miR-181a-5p, an inducer of Wnt-signaling, facilitates cell proliferation in acute lymphoblastic leukemia

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Abstract. Uncontrolled Wnt signaling causes leukemia. Inactivation of Wnt antagonists could play an important role in leukemia progression by activating the Wnt/β-catenin pathway. Wnt inhibitory factor-1 (WIF1) is one of the important Wnt antagonists. Few miRNAs have been reported to directly target this gene in hematopoiesis. Here, we observed that miR-181a-5p expression was markedly overexpressed in several leukemia cell lines and acute lymphoblastic leukemia (ALL) samples compared with that noted in normal peripheral blood mononuclear cells. MTT assays, soft agar colony formation assays and flow cytometry analysis collectively showed that ectopic expression of miR-181a-5p induced ALL cell growth and proliferation. Furthermore, a mechanistic study disclosed that miR-181a-5p directly downregulated WIF1 expression by binding to its 3'-UTR, and further activated Wnt/β-catenin signaling. These findings provide a novel mechanistic insight into the role of miR-181a-5p in ALL cell growth and proliferation and implicate miR-181a-5p as an attractive candidate for ALL therapy.

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid progenitor cells. The steady progress in the

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development of effective treatments has led to a cure rate of more than 80% in children with ALL, most of whom will lead healthy productive lives as long-term cancer survivors (1,2). Yet, studies are underway to ascertain the precise events that take place in the genesis of ALL, to enhance the clinical application of known risk factors and anti-leukemic agents, and to identify treatment regimens that may boost the generally low cure rates in adults and subgroups of children with high-risk leukemia (3-5). Approaches aimed at blocking associated miRNAs may serve as effective therapeutic strategies for treating ALL patients and may be valuable biomarkers for diagnosis and treatment.

Dysregulated Wnt signaling has been reported as a hallmark of particular types of solid tumors (6-9). Several studies have also indicated that Wnt signal transduction plays a key role in certain stages of lymphocyte development and the self-renewal of hematopoietic stem cells (10-16), suggesting its dysregulation as a mechanism underlying lymphoid leukemogenesis. Recent studies concerning acute or chronic lymphoid leukemias have provided further evidence for the role of Wnt signaling in malignant hematopoiesis (10-12,17,18).

The deficiency of Wnt antagonists can contribute to activation of the Wnt pathway resulting in carcinogenesis via deregulation of cell proliferation and differentiation. Numerous studies have shown that impaired regulation of Wnt antagonists such as Wnt inhibitory factor-1 (WIF1), sFRP, HDPR1 and DKK3 by promoter hypermethylation is present in several human malignancies including ALL (17-22). However, it is noteworthy that the frequency of methylation of WIF1 is less than 30% in ALL (20), which implies that there may be additional regulatory mechanisms responsible for downregulating WIF1 expression.

miRNAs are short (19-25 nucleotides) RNA molecules that can modulate the expression of a wide range of target genes by pairing homologous sequences within the 3'-UTR of mRNAs, thus impairing their translation or promoting RNA degradation (23,24) in multiple diseases including cancers.

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miR-181a has been proposed to play multi-roles in neoplasia and progression. miR-181a-5p is an oncogenic miRNA found to be deregulated in multiple types of tumors [e.g., breast cancer (25-27), osteosarcoma (28), colorectal carcinoma (29), gastric cancer (30), salivary adenoid cystic carcinoma (31) and lung cancer (32)]. In contrast, miR-181a was downregulated in many other tumors and thus served as a tumor-suppressor gene (33,34). In previous studies of hematologic malignancies, miR-181a was found to be upregulated in acute myeloid leukemia (35) and myelodysplastic syndromes (36), but downregulated in multiple myeloma (37) and chronic lymphocyte leukemia (38).

In this study, we demonstrated that upregulation of miR-181a-5p directly targets WIF1 in ALL cells, suggesting that miR-181a-5p-mediated Wnt-signaling activation may be implicated in the pathogenesis of ALL.

Materials and methods

Cell lines and clinical samples. Two ALL-derived cell lines (Jurkat and MOLT-4) and other hematopoietic tumor cell lineages (HL60, NB4 and KG-1) were obtained from the Cancer Research Institute, Southern Medical University, Guangzhou, China. Cells were cultured at 37° C under 5% CO₂ in humidified air in RPMI-1640 medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA). Thirty-seven primary ALL patients and 28 ALL-complete remission bone marrow samples were collected from The Third Affiliated Hospital and Zhujiang Hospital, Southern Medical University. Twenty-three samples of normal peripheral blood mononuclear cells (PBMCs) were obtained from Guangzhou Blood Center.

The study was approved by the Human Ethics Committee at The Third Affiliated Hospital of Southern Medical University. Written informed consent was obtained from all participants.

Extraction of total RNA and quantitative RT-PCR (qRT-PCR). Total RNA was extracted from tissues and cell lines with TRIzol (Invitrogen, Carlsbad, CA, USA) according to the user manual. For mRNA expression analysis, 1 μ g of total RNA was used for RT using PrimerScript[™] RT reagent kit following the manufacturer's instructions (lot. no. BK3001), and real-time PCR was performed using SYBR[®] Premix Ex Taq[™] Real-Time PCR kit (code no. DRR041A) (both from Takara Bio) on an Mx3005P Stratagene. All data were normalized to GAPDH expression and further normalized to the negative control unless otherwise indicated. Primer sequences for WIF-1 (forward, 5'-TCTCCAAACACCTCAAAATGCT-3' and reverse, 5'-GACACTCGCAGATGCGTCT-3') and GAPDH (forward, 5'-CCATGAGAAGTATGACAACAGCC-3' and reverse, 5'-GGGTGCTAAGCAGTTGGTG-3') were directly acquired from PrimerBank (http://pga.mgh.harvard. edu/primerbank/). For miRNA expression analysis, mature miRNAs were reverse-transcribed, and real-time PCR was performed using All-in-One[™] miRNA qRT-PCR detection kit following the manufacturer's instructions (cat. no. AOMD-Q020; GeneCopoeia, Inc., Rockville, MD, USA). All data were normalized to U6 expression. The fold changes were calculated by relative quantification (2^{- $\Delta\Delta Ct$}). qRT-PCR was conducted for each sample in triplicate.

MTT assay. MOLT-4 $(5x10^3)$ and Jurkat cells $(3x10^3)$ were plated onto 96-well plates (NEST Biotechnology, Wuxi, China) respectively in 100 μ l growth medium and allowed to adhere overnight. The cells were then transfected with 50 nM of miRNA mimics (GenePharma, Shanghai, China) or siRNA (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and inhibitor (GenePharma), respectively. At different time-points (24, 48 and 72 h), the culture medium was removed and replaced with culture medium containing $10 \,\mu l$ of sterile 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye (5 mg/ml). After incubation at 37°C for 4 h, the MTT solution was removed, and 150 µl dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Spectrometric absorbance at 490 nm was measured using a BioTek ELx800 microplate photometer (BioTek ELx800, SN211805; BioTek, Winooski, VT, USA).

Anchorage-independent growth assay. Cells were blowed gently, and $1x10^5$ cells were resuspended in 2 ml complete medium plus 0.3% agar (Sigma, St. Louis, MO, USA). The agar-cell mixture was plated on top of a bottom layer consisting of 1% agar in complete medium. After 15 days, colony size was measured using an ocular micrometer and colonies larger than 0.1 mm in diameter were counted. The experiment was performed three times for each cell line.

5-Ethynyl-2'-deoxyuridine assay. Cells were transfected with miRNA mimics in 96-well plates. Forty-eight hours after transfection, 5-ethynyl-2'-deoxyuridine (EdU) (100 μ M) (Cell Light EdU DNA imaging kit; Guangzhou RiboBio Co., Ltd., Guangzhou, China) was added, and the cells were cultured for an additional 2 h. The cells were then stained according to the production manual (Guangzhou RiboBio Co., Ltd.). Images were captured and analyzed using fluorescence microscopy (Nikon Eclipse 80i; Nikon, Tokyo, Japan). EdU-positive cells were determined with the formula: (EdU-treated cells/DAPI stained cells) x 100%.

Flow cytometry. The cells were fixed in 70% ice-cold ethanol for 48 h at 4°C and stained by incubation with PBS containing 10 μ g/ml propidium iodide and 0.5 mg/ml RNaseA for 15 min at 37°C. The cells were analyzed for the DNA content of labeled cells by FACSCaliber cytometry (BD Biosciences, Franklin Lakes, NJ, USA). Each experiment was conducted in triplicate.

Dual-luciferase assay. The luciferase reporter constructs pEZX-MT01-WIF1 3'-UTR WT and miRNA target clone control vector for pEZX-MT01 were purchased from GeneCopoeia, Inc. (HmiT001390-MT01 and CmiT000001-MT01). Mutant reporter plasmids were obtained from this plasmid using a KOD-Plus Mutagenesis kit (SMK-101; Toyobo Co., Ltd., Life Science Department, Osaka, Japan). Luciferase assays were conducted using 293T cells plated in a 24-well plate (NEST Biotechnology). Transfections were performed using Lipofectamine[™] 2000 (11668-019; Invitrogen) in Opti-MEM serum-free media (cat. no. 10742; Gibco). Luciferase and *Renilla* signals were measured 48 h after transfection using the Dual-Luciferase Reporter Assay kit (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. Three



Figure 1. miR-181a-5p is overexpression in leukemia cell lines and primary acute lymphoblastic leukemia (ALL) patients. (A) Real-time PCR analysis of miR-181a-5p expression in five leukemia cell lines (HL60, MOLT-4, JURKAT, NB4 and KG-1), compared with the normal peripheral blood mononuclear cells (PBMCs) from healthy voluntary individuals. (B) miR-181a-5p expression detected by qRT-PCR in clinical samples. N-PBMC, normal PBMCs; P-ALL and R-ALL, primary or complete remission ALL patient' bone marrow samples. Values represent mean \pm SD; n=3, **P<0.01.

independent experiments were performed, and the data are presented as the mean \pm SD.

Western blot assay. Cell lysate was prepared using RIPA buffer with protease inhibitors and quantified using the BCA protein assay (BioTek China, Beijing, China). The Nuclear Protein Extraction kit (BSP009; Sangon Biotech Co., Ltd., Shanghai, China) was used for extracting nuclear proteina of the ALL cells. Protein (20 μ g) was loaded onto a 10% SDS-PAGE gel that was then transferred onto a PVDF membrane and incubated with anti-WIF1 (1:1,000, ab2064; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-C-myc (1:500, SC-40), anti-E2F1 (1:1,000, SC-22820), anti-CDK4 (1:1,000, SC-260), anti-CCND1 (1:500, SC-753) and anti-P21 (1:1,000, SC-397) (all from Santa Cruz Biotechnology, Inc.) at 4°C overnight in blocker (3% non-fat dry milk/BSA in TTBS) followed by incubation with HRP-conjugated secondary anti-mouse antibody (1:2,000, ZB2305; ZSGB-BIO, Beijing, China). Protein was normalized with GAPDH (1:5,000, no. P30008; Abmart, Shanghai, China) and histone-H3 (1:2,000, 17168-1-AP; ProteinTech Group, Inc., Chicago, IL, USA).

Statistical analysis. All statistical analyses were performed using the SPSS 13.0 Statistical Software Package (SPSS, Inc., Chicago, IL, USA). Two-tailed Student's t-test was used to determine the differences between groups for *in vitro* and *in vivo* analyses. The differences were considered to be statistically significant at a P-value <0.05. All data are presented as mean \pm SD or SEM unless otherwise noted.

Results

miR-181a-5p is highly expressed in various leukemia cell lines and primary ALL. RT-qPCR analyses initially showed that the expression of miR-181a-5p was markedly upregulated in 5 cell lines (HL60, MOLT-4, JURKAT, NB4 and KG-1) as compared with that in the normal PBMCs from healthy voluntary individuals (Fig. 1A). To validate the clinical relevance of miR-181a-5p to ALL patients, we further examined the miR-181a-5p expression level in 37 primary ALL samples and 28 ALL-complete remission samples compared with 23 PBMC samples. As shown in Fig. 1B, miR-181a-5p was highly expressed in the primary ALL patient samples. Notably, the expression level of miR-181a-5p was significantly reduced in the ALL-complete remission patient samples compared with primary ALL patients. These data suggest that miR-181a-5p may play an important role in the pathogenesis of ALL, and is associated with prognosis and may be valuable in the evaluation of therapeutic effects.

miR-181a-5p promotes ALL cell growth and proliferation in vitro. To explore the role of miR-181a-5p in the development and progression of ALL, we next examined its role in cellular proliferation. MOLT-4 cells with lower miR-181a-5p expression were used in the gain-of-function studies, whereas Jurkat cells with higher miR-181a-5p expression (Fig. 1A) were applied in the loss-of-function analyses. MTT and soft agar colony formation assays were conducted in the MOLT-4 cells transfected with the miR-181a-5p mimic and in Jurkat cells transfected with the miR-181a-5p mimic and in Jurkat cells transfected with the miR-181a-5p inhibitor. The miR-181a-5p mimic increased cell growth and anchorage-independent growth ability of the MOLT-4 cells (Fig. 2A and B), while the miR-181a-5p inhibitor markedly reduced cell growth and anchorage-independent growth ability of the Jurkat cells (Fig. 3A and B).

Using flow cytometric analysis, we further found that MOLT-4 cells transfected with the miR-181a-5p mimic exhibited a significantly reduced cell proportion in the G1 phase by 9.22±0.34% (P<0.001) and increased proliferation (S/G2/M cell proportion) by 9.18±0.30% (P<0.001) (Fig. 2C), whereas the miR-181a-5p inhibitor increased the percentage of cells in the G1 phase by 9.02±0.83% (P<0.001) and reduced the proliferation of Jurkat cells (S/G2/M cell proportion) by 9.08±0.47% (P<0.001) (Fig. 3C). Consistently, EdU incorporation assay showed that the percentage of cells in S phase was significantly increased in the miR-181a-5p-overexpressing MOLT-4 cells compared with the miR-control MOLT-4 cells (Fig. 2D), but the miR-181a-5p inhibitor reduced the percentage of Jurkat cells in the S phase (Fig. 3D). These results suggest that miR-181a-5p modulates ALL cell proliferation through regulation of G1/S transition.

WIF1 is a major target gene of miR-181a-5p in ALL cells. WIF1 is a Wnt antagonist that inhibits Wnt signaling by direct



Figure 2. miR-181a-5p promotes acute lymphoblastic leukemia (ALL) cell growth and proliferation. (A) Effect of miR-181a-5p mimics on the proliferation of MOLT-4 as detected by MTT assay in triplicate. (B) Quantification of colony formation results of MOLT-4 cells in complete medium consisting of 1% agar. (C) The cell cycle distribution was detected in the MOLT-4 cells following transfection with miR-181a-5p mimics (miR-181a-5p) or negative control (miR-Ctrl) by FACSCaliber cytometry. Data are presented as mean \pm SEM for three independent experiments. (D) Representative micrographs (left, 100x magnification; scale bar, 20 μ m) and quantification of EdU-positive MOLT-4 cells (right) following transfection with miR-181a-5p mimics (miR-181a-5p) or negative control (miR-Ctrl). Values represent mean \pm SEM; **P<0.001.



Figure 3. Inhibition of miR-181a-5p suppresses acute lymphoblastic leukemia (ALL) cell growth and proliferation. (A) Effect of miR-181a-5p inhibitor (miR-181a-5p-in) on Jurkat cell proliferation as detected by MTT assay in triplicate. (B) Quantification of colony formation results of Jurkat cells in complete medium consisting 1% agar. (C) The cell cycle distribution was detected in the Jurket cells following transfection with miR-181a-5p-in or the inhibitor negative control (miR-in-Crl) by FACSCaliber cytometry. Data are presented as mean \pm SEM for three independent experiments. (D) Representative micrographs (left, 100x magnification; scale bar, 20 μ m) and quantification of EdU-positive Jurkat cells (right) following transfection with miR-181a-5p-in or inhibitor negative control (miR-in-Crl). Values represent mean \pm SEM; **P<0.001.



Figure 4. miR-181a-5p suppresses Wnt inhibitory factor-1 (WIF1) expression by directly targeting the WIF1 3'-UTR. (A) RNA hybrid prediction of the binding sites of miR-181a-5p in the 3'-UTR of WIF1. (B) WIF1 protein expression was detected by western blot analysis in the MOLT-4 and Jurkat cells transfected with the miR-181a-5p mimic and inhibitor, respectively. (C) Luciferase activities of dual-luciferase reporter carrying a complete wild-type or mutant of WIF1 3'-UTR were assessed in the indicated cells in triplicate. Values represent mean \pm SEM, ***P<0.001.

Table I. Predicted microRNA according to mRNA selected regions (minimum seed length, 7; P<0.001).

	Gene	KeiseqiD	Seed length	Start	Seed sequence	End	P-value
hsa-miR-181a	CPOX	NM_000097	11	2596	AACAUUCAACG	2586	0.0003
hsa-miR-181a	CHST9	NM_031422	10	3110	AACAUUCAAC	3101	0.0006
hsa-miR-181a	HMGB2	NM_002129	10	1055	AACAUUCAAC	1046	0.0007
hsa-miR-181a	IL2	NM_000586	10	739	AACAUUCAAC	730	0.0003
hsa-miR-181a	SYNE1	NM_182961	10	27647	AACAUUCAAC	27638	0.0007
hsa-miR-181a	WIF1	NM_007191	10	1357	AACAUUCAAC	1348	0.0007
hsa-miR-181a	C12orf56	NM_001099676	9	1529	AACAUUCAA	1521	0.0009
hsa-miR-181a	DNAJC3	NM_006260	9	5051	ACAUUCAAC	5043	0.0000
hsa-miR-181a	ELAVL4	NM_021952	9	1664	AACAUUCAA	1656	0.0009
hsa-miR-181a	HIGD2A	NM_138820	9	548	AACAUUCAA	540	0.0010
hsa-miR-181a	PIH1D2	NM_001082619	9	1095	AACAUUCAA	1087	0.0007
hsa-miR-181a	SFT2D1	NM_145169	9	546	AACAUUCAA	538	0.0008
hsa-miR-181a	CENPI	NM_006733	8	2550	AACAUUCA	2543	0.0005
hsa-miR-181a	KRTCAP2	NM_173852	8	526	AACAUUCA	519	0.0009
hsa-miR-181a	NCR2	NM_004828	8	940	AACAUUCA	933	0.0004
hsa-miR-181a	WDR79	NM_018081	8	1836	ACAUUCAA	1829	0.0002

binding to Wnt molecules. Using miRwalk, a publicly available algorithm, we found that WIF1 is theoretically the target gene of miR-181a-5p (Fig. 4A and Table I). To validate whether WIF1 is a direct target of miR-181a-5p, a wild-type or mutant 3'-UTR fragment of WIF1 was cloned downstream of the firefly luciferase gene. Dual-luciferase reporter assays revealed that miR-181a-5p significantly attenuated the activity of firefly luciferase with the wild-type 3'-UTR of WIF1, whereas this effect was abolished when the predicted 3'-UTR-binding site was mutated (Fig. 4B). Subsequent western blot analysis confirmed that miR-181a-5p attenuated the expression of cellular WIF1 in MOLT-4 cells and silencing of miR-181a-5p increased the level of cellular endogenous WIF1 protein in the Jurkat cells (Fig. 4C). These data suggest that miR-181a-5p may inhibit the expression of WIF1 by directly binding to its 3'-UTR.

WIF1 suppression is required for miR-181a-5p-induced ALL cell proliferation. To further confirm the role of WIF1 suppression in miR-181a-5p-induced ALL cell proliferation, we knocked down WIF1 expression in ALL cells (MOLT-4) using specific siRNA (Fig. 5E) and then observed the alteration of cell proliferation. Similar to miR-181a-5p, WIF1 siRNA enabled an obviously reduced WIF1 expression followed by increased ALL cell growth and proliferation (Fig. 5A-E). In addition, following treatment of Jurkat cells with the miR-181a-5p inhibitor, significantly increased WIF1 expression and restricted cell proliferation were observed. Furthermore, we transfected WIF1-siRNA into the miR-181a-5p inhibitor-treated cells and observed that G1/S phase cell cycle arrest due to miR-181a-5p inhibition was abrogated (Fig. 5F and G). Taken together, these data suggest that WIF1 suppression is required for miR-181a-5p-induced ALL cell proliferation.



Figure 5. Wnt inhibitory factor-1 (WIF1) downregulation is required for miR-181a-5p-induced proliferation of ALL cells. (A) Effect of small interfering RNA against WIF1 (siRNA-WIF1 or WIF-1-siRNA) on MOLT-4 cell proliferation as detected by MTT assay in triplicate. (B) Quantification of colony formation results of MOLT-4 cells treated with small interfering RNA against WIF1 and negative control in complete medium consisting 1% agar. (C) The cell cycle distribution was detected in the indicated MOLT-4 cells by FACSCaliber cytometry. (D) Representative micrographs (left, 100x magnification; scale bar, 20 μ m) and quantification of EdU-incorporating MOLT-4 cells (right) following transfection with small interfering RNA against WIF1 and negative control. (E and F) Real-time PCR analysis for the relative expression of WIF1 in the indicated cells. (G) The cell cycle distribution was detected in the indicated Jurkat cells by FACSCaliber cytometry. Data are presented as mean ± SEM for three independent experiments, ***P<0.001.

miR-181a-5p activates Wnt/ β -catenin signaling through suppression of WIF1. The cellular fractionation and western blotting showed that miR-181a-5p overexpression promoted nuclear accumulation of β -catenin, indicating that miR-181a-5p may activate the Wnt/ β -catenin pathway through promoting nuclear β -catenin accumulation (Fig. 6A). Accordingly, we investigated whether miR-181a-5p influences downsteam signaling of the Wnt/ β -catenin pathway. As expected, the expression levels of c-myc, E2F1, cyclin D1, and CDK4 were increased while p21 expression was decreased following overexpression of miR-181a-5p. Opposite results were found in the miR-181a-5p-inhibited ALL cells (Fig. 6B).

Discussion

The expression and functions of specific miRNAs generally differ distinctly depending on the cancer cell type. In the present study, we observed that miR-181a-5p expression was highly expressed in several leukemia cell lines compared with that in normal PBMCs. Notably, we found that miR-181a-5p



Figure 6. miR-181a-5p activates the Wnt/ β -catenin pathway. (A) Western blot analysis of β -catenin expression in nucleus of MOLT-4 and Jurkat cells transfected with miR-181a-5p mimic (miR-181a-5p) and inhibitor (miR-181a-5p-in) respectively. (B) Western blot analyses of *c*-myc, *E2F1*, *cyclin D1*, *CDK4* and *p21* in the indicated cell groups.

was downregulated in ALL-complete remission samples compared with that in primary ALL samples, indicating that miR-181a-5p may be a potential biomarker for the evaluation of clinical efficacy and prognosis of ALL. This is consistent with a previous study that increased expression of miR-181a is associated with a poor outcome of ALL (39).

miR-181a can serve as an oncomiR or tumor suppressor, also implicating its complexity and muti-functions in the regulation of its target genes or signaling pathways in cancer. In the present study, we found that the ectopic expression of miR-181a enhanced ALL cell proliferation and silencing of miR-181a expression displayed the opposite effect, suggesting that abnormal miR-181a expression may contribute to the pathogenesis of ALL. Furthermore, we used a bioinformatics approach to predict the target genes of hsa-miR-181a-5p. Among the potential candidates, WIF1 attracted our attention since it is a classic tumor-suppressor gene involved in the cell proliferation in various cancers (40-42). Importantly, Wnt/ β -catenin signaling is frequently activated in ALL (43,44), but its precise mechanism of action is not well understood. WIF1 has proved to act as a secreted Wnt inhibitor that binds directly to Wnt, and thus constraining the binding of Wnt ligands to the Frizzled receptor (45). Thus, the downregulation of WIF1 contributes to activation of the Wnt pathway resulting in carcinogenesis through dysregulation of cell proliferation and differentiation. Luciferase reporter assays and western blotting further confirmed that miR-181a-5p directly suppressed WIF1 expression via directly targeting the 3'-UTR of WIF1. Although it has been reported that WIF1 is a target gene of miR-181a in colorectal cancer (46), our study suggests that WIF1 is a major target of miR-181a-5p and inactivation induced by miR-181a-5p may play an important role in promoting the carcinogenesis of ALL.

 β -catenin is a major cellular effector of Wnt signaling. It is normally targeted by a complex of axin, APC, glycogen synthase kinase-3 β and casein kinase 1 α for proteasomemediated degradation following phosphorylation and ubiquitination (47,48). β -catenin accumulation and nuclear translocation to regulate genes are important for cell proliferation. Notably, we observed that high expression of miR-181a-5p led to increased nuclear accumulation of β -catenin, indicating that miR-181a-5p can activate Wnt/ β -catenin signaling in ALL. Subsequently, we detected the downstream targets of activated Wnt pathway signaling, confirming the activation of Wnt/ β -catenin signaling in the presence of WIF1 inactivation via miR-181a-5p in ALL. Therefore, *in vivo* antagomir-based strategies may be researched for further understanding the basic and translational significance of miR-181a-5p-Wnt/ β -catenin signaling in ALL.

In conclusion, we demonstrated that WIF1 is a major target of miR-181a-5p in ALL and downregulation induced by miR-181a-5p activated Wnt/ β -catenin signaling, promoting the proliferation of ALL. These findings uncover a crucial molecular mechanism that maintains the constitutive activation of the Wnt/ β -catenin pathway and may prove to be clinically useful for developing a new curative and effective biomarker and therapeutic target for ALL.

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