.1-Wnt1 group showed no differences than the blank group in cell cycle by flow cytometry (both P>0.05). Compared with the blank group, the ratio of G1 phase cells increased and the ratio of S phase cells decreased in the miR-140-5p mimic group (both P<0.05). While in the pcDNA3.1-Wnt1 group, the ratio of G1 phase cells decreased and the ratio of S phase cells increased (both P>0.05). Compared with the miR-140-5p mimics group, the miR-140-5p mimic + pcDNA3.1-Wnt1 group had lower G1 phase ratio and higher S phase ratio (both P<0.05). See Figure 6.
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Table 2. Bioinformatics showed miR-140-5p specifically inhibited the expression of Wnt1. A: The putative binding site between miR-140-5p and Wnt1 3’-UTR; B: Relative luciferase activity; *P<0.05, compared with miR-140-5p mimic NC.

Figure 3. The expression of miR-140-5p, Wnt1, β-catenin and TCF-4 by qRT-PCR and Western blot. A: mRNA expression of miR-140-5p, Wnt1, β-catenin and TCF-4 by qRT-PCR; B, C: Protein expression of miR-140-5p, Wnt1, β-catenin and TCF-4 by Western blot; *P<0.05, compared with the blank group; †P<0.05, compared with the NC group; &P<0.05, compared with the miR-140-5p mimic group; @P<0.05, compared with the pcDNA3.1-Wnt1 group.

Figure 4. The expression of PCNA, N-cadherin, E-cadherin, Bcl-2, and Bax by qRT-PCR and Western blot. A: mRNA expression of PCNA, N-cadherin, E-cadherin, Bcl-2, and Bax by qRT-PCR; B, C: Protein expression of PCNA, N-cadherin, E-cadherin, Bcl-2, and Bax by Western blot; *P<0.05, compared with the blank group; †P<0.05, compared with the NC group; &P<0.05, compared with the miR-140-5p mimic group; @P<0.05, compared with the pcDNA3.1-Wnt1 group.
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The NC group and the miR-140-5p mimic + pcDNA3.1-Wnt1 group showed no differences than the blank group in Transwell invasion assay (both P>0.05). Compared with the blank group, cell invasion potential decreased in the miR-140-5p mimic group, and increased in the pcDNA3.1-Wnt1 group (both P<0.05). The miR-140-5p mimic + pcDNA3.1-Wnt1 group had significantly higher invasion potential than the miR-140-5p mimic group (P<0.05). See Figure 8.

Discussion

NSCLC includes the most common types of lung cancer, and the number of NSCLC patients accounts for more than 80% of all lung cancer patients. The morbidity and mortality of patients with NSCLC are extremely high, which poses a great threat to the patient’s life [24-26]. Therefore, exploring the regulatory network and pathogenesis of NSCLC will provide a theoretical basis for the development of more effective clinical treatment methods.

As a member of the microRNA family, miR-140-5p regulates a variety of life activities. It was reported that miR-140-5p inhibited the proliferation and invasion of tumor cells as well as the occurrence of NSCLC [27, 28]. In this study, we cultured NSCLC cell line H1975 and transfected cells with miR-140-5p mimic to detect cell proliferation, invasion, and apoptosis in each group. The results showed that compared with the blank group, the cell proliferation and invasion ability of the miR-140-5p mimic group were significantly decreased, and the apoptosis rate was significantly increased; in addition, cell cycle was more hindered in the miR-140-5p mimic group. The expression of PCNA, N-cadherin, and Bcl-2 were higher, and the expression of E-cadherin and Bax were lower in the miR-140-5p mimic group than in the blank group. The above results indicated that overexpression of miR-140-5p could inhibit proliferation and invasion, and promote apoptosis of NSCLC cells.

MicroRNAs primarily regulate life activities by targeting downstream genes [29, 30]. We found through bioinformatics that there is a putative binding site between miR-140-5p and Wnt1 3’UTR. As a member of Wnt signaling pathway, when overexpressed, Wnt1 could lead to recruitment factors such as β-catenin and TCF-4, thus triggering the expression of downstream translation related factors. As a result, tumor cell proliferation and invasion was promoted and apoptosis was inhibited [31, 32]. In this study, we transfected H1975 cells with pcDNA3.1-Wnt1 to verify whether the activation of Wnt signaling pathway would affect the proliferation, invasion, apoptosis and cell cycle of NSCLC. The results confirmed that after Wnt1 overexpression, the proliferation and invasion ability of NSCLC was significantly enhanced, cell apoptosis was reduced, and cell cycle was also accelerated. To further explore the link between miR-140-5p and Wnt signaling pathway, we performed a dual luciferase reporter assay to confirm that Wnt1 is a binding target of miR-140-5p; overexpression of miR-140-5p significantly inhibited the expression of Wnt signaling pathway members Wnt1, β-catenin and TCF-4. To determine whether the overexpression of Wnt1 would reverse the effects of miR-
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Figure 6. Comparison of cell cycle. A: Cell cycle detected by flow cytometry; B: Cell cycle percentage; *P<0.05, compared with the blank group; #P<0.05, compared with the NC group; &P<0.05, compared with the miR-140-5p mimic group; @P<0.05, compared with the pcDNA3.1-Wnt1 group.
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Figure 7. Comparison of cell apoptosis. A: Cell apoptosis detected by flow cytometry; B: Cell apoptosis rate; *P<0.05, compared with the blank group; #P<0.05, compared with the NC group; &P<0.05, compared with the miR-140-5p mimic group; @P<0.05, compared with the pcDNA3.1-Wnt1 group.
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140-5p overexpression, we co-transfected H1975 cells with miR-140-5p mimic and pcDNA3.1-Wnt1. The results showed that compared with the miR-140-5p mimic group, the miR-140-5p mimic + pcDNA3.1-Wnt1 group had enhanced invasion and proliferation, decreased apoptosis, and accelerated cell cycle. These results confirmed that miR-140-5p could inhibit the proliferation and invasion, and promote apoptosis of NSCLC cells by inhibiting the Wnt signaling pathway.

Our study confirmed the effects of miR-140-5p and Wnt signaling pathway on the biological characteristics, including proliferation, invasion and apoptosis of NSCLC. We also confirmed the targeted negative inter-regulation between miR-140-5p and Wnt1. The findings of this study further improved our understanding of molecular mechanism of NSCLC, provided a theoretical basis for exploring more effective and more targeted treatment method. However, further experimentation is still needed to transfer biological study into clinical application.

Disclosure of conflict of interest

None.

Address correspondence to: Shijie Liu, Department of Emergency, Yantai Municipal Laiyang Central Hospital, No. 111 Changshan Road, Yantai 265200, Shandong Province, China. Tel: +86-0535-7232141; Fax: +86-0535-7232141; E-mail: liushijie19j@163.com

References


Figure 8. Comparison of cell invasion. A: Pictures of Transwell invasion assay; B: Cell counts of each group; *P<0.05, compared with the blank group; #P<0.05, compared with the NC group; &P<0.05, compared with the miR-140-5p mimic group; @P<0.05, compared with the pcDNA3.1-Wnt1 group.


miR-140-5p inhibits Wnt signaling pathway in NSCLC