European Review for Medical and Pharmacological Sciences 2018; 22: 7671-7678

MiR-144-3p inhibits the proliferation and metastasis of pediatric Wilms' tumor cells by regulating Girdin

C.-L. LIU[1], W.-H. WANG[1], Y.-L. SUN[2], H.-W. ZHUANG[1], M. XU[1], H.-F. CHEN[1], J.-X. LIU[1]

[1]Department of Pediatric Surgery, Linyi Central Hospital, Linyi, China
[2]Department of Gynecology, Yishui People's Hospital, Linyi, China

Chengliang Liu and *Wenhua Wang* contributed equally to this work

Abstract. – OBJECTIVE: The aim of this study was to investigate the role of miR-144-3p in the proliferation and metastasis capacity of pediatric Wilms' tumor (WT) cells and to explore the underlying mechanism.

PATIENTS AND METHODS: The quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was performed to measure the expression level of miR-144-3p in pediatric WT tissues and cell lines (G401). A bioinformatics software was utilized to predict the interaction between miR-144-3p and Girdin. Subsequently, the interaction was further verified by dual luciferase reporter (DLR) gene assay and Western blot. The proliferation and colony formation ability of G401 cells were examined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and colony formation assay, respectively. Finally, the effect of miR-144-3p on cell invasion and migration was analyzed by transwell assay.

RESULTS: In the current study, we found that the expression level of miR-144-3p was significantly reduced in pediatric WT tissues and cells, whereas Girdin expression was upregulated. On-line target gene prediction software was applied to screen Girdin, which was considered as a downstream target gene of miR-144-3p. The interaction between miR-144-3p and Girdin was further verified by dual Luciferase reporter gene assay and Western blot. Subsequent experiments demonstrated that the proliferation and metastasis ability of cells was remarkably suppressed after up-regulating the expression of miR-144-3p. However, an addition of Girdin could reverse the effect of miR-144-3p.

CONCLUSIONS: MiR-144-3p, which was up-regulated in pediatric WT, might inhibit the proliferation and metastasis of the cells by directly targeting Girdin. This further indicated that miR-144-3p could be a potential therapeutic target for the treatment of pediatric WT.

Key Words:
Wilms' tumor (WT), MiR-144-3p, Girdin, Proliferation, Invasion, Migration.

Introduction

Wilms' tumor (WT) is the most common malignant renal tumor in children, the incidence of which is approximately 1/100,000. WT ranks 2nd in primary childhood malignant abdominal tumor and 5th in all childhood malignant tumors[1]. Meanwhile, WT accounts for 95% of renal tumor in children. With the rapid development of comprehensive therapies such as surgery, radiotherapy and chemotherapy[2], the overall survival rate of WT has been greatly increased by up to 90%. However, the risk of recurrent malignant tumor and distant metastasis in pediatric WT patients is still high[3].

The occurrence and development of tumors are accompanied by molecular changes. Although histological classification helps guide the treatment and prognosis evaluation of pediatric WT patients, deepening the understanding of tumor at the molecular level can help us to know more about the biological characteristics of WT. Meanwhile, distinguishing different tumors through molecular changes may also help to achieve more accurate molecular classification[4]. Currently, it has been found that some molecular markers can be served as markers for WT molecular classification, eventually helping to guide the diagnosis, treatment and prognosis evaluation of WT.

Micro-ribonucleic acid (miRNA) is a kind of non-coding RNA with about 20-25 nucleotides

Corresponding Author: Jinxiang Liu, MM; e-mail: 461900909@qq.com

in length. MiRNAs also exert an endogenous regulatory function in eukaryotes, It's known that miRNAs are produced by longer primary transcripts *via* cleaving and processing a series of nucleases. MiRNAs can specifically silence the expression of certain proteins in cells, thereby exerting a corresponding biological effect[5]. Multiple studies have demonstrated that miRNA is closely related to the occurrence and development of tumors. Meanwhile, miRNA also involves in the regulation of tumor biological characteristics, such as proliferation, apoptosis, invasion and metastasis. MiRNAs can also play a similar regulatory role as tumor suppressor genes or oncogenes, indicating that miRNA may serve as a potential therapeutic target for the treatment of tumors[6,7]. At the same time, miRNA also plays an important role in normal kidney development and tumor progression, which provides a new intervention target for the treatment of renal tumors[8-10].

Currently, multiple studies have demonstrated that some miRNAs play a vital role in the malignant progression of WT. Koller et al[11] have found that low expression of miR-204 in WT may increase the expression of oncogene MESI1. Meanwhile, low expression of miR-23a leads to an increased expression of oncogene HOXB4 and promotes the malignant progression of the tumor[12]. Besides, Cao et al[13] have also found that the transcription factor STAT3 can inhibit the expression of tumor suppressor gene WTX through up-regulating miR-370 expression, thus promoting the malignant progression of the tumor.

In this study, we investigated the regulatory effect of miR-144-3p on the development of WT. Bioinformatics predicted that girders of actin filaments (Girdin) might be a potential target gene of miR-144-3p. Subsequently, we further investigated whether miR-144-3p could affect the biological function of WT cells by targeting Girdin. Our study aimed at laying the foundation for the clinical use of miRNA in the regulation of WT biological behaviors and providing valuable clues for the treatment of pediatric WT.

Patients and Methods

Tissue Samples and Cell Lines

A total of 80 surgically resected specimens, including 40 pediatric WT tissues and 40 adjacent normal tissues, were collected from July 2012 to July 2017 in Linyi Central Hospital. All the cases enrolled in this study were inpatient children. Adjacent normal tissues were confirmed by biological biopsy to ensure that they did not include WT cells. After isolation, liquid nitrogen was used to freeze WT tissues and adjacent normal tissues. After all, Declaration of Helsinki should be mentioned and respected. This study was approved by the Ethics Committee of Linyi Central Hospital. Signed written informed consents were obtained from all participants before the study.

Human WT cell line (G401) and human embryonic kidney cell line (HEK-293T) were purchased from Shanghai Baili Biotech Co., Ltd. (Shanghai, China). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) in a 37°C, 5% CO_2 incubator. Experimental cells were collected in the logarithmic growth phase.

Luciferase Reporter Gene Assay

In TargetScan, miRDB and microRNA websites, we found that Girdin was a downstream target gene of miR-144-3p. In the current study, wild-type Girdin (Wt-Girdin-3'-UTR) and mutant Girdin (Mut-Girdin-3'-UTR) were transfected into G401 cells together with an empty plasmid and the miR-144-3p overexpression plasmid. Dual-Luciferase reporter gene assay kit was performed for activity detection by a multi-function microplate reader after culture for 48 h.

Cell Transfection

MiR-144-3p mimics and si-Girdin were synthesized and transfected into WT cells (G401) to analyze the biological function of miR-144-3p. Next, three groups were established to elucidate the relationship between miR-144-3p and G401 cells, including the NC group (negative control), the miR-144-3p mimics group (G401 cells transfected with miR-144-3p mimics) and the mimics + Girdin group (G401 cells transfected with miR-144-3p mimics and si-Girdin). All the stuff was purchased from RiboBio (Guangzhou, China). Cell transfection was performed according to the instructions of Lipofectamine RNAiMAX (Life Technologies, Gaithersburg, MD, USA).

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted according to the instructions of TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). SYBR green qPCR assay was used to measure the expression level of Girdin. Glycer-

aldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. TaqMan miRNA assay (Applied Biosystems, Foster City, CA, USA) was performed to measure the expression of miR-144-3p normalized to U6. Primers used in this study were as follows: Girdin, F: 5'- CTACTACAGCCATCAACTT-3', R: 5'-AGAATCATCACTAACACCTT-3'; miR-144-3p, F: 5'-TACTGCATCAGGAACTGACTGGA-3', R: 5'-GTGCAGGGTCCGAGGT-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot

Radio-immunoprecipitation assay (RIPA) lysate (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used to extract total protein of WT cells. The concentration of extracted protein was detected by the bicinchoninic acid (BCA) method (Merck, Billerica, MA, USA). A total of 20 μL extracted proteins were separated by sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gel electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, the membranes were incubated with 5% milk for non-specific binding. Then the membranes were incubated with primary antibodies of anti-Girdin and anti-GADPH (diluted at 1:1000, Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. After washing with TBST 3 times, the membranes were incubated with corresponding secondary antibody at room temperature for 2 h. Immunoreactive bands were exposed by Enhanced chemiluminescence method (Thermo Fisher Scientific, Waltham, MA, USA). GAPDH was used as an internal reference, and relative changes in protein expression were calculated by Image-J Software.

Cell Proliferation

After 2 h of transfection, G401 cells were seeded into 96-well plates at a density of 7×10^3 cells/well. Cell viability was determined *via* MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA). Briefly, 20 μL MTT reagent (5 mg /mL) was added into each well at 24, 48, 72, and 96 h, respectively, followed by incubation for another 2 h. The absorbance was determined by a microplate reader at the wavelength of 450 nm.

Colony Formation Assay

Transfected cells were seeded into 6-well plates at a density of 10^3 cells/well and incubated for 2 weeks. The cells were washed and fixed, followed by staining with 0.5% crystal violet. 10 randomly selected fields were observed under a light microscope.

Cell Migration and Invasion Assay

Transwell lower chamber was supplemented with Dulbecco's modified eagle medium (DMEM) containing 15% FBS as a migration-inducing factor. Cells (5×10^4/well) were added into the upper chamber and then incubated in a 37°C incubator. The upper chamber was removed after incubation for 16 h. Subsequently, the upper chamber was washed with PBS and fixed with 95% anhydrous ethanol, followed by staining with 0.1% crystal violet at room temperature for 20 min. After drying, 5 randomly selected fields were observed under an inverted microscope (×200). Finally, the number of migrated cells were counted and calculated.

Cell Invasion

Matrigel was diluted to a final concentration of 1 mg/ml in 4°C pre-cooled serum-free medium. Then, 50 μL diluted Matrigel was added vertically to the center of the upper chamber, followed by incubation at 37 °C for 0.5 h for gelatinization. Complete culture medium containing 15% FBS was added to the lower chamber. Subsequently, 5×10^4 cells were added to the upper chamber and cultured for 36 h. The remaining steps were the same as the cell migration assay.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 Software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. The *t*-test was applied to compare the difference between the two groups. One-way ANOVA was used for comparison among the different groups, followed by Post-Hoc Test (Least Significant Difference). $p < 0.05$ was considered statistically significant.

Results

Expression of miR-144-3p and Girdin in WT Tissues and Cells

QRT-PCR results illustrated that the expression level of miR-144-3p in WT tissues was

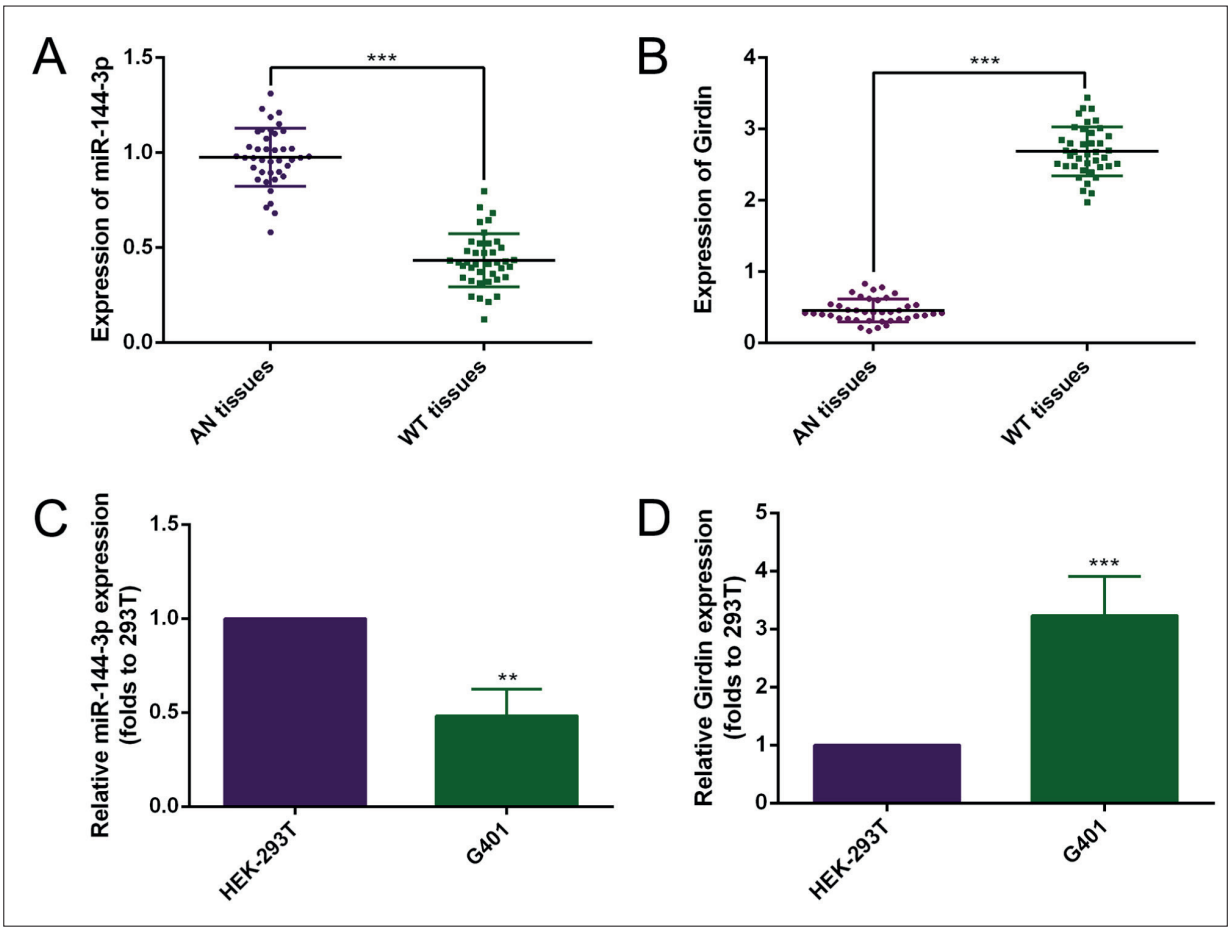

Figure 1. The expression levels of miR-144-3p and Girdin in WT tissues and cell lines. ***A,*** Difference in the expression of miR-144-3p and Girdin between WT tissues and adjacent normal tissues (***$p < 0.001$ compared with adjacent normal tissues). ***B,*** The expression of miR-144-3p and Girdin in WT cell line (G401) and human embryonic kidney cell line (HEK-293T) (***$p < 0.0001$ compared with 293T).

significantly lower than that of adjacent tissues (Figure 1A). However, the expression of Girdin was significantly increased in WT tissues (Figure 1B). Same results were obtained at the cellular level. Results indicated that miR-144-3p was remarkably down-regulated in WT cells (Figure 1C), whereas Girdin was abnormally up-regulated (Figure 1D). These results demonstrated that miR-144-3p might correlate with Girdin during the progression of WT.

Transfection Efficiency of miR-144-3p

As shown in Figure 2, the expression of miR-144-3p in G401 cells was obviously up-regulated after transfection of miR-144-3p mimics. These data confirmed that miR-144-3p mimics could effectively increase the expression level of miR-144-3p in G410 cells.

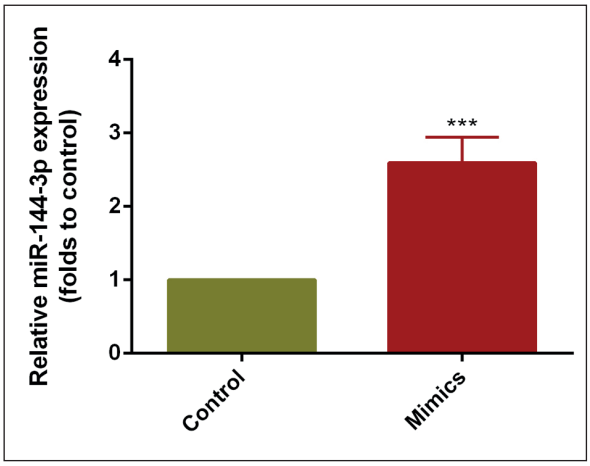

Figure 2. Transfection efficiency detected by qRT-PCR (***$p < 0.001$).

Girdin was a Direct Target of miR-144-3p

MicroRNA target gene prediction software showed that miR-144-3p could act on the 3'-UTR of Girdin (Figure 3A). In addition, qRT-PCR results demonstrated that the expression of miR-144-3p was correlated with Girdin expression in WT tissues and cells (Figure 1).

Luciferase reporter gene assay suggested the Girdin-WT luciferase activity was obviously suppressed after overexpression of miR-144-3p. However, there was no statistical difference in the Girdin-Mut luciferase activity compared with the NC group. The above results indicated that miR-144-3p could negatively regulate the expression of Girdin through binding to Girdin 3'-UTR (Figure 3A).

Taken all, it was confirmed that Girdin was a functional target gene of miR-144-3p in the development of WT.

MiR-144-3p Decreased the Expression Level of Girdin

Three groups were established to perform similar experiments in G401 cells, including the miR-NC group, the miR-144-3p mimics group and the mimics + si-Girdin group.

Western blot showed that the expression level of Girdin was significantly decreased after up-regulating the expression of miR-144-3p in G401 cells. These data further illustrated the regulatory effect of miR-144-3p on the expression of Girdin (Figure 3B).

MiR-144-3p Inhibited the Proliferation of WT Cells

MTT results showed that the proliferative inhibition rate of the miR-144-3p mimics group was significantly higher than that of the NC group and the mimics + si-Girdin group. This indicated that miR-144-3p had a conspicuous inhibitory effect on cell proliferation. However, the addition of Girdin could reverse the inhibitory effect (Figure 4A). Moreover, the colony formation ability of G401 cells was significantly suppressed by miR-144-3p (Figure 4B). After transfection of miR-144-3p mimics, colonies formed by G401 cells were less in number and smaller in size.

MiR-144-3p Inhibited the Migration and Invasion of WT Cells

Migration and invasion are two key factors in cancer cell proliferation. Transwell experiment indicated that the migration and invasion ability of G401 cells was significantly restricted by the

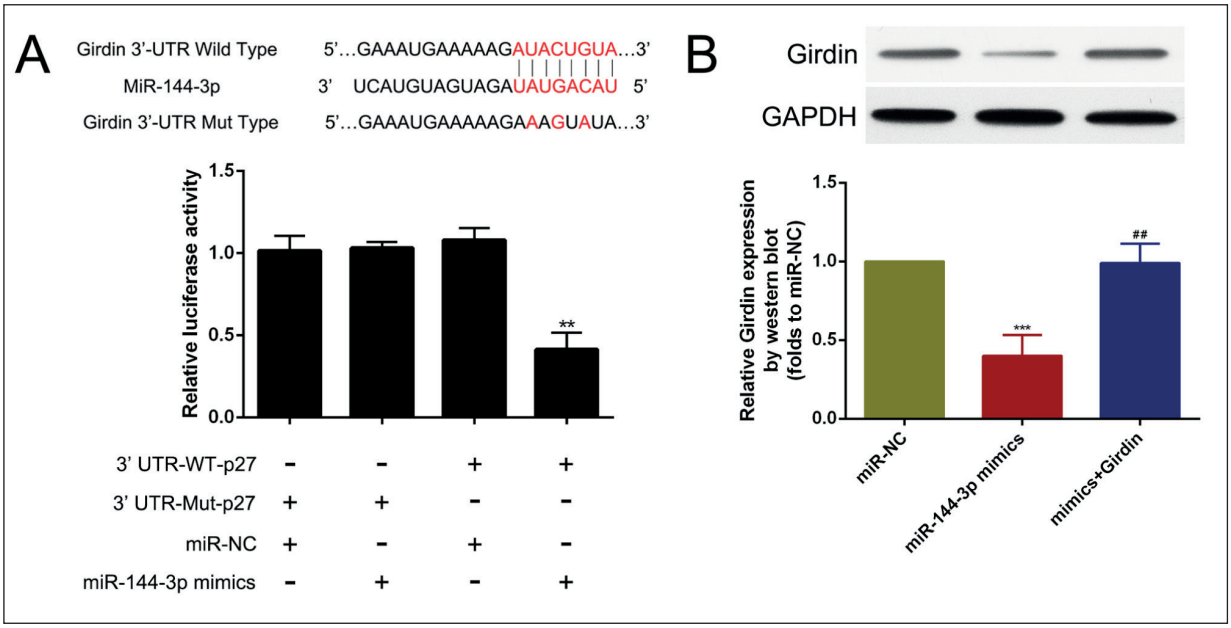

Figure 3. Girdin was a direct and functional target of miR-144-3p. G401 cells were transfected with miR-144-3p mimics and inhibitor. ***A,*** Diagram of putative miR-144-3p binding sites of Girdin and relative activities of Luciferase reporters (**$p < 0.01$). ***B,*** Western blot indicated that miR-144 decreased the expression level of Girdin (***$p < 0.001$ *vs.* NC group; ##$p < 0.01$ *vs.* mimics group).

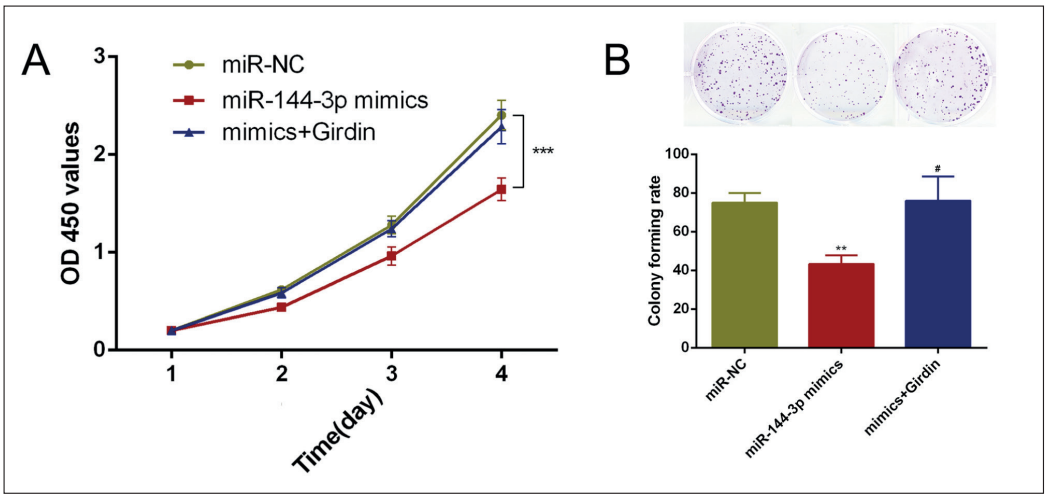

Figure 4. MiR-144-3p decreased the proliferation of WT cells. ***A,*** Cell proliferation detected by MTT assay (***$p < 0.001$). ***B,*** Colony formation ability detected by colony formation assay (**$p < 0.01$ *vs.* NC group; #$p < 0.05$ *vs.* mimics group).

up-regulation of miR-144-3p. However, the addition of Girdin resulted in increased malignant metastasis of G401 cells (Figure 5).

Discussion

Scholars have found that the changes in miRNA expression profile in tissue and blood samples can be used as an auxiliary marker for the diagnosis of WT. The expression levels of these indicators can assist clinicians to classify risks before chemotherapy, which may also guide the development of appropriate therapeutic therapy[14]. Murray et al[15] have revealed serum miRNA expression profiles have a significant difference in childhood solid tumor patients, in which miR-129-5p and miR-143-3p can be used as potential

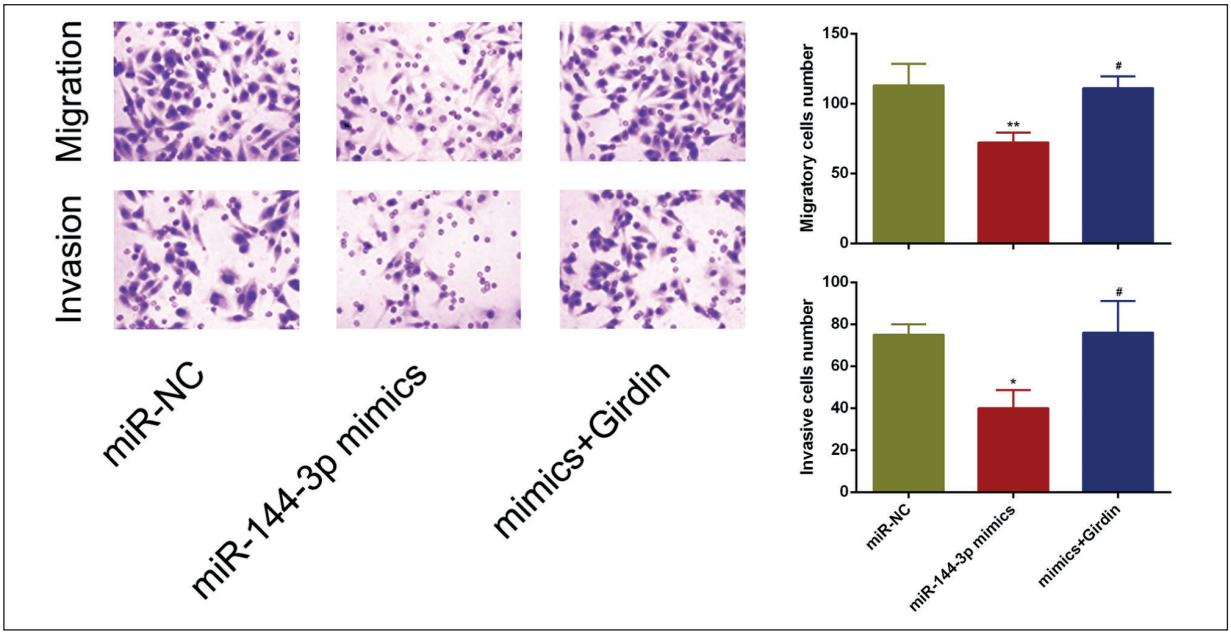

Figure 5. The miR-144-3p/Girdin axis inhibited the invasion and migration of WT cells. Girdin overexpression attenuated the inhibitory effect of miR-144-3p on WT cells. Cell invasion and migration detected by transwell assay. All data were presented as means ± standard deviations (*$p < 0.05$, **$p < 0.01$ *vs.* NC group; #$p < 0.05$ *vs.* mimics group).

diagnostic markers for WT. Moreover, Luwdig et al[16] have found that 14 miRNAs are differentially expressed between WT and normal serum samples, among which miR-100-5p and miR-130-3p can serve as potential biomarkers for WT. In the current study, results indicated that the expression of miR-144-3p was decreased in pediatric WT tissues and cell lines. Therefore, miR-144-3p, as a tumor suppressor gene, might be involved in the occurrence and development of WT.

MiRNAs generally exert their function through binding to the 3'-UTR of target genes in line with the principle of complete or incomplete complementary base pairing. This may lead to the direct degradation of target gene mRNA or inhibit the protein translation process of target genes, eventually suppressing the expression of corresponding proteins[17,18]. Therefore, searching and determining the functional targeting of miRNAs is of vital significance in clearing the mechanism of corresponding miRNAs in tumor biological function. In this work, online prediction software predicted that Girdin was a potential target gene of miR-144-3p, which was then confirmed *via* dual luciferase reporter gene assay and Western blot. Results demonstrated that Girdin could bind to the 3'-UTR of miR-144-3p, proving that the expression of Girdin was negatively regulated by miR-144-3p.

Girdin, a macromolecular protein with a relative molecular weight of 220 kDa, consists of 1870 amino acid residues. CCDC88A is the gene encoding human Girdin protein, which is located on chromosome 2. A large number of studies have found that Girdin is abnormally expressed in a variety of tumor tissues, such as gastric cancer[19], esophageal cancer[20], colorectal cancer[21], glioblastoma[22], breast cancer[23], and non-small cell lung cancer[24], thereby playing a vital role in tumor biology. For example, its phosphorylation can promote the synthesis of DNA in tumor cells[25], and facilitate the growth, proliferation, invasion and metastasis of tumor cells[21,26,27]. Meanwhile, it may encourage the migration of VEGF-dependent endothelial cells, the formation of tubular structure and microvascular remodeling after birth[28-31]. However, no reports have investigated the role of Girdin protein in pediatric WT patients, as well as its correlation with the proliferation and metastasis of WT cells.

As expected, we found that the protein expression of Girdin in pediatric WT tissues and cells was significantly higher than that of the control group, indicating Girdin played an important role in the pathological process of pediatric WT. Furthermore, after overexpression of miR-144-3p in WT cells, the proliferation and metastasis ability of cells were inhibited. However, the inhibitory effect of miR-144-3p was obviously weakened after the up-regulation of Girdin expression. The above results suggested that miR-144-3p could achieve its inhibitory effect on the malignancy of WT cells *via* targeting Girdin expression.

Conclusions

We firstly revealed the molecular mechanism of miR-144-3p in pediatric WT, which was achieved through targeted regulation of Girdin. Our study might provide an important theoretical basis for researching the pathogenesis of pediatric WT, which could also serve as an important reference value for further study of the biological targeting gene therapy of pediatric WT.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.
2) Dome JS, Graf N, Geller JI, Fernandez CV, Mullen EA, Spreafico F, Van den Heuvel-Eibrink M, Pritchard-Jones K. Advances in Wilms tumor treatment and biology: progress through international collaboration. J Clin Oncol 2015; 33: 2999-3007.
3) Lee JS, Padilla B, DuBois SG, Oates A, Boscardin J, Goldsby RE. Second malignant neo-plasms among children, adolescents and young adults with Wilms tumor. Pediatr Blood Cancer 2015; 62: 1259-1264.
4) Sredni ST, Gadd S, Huang CC, Breslow N, Grundy P, Green DM, Dome JS, Shamberger RC, Beckwith JB, Perlman EJ. Subsets of very low risk Wilms tumor show distinctive gene expression, histologic, and clinical features. Clin Cancer Res 2009; 15: 6800-6809.
5) Liu Q, Yang W, Luo Y, Hu S, Zhu L. Correlation between miR-21 and miR-145 and the incidence and prognosis of colorectal cancer. J BUON 2018; 23: 29-35.
6) Qi R, Wang DT, Xing LF, Wu ZJ. MiRNA-21 promotes gastric cancer growth by adjust-ing prostaglandin E2. Eur Rev Med Pharmacol Sci 2018; 22: 1929-1936.

7) Wang X, Shi Z, Liu X, Su Y, Li W, Dong H, Zhao L, Li M, Wang Y, Jin X, Huo Z. Upregulation of miR-191 promotes cell growth and invasion via targeting TIMP3 in prostate cancer. J BUON 2018; 23: 444-452.

8) Urbach A, Yermalovich A, Zhang J, Spina CS, Zhu H, Perez-Atayde AR, Shukrun R, Charlton J, Sebire N, Mifsud W, Dekel B, Pritchard-Jones K, Daley GQ. Lin28 sustains early renal progenitors and induces Wilms tumor. Genes Dev 2014; 28: 971-982.

9) Hohenstein P, Pritchard-Jones K, Charlton J. The yin and yang of kidney development and Wilms' Tumors. Genes Dev 2015; 29: 467-482.

10) Saal S, Harvey SJ. MicroRNAs and the kidney: coming of age. Curr Opin Nephrol Hyper-tens 2009; 18: 317-323.

11) Koller K, Pichler M, Koch K, Zandl M, Stiegelbauer V, Leuschner I, Hoefler G, Guertl B. Nephroblastomas show low expression of microR-204 and high expression of its target, the oncogenic transcription factor MEIS1. Pediatr Dev Pathol 2014; 17: 169-175.

12) Koller K, Das S, Leuschner I, Korbelius M, Hoefler G, Guertl B. Identification of the transcription factor HOXB4 as a novel target of miR-23a. Genes Chromosomes Cancer 2013; 52: 709-715.

13) Cao X, Liu D, Yan X, Zhang Y, Yuan L, Zhang T, Fu M, Zhou Y, Wang J. Stat3 inhibits WTX expression through up-regulation of microRNA-370 in Wilms Tumor. FEBS Lett 2013; 587: 639-644.

14) Watson JA, Bryan K, Williams R, Popov S, Vujanic G, Coulomb A, Boccon-Gibod L, Graf N, Pritchard-Jones K, O'Sullivan M. MiRNA profiles as a predictor of chemorespon-siveness in Wilms' tumor blastema. PLoS One 2013; 8: e53417.

15) Murray MJ, Raby KL, Saini HK, Bailey S, Wool SV, Tunnacliffe JM, Enright AJ, Nichol-son JC, Coleman N. Solid tumors of childhood display specific serum microRNA profiles. Cancer Epidemiol Biomarkers Prev 2015; 24: 350-360.

16) Ludwig N, Nourkami-Tutdibi N, Backes C, Lenhof HP, Graf N, Keller A, Meese E. Circulating serum mirnas as potential biomarkers for nephroblastoma. Pediatr Blood Cancer 2015; 62: 1360-1367.

17) Doench JG, Petersen CP, Sharp PA. SiRNAs can function as miRNAs. Genes Dev 2003; 17: 438-442.

18) Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, in-dicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15-20.

19) Wang C, Lin J, Li L, Wang Y. Expression and clinical significance of girdin in gastric cancer. Mol Clin Oncol 2014; 2: 425-428.

20) Shibata T, Matsuo Y, Shamoto T, Hirokawa T, Tsuboi K, Takahashi H, Ishiguro H, Ki-mura M, Takeyama H, Inagaki H. Girdin, a regulator of cell motility, is a potential prognos-tic marker for esophageal squamous cell carcinoma. Oncol Rep 2013; 29: 2127-2132.

21) Jun BY, Kim SW, Jung CK, Cho YK, Lee IS, Choi MG, Choi KY, Oh ST. Expression of girdin in human colorectal cancer and its association with tumor progression. Dis Colon Rectum 2013; 56: 51-57.

22) Natsume A, Kato T, Kinjo S, Enomoto A, Toda H, Shimato S, Ohka F, Motomura K, Kondo Y, Miyata T, Takahashi M, Wakabayashi T. Girdin maintains the stemness of glioblastoma stem cells. Oncogene 2012; 31: 2715-2724.

23) Liu C, Zhang Y, Xu H, Zhang R, Li H, Lu P, Jin F. Girdin protein: a new potential distant metastasis predictor of breast cancer. Med Oncol 2012; 29: 1554-1560.

24) Song JY, Jiang P, Li N, Wang FH, Luo J. Clinical significance of Girdin expression de-tected by immunohistochemistry in non-small cell lung cancer. Oncol Lett 2014; 7: 337-341.

25) Anai M, Shojima N, Katagiri H, Ogihara T, Sakoda H, Onishi Y, Ono H, Fujishiro M, Fukushima Y, Horike N, Viana A, Kikuchi M, Noguchi N, Takahashi S, Takata K, Oka Y, Uchijima Y, Kurihara H, Asano T. A novel protein kinase B (PKB)/AKT-binding protein enhances PKB kinase activity and regulates DNA synthesis. J Biol Chem 2005; 280: 18525-18535.

26) Ohara K, Enomoto A, Kato T, Hashimoto T, Isotani-Sakakibara M, Asai N, Ishida-Takagishi M, Weng L, Nakayama M, Watanabe T, Kato K, Kaibuchi K, Murakumo Y, Hi-rooka Y, Goto H, Takahashi M. Involvement of Girdin in the determination of cell polarity during cell migration. PLoS One 2012; 7: e36681.

27) Garcia-Marcos M, Jung BH, Ear J, Cabrera B, Carethers JM, Ghosh P. Expression of GIV/Girdin, a metastasis-related protein, predicts patient survival in colon cancer. FASEB J 2011; 25: 590-599.

28) Miyake H, Maeda K, Asai N, Shibata R, Ichimiya H, Isotani-Sakakibara M, Yamamura Y, Kato K, Enomoto A, Takahashi M, Murohara T. The actin-binding protein Girdin and its Akt-mediated phosphorylation regulate neointima formation after vascular injury. Circ Res 2011; 108: 1170-1179.

29) Kitamura T, Asai N, Enomoto A, Maeda K, Kato T, Ishida M, Jiang P, Watanabe T, Usukura J, Kondo T, Costantini F, Murohara T, Takahashi M. Regulation of VEGF-mediated angiogenesis by the Akt/PKB substrate Girdin. Nat Cell Biol 2008; 10: 329-337.

30) Ito T, Komeima K, Yasuma T, Enomoto A, Asai N, Asai M, Iwase S, Takahashi M, Tera-saki H. Girdin and its phosphorylation dynamically regulate neonatal vascular development and pathological neovascularization in the retina. Am J Pathol 2013; 182: 586-596.

31) Weng L, Enomoto A, Ishida-Takagishi M, Asai N, Takahashi M. Girding for migratory cues: roles of the AKT substrate Girdin in cancer progression and angiogenesis. Cancer Sci 2010; 101: 836-842.