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miR-483-5p Targets MKNK1 to Suppress Wilms' Tumor Cell Proliferation and Apoptosis *In Vitro* and *In Vivo*

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Background: Wilms' tumor (WT) is the most common type of renal tumor in children and it has high mortality rates. MicroRNAs (miRNAs) are important regulators of cellular differentiation processes that have been discovered to contribute to the development of various kinds of tumors.

Material/Methods: The Wilms' tumor tissues and adjacent tissues were obtained from 28 patients to quantity miR-483-5p expression level. The miR-483-5p mimics and scrambles were transfected into the human kidney WT cell line GHINK-1 to evaluate the effect of miR-483-5p on Wilms' tumor cell proliferation and apoptosis *in vitro*. A total of 18 female BALB/c nu/nu mice were used to further confirm how miR-483-5p affects Wilms' tumor *in vivo*.

Results: In the present study, miR-483-5p was identified to be downregulated in Wilms' tumor tissues compared with the normal adjacent tissues. Additionally, low expression of mir-483-5p was significantly correlated with unfavorable histology subtypes, lymphatic metastasis, and late clinical stage (stage III and IV). Overexpression of miR-483-5p inhibited the proliferation and colony formation of GHINK-1 (Wilms' tumor) cells compared with the control group due to enhanced cell apoptosis. Furthermore, miR-483-5p upregulated the protein expression level of caspase-3. Finally, MAP kinase-interacting serine/threonine-protein kinase 1 was identified as a direct target of miR-483-5p, which was confirmed by luciferase reporter assay and Western blotting.

Conclusions: MiR-483-5p suppressed WT cell proliferation via inducing apoptosis through targeting MKNK1. This may provide novel insights into the mechanisms underlying WT and a potential therapeutic candidate for the treatment of WT in the future.

MeSH Keywords: Apoptosis • Genes, Wilms Tumor • MicroRNAs

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Background

MicroRNAs are a class of highly conserved and endogenously synthesized gene expression regulators that are single-stranded, non-coding RNA molecules, typically 18–22 nucleotides long [1]. Through binding to the 3' untranslated region (3'UTR) of specific genes and triggering either mRNA degradation or translational repression, miRNAs exert their role of regulating gene expression [2]. Previous studies have identified that deregulated miRNAs serve crucial roles in numerous cancer types, including gastric, ovarian, and lung cancer, and may be regarded as tumor suppressors or oncogenes in cancer development [3–6].

Wilms' tumor is the most common type of renal tumor and it has a high mortality rate in children [7]. It is an embryological tumor that histologically mimics renal embryogenesis and is composed of stromal, blastemal, and epithelial elements [8]. It is estimated that about 10 000 children globally have WT, and its incidence is increasing [9]. Surgical resection with chemotherapy and radiotherapy remain the most effective treatment methods for WT patients [10]. However, the intensity burden of chemotherapy and the high recurrence rate emphasize an urgent requirement for development of novel therapeutic approaches. By miRNA profiling and quantitative reverse transcription-quantitative polymerase chain reaction, many studies have identified key dysregulated miRNAs in the tissue and serum of patients with WT. miRNAs may participate in WT development and progression through regulating cell proliferation, migration, and apoptosis. miR-590 [11] and miR-21 [12], which promote cancer cell proliferation, were identified to be upregulated in WT tissues. The Wilms' tumor 1 (*WT1*) gene, which plays multiple roles in WT disease development, can be regulated by miR-590 directly [11,13]. On the contrary, miR-204 [14] and miR-185 [15] expression have been demonstrated to be significantly reduced in WT samples, which indicates that these miRNAs may be used to treat WT. However, studies on the functional roles of miRNAs in WT are limited.

The present study revealed that the expression level of miR-483-5p was decreased in WT tissue, and the level was associated with unfavorable histology subtypes, lymphatic metastasis, and late clinical stage. In addition, miR-483-5p was demonstrated to inhibit GHINK-1 cell proliferation and colony formation via inducing apoptosis. The miR-483-5p targets MKNK1 directly, which was confirmed by luciferase reporter gene assay, Western blotting, and RT-qPCR. It was demonstrated that the function of eIF4E (eukaryotic initiation factor 4E) is modulated through phosphorylation of a conserved serine (Ser209) by MKNK1 and MKNK2, downstream of ERK [16]. Pharmacological inhibition of MKNKs may be an ideal therapeutic strategy for cancer treatment.

Material and Methods

Clinical samples

Healthy adjacent tissue samples and tumor tissue samples of Wilms' tumor patients were collected from 28 patients between ages 25–45 years. These patients were recruited in the First People's Hospital of Yunnan Province between 2013 and 2017. The sample consisted of 15 male patients and 13 female patients. All patients recruited in this study had already provided informed consent. All operations in this study were approved by the Ethics Committee of the First People's Hospital of Yunnan Province (Kunming, China).

Cell culture and miRNA transfection

The human kidney (Wilms' Tumor) cell line GHINK-1 was obtained from RIKEN (Wako, Japan). The cells were cultivated in Maccyo'5 media (Invitrogen) supplemented with 15% fetal bovine serum (Gibco) in a humidified incubator with 5% CO_2 at 37°C. Mature miR-483-5p mimics and a non-targeting scrambled control sequence were purchased from Invitrogen. The sequence of mimics and miR-483-5p mimics were as follows: mimics, 3'-AGCCAGGAGGGCAGCCCAGGA-5'; miR-483-5p, 3'-AGGGAAGAAAGGAGGGCAGAA-5'. GHINK-1 cells were seeded and cultured for 1 day prior to transfection. DMSO (control), 10 nM scrambles, or 10 nM miR-483-5p mimics were transfected into cells (5×10^3/well) using Lipofectamine 2000 (Invitrogen). Then, cells were harvested 24, 48, or 72 h after incubation for subsequent assays.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

TRIzol (Invitrogen) was used to extract total RNA from tissues or cells, in accordance with the manufacturer's protocol. The reverse transcription into cDNA was performed with M-MLV reverse transcriptase (Promega) according to the manufacturer's instruction. RT-qPCR was conducted on the CFX96 sequence detection system (Bio-Rad) with 20 ng cDNA using the 2X Ultra SYBR Mixture kit (Takara Bio, Inc., Otsu, Japan). The thermocycling was conducted as follows: Initial denaturation at 95°C for 1 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 10 s, followed by 72°C for 2 min and 16°C for 5 min. The primer sequences were as follows: GAPDH, forward, 5'-CGGAGTCAACGGATTTGGTCGTAT-3', reverse, 5'-AGCCTTCTCCATGGTGGTGAAGAC-3'; miR-483-5p, forward, 5'-AGTTGGCTCACGGTTCTTTCAA-3', reverse, 5'-ATCGCCaTGGCCCGCATGTCGG-3.

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CCK-8 assay

The proliferative potential of GHINK-1 cells transfected miR-483-5p mimics or scrambled control were measured using the Cell Counting kit-8 (Dojindo) following the manufacturer's instructions. The cells at a density of 3×10^3 cells per well were seeded into 96-well plates and transfected as aforementioned for 24, 48, and 72 h. Next, 10 µl CCK-8 solution was added to each well and incubated at 37°C for an additional 3 h. Optical density (OD) was tested at a wavelength of 450 nm with a microplate reader.

Colony formation assay

GHINK-1 cells were transfected as aforementioned at 24 h prior to reseeding in 6-well plates at 5000 cells/well. After 2-week cultivation, the surviving colonies were fixed with paraformaldehyde, followed by staining with 0.5% crystal violet for 1 h at 37°C. Surviving cells were counted. Photographs were captured using a digital camera.

Apoptosis measured by flow cytometry

Cellular apoptosis was evaluated using R&D TACS® Annexin V-FITC kit (R&D Systems) based on the manufacturer's instructions. GHINK-1 cells were seeded into 6-well plates for 1 day before transfection. After 48 h, cells were harvested by trypsinization, washed twice with cold PBS, and then resuspended in 1× binding buffer. Next, 5 µl Annexin V and propidium iodide solution was added per 100 µl cell suspension (1×10^5 cells). The cells were gently mixed and incubated at room temperature in the dark for 15 min. Each sample was added to 300 µl 1× binding buffer, and the cells were examined using a flow cytometer (FACScan) within 1 h. Data were analyzed using FlowJo software (version 10; FlowJo LLC, Ashland, OR, USA). Each experiment was independently repeated 3 times.

Luciferase reporter assay

TargetScan, PicTar, and miRbase (*http://www.mirbase.org/*) were used to predict targets of miR-483-5p. In the luciferase reporter assay, the 3'-untranslated regions (UTRs) were designed according to bioinformatics software [18]. TargetScan, PicTar, and miRbase were used to design 3'-UTRs. MKNK1 sequence used was as follows: 5'-CGGAUGUCCUCUUUGAAACUCUCC-3'. The MKNK1 UTRs containing the predicted miR-483-5p binding sites and corresponding mutant sites were amplified, and were then inserted with the downstream luciferase gene in the psi-CHECKTM vector (Promega Corporation). GHINK-1 cells were cultured in a 24-well plate at a density of 2.5×10^5 cells/well 1 day prior to transfection. The final concentration (10 nM) of MKNK1 wild-type or mutant reporter plasmids, the p-TK *Renilla* plasmid (Promega Corporation), plus miR-483-5p mimics or controls, were used for co-transfection of GHINK-1 cells (4×10^5 cells/well) using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocols. Luciferase activities were determined with a dual-luciferase assay kit (Promega Corp.) 48 h subsequent to transfection. The Renilla luciferase activity was measured as an internal control for each well.

Western blotting

GHINK-1 cells were harvested and lysed in radioimmunoprecipitation assay buffer with protease inhibitors (Sigma-Aldrich) added. Total protein concentration was quantified with a bicinchoninic acid assay protein kit (Beyotime Institute of Biotechnology). Equal amounts of 50 µg total protein were separated by 10% SDS-PAGE and transferred to PVDF membranes (Bio-Rad Laboratories). After transfer to pure PVDF membranes, the separated proteins were blocked with 5% fat-free dry milk in PBS for 1 h at room temperature. After blocking with fat-free dry milk, the membranes were incubated at 4°C overnight with the primary antibodies as follows: anti-MKNK1 (dilution, 1: 1,000; cat. no., 2195), anti-cleaved caspase 3 (dilution, 1: 1,000; cat. no., 9664), anti-cleaved PARP (dilution, 1: 1,000; cat. no., 9548), and β-actin (dilution, 1: 5,000; cat. no., 4970). After washing 3 times with PBS-Tween, the membranes were incubated with a horseradish peroxidase-conjugated secondary rabbit antibody (dilution, 1: 5,000; cat. no., 15180) for 1 h at 4°C. All antibodies were obtained from Cell Signaling Technology, Inc. The target proteins expression levels were visualized with electrochemiluminescence (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The densitometry of proteins was analyzed with Quantity One software (version 4.6.2; Bio-Rad Laboratories).

Animal study

Eighteen female BALB/c nu/nu mice (8 weeks old) were provided by Beijing Vital River Laboratory Animal Technology Company (Beijing, China), and kept under specific pathogen-free conditions. The animals were maintained on a 12 h/12 h light/dark cycle at a constant temperature of 22°C and a humidity of 50–60%. Free access to chow and water were provided. All experiments were approved by the Committee on Animal Experimentation of the First People's Hospital of Yunnan Province (Kunming, China). Each mouse was subcutaneously injected with 5×10^6 GHINK-1 cells, with 5 mice in each group. The volume of tumors was evaluated by micrometer calipers weekly. When tumor volume reached 150 mm^3, the mice underwent an intratumoral injection with 50 µg of miR-483-5p mimics or scrambled control dissolved in 100 µl of DMEM with 5 µl of Lipofectamine 2000 added. At week 4, the mice were sacrificed with 100% CO_2 at a flow rate of 5 L/min, and the tumors were weighed.

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TUNEL assay

The terminal deoxynucleotidyl transferase dUTP nick-end labeling assay kit (TUNEL, Sigma-Aldrich) was used to evaluate apoptosis in tumor tissues. Briefly, tumor sections were dewaxed and hydrated. After that, they were digested with proteinase K for 30 min and labeled with a TUNEL reaction mixture for 2 h at 37°C. TUNEL-positive cells in 5 views of each tumor section were calculated (×200 or ×400).

Statistical analysis

Statistical analysis was conducted with SPSS 13.0 (SPSS). Data are expressed as the mean ± standard deviation. Significant differences between 2 groups were analyzed with two-tailed unpaired *t* test. P<0.05 was considered a significant difference. All of the experimental values are expressed as the means of 3 independent repeats.

Results

The expression level of miR-483-5p was significantly lower in tumor tissues than in adjacent tissues.

In this study, the expression levels of miR-483-5p in 28 Wilms' tumor tissues were quantified using RT-qPCR to measure the involvement of miR-483-5p in WT. The relative expression levels of miR-483-5p in healthy adjacent tissues and in WT tissues were 2.0 and 1.2, respectively (P<0.01; Figure 1A). This result confirmed that the expression level of miR-483-5p in tumor tissues was significantly lower than in adjacent tissues.

miR-483-5p expression was correlated with clinicopathological parameters

To examine the association between miR-483-5p expression and clinicopathological parameters of the WT patients, the samples were divided into high and low miR-483-5p expression groups,

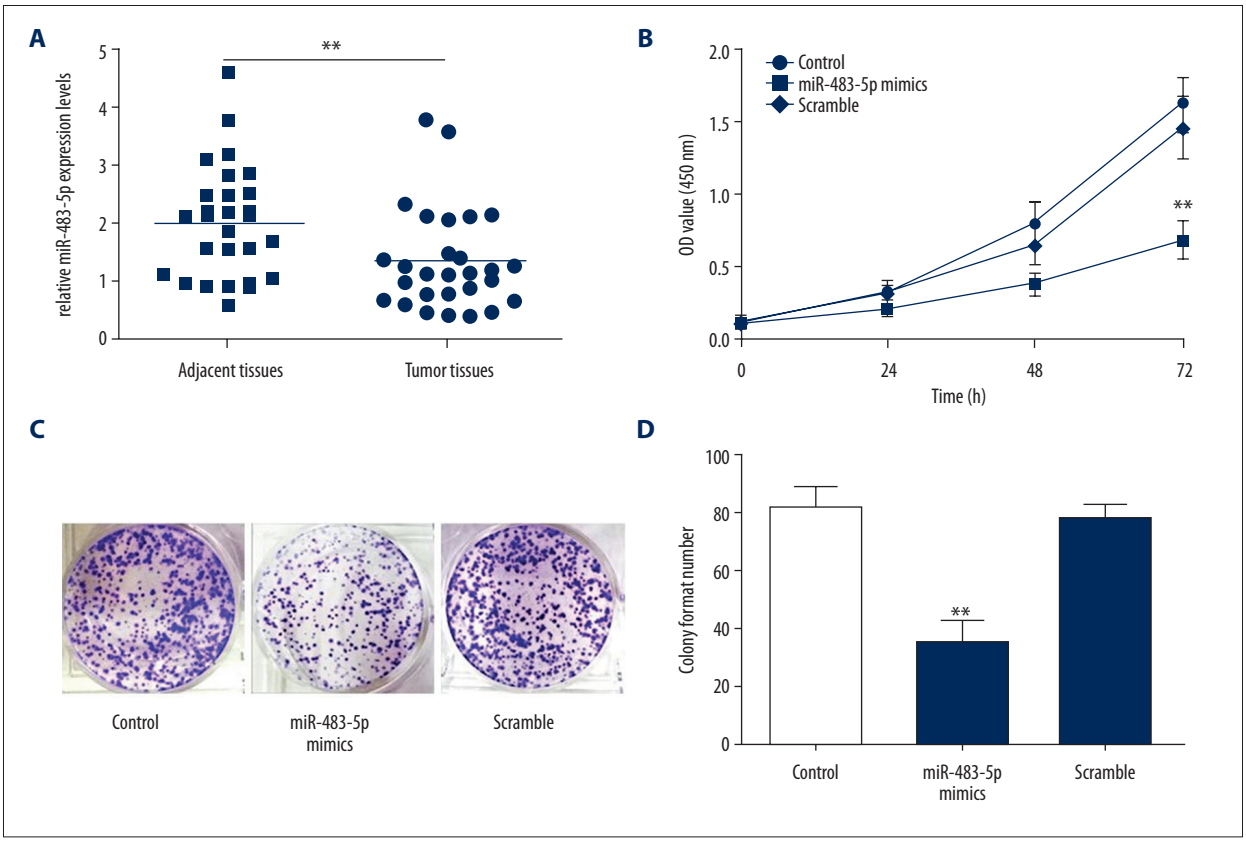

Figure 1. Downregulation of miR-483-5p expression in Wilms' tumor tissues suppresses the proliferation of GHINK-1 cells. (**A**) Relative miR-483-5p expression levels in Wilms' tumor tissues (n=28) and adjacent tissues (n=28). ** P<0.01 compared with adjacent tissues. (**B**) Viability of GHINK-1 cells was examined after treatment with control (DMSO), miR-483-5p mimics, or scrambled control for 24, 48, or 72 h. Experiments were repeated 3 times. ** P<0.01 compared with control, n=3. (**C**) GHINK-1 cells treated with control (DMSO) or miR-483-5p mimics underwent a colony formation assay for 2 weeks, and surviving colonies were counted. (**D**) Colony number was significantly reduced after miR-483-5p treatment. ** P<0.01 compared with control, n=3. miR – microRNA.

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Table 1. miR-483-5p expression levels in patients with Wilm's tumor.

Parameters	n	miR-483-5p		*P* value
		Low	High	
Gender				0.716
Male	11	5	6	
Female	17	11	6	
Age(months)				0.675
<36	16	9	7	
≥36	12	7	5	
Histopathological type				0.039*
Favorable (FH)	19	7	12	
Unfavorable(UH)	9	6	3	
Lymphatic metastasis				0.042*
Yes	5	3	2	
No	23	8	15	
NWTS stage				0.019*
I, II	7	2	5	
III, IV	21	13	8	

miR – mciro RNA; NWTS – National Wilms Tumor Study; * P<0.05.

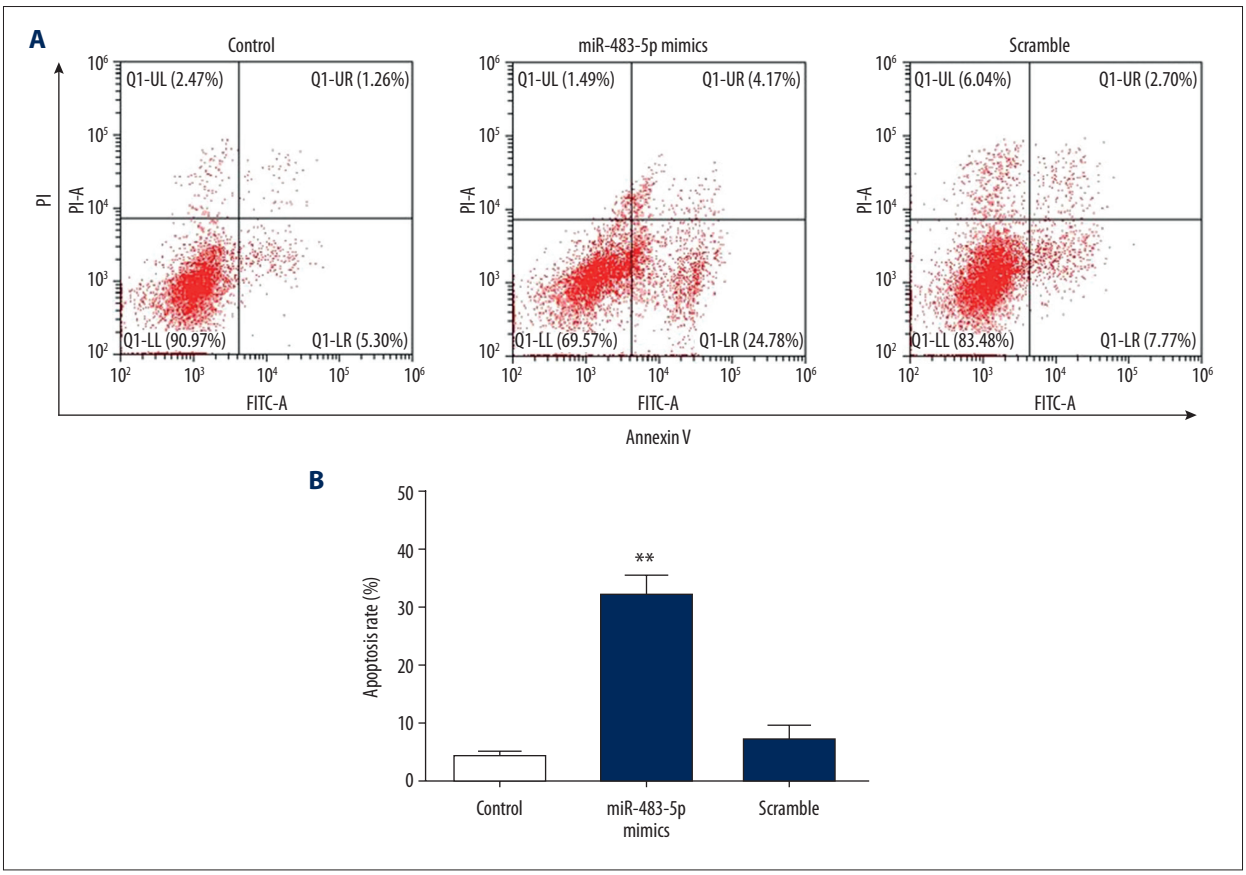

Figure 2. The apoptotic rate of GHINK-1 cells after treatment with control (DMSO), miR-483-5p mimics, or scrambled control for 48 h. (**A**) Flow cytometry analysis of apoptosis. (**B**) Apoptotic rate of early apoptotic and late apoptotic cells. All data are presented as the mean ± standard deviation of 3 independent experiments. ** P<0.01 *vs.* control, n=3. miR – microRNA.

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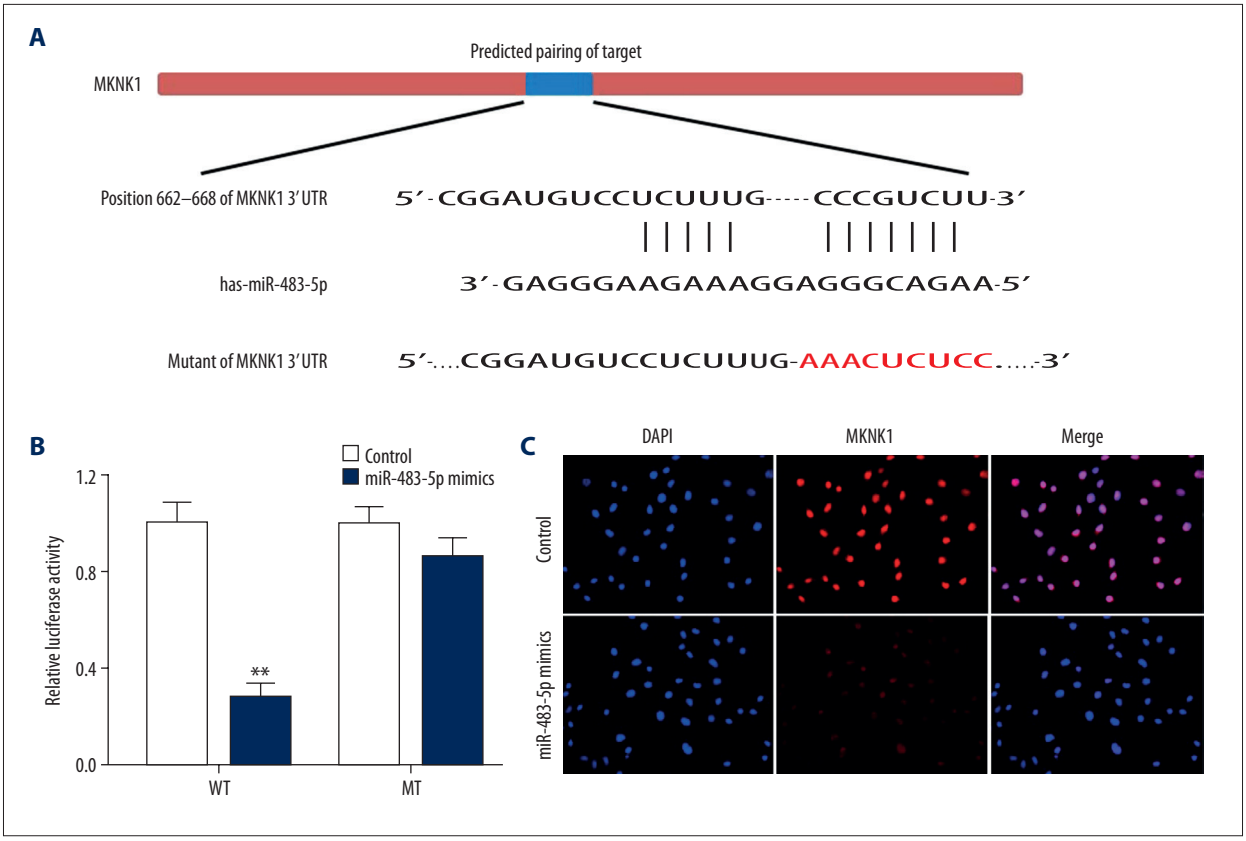

Figure 3. MKNK1 is a direct transcriptional target of miR-483-5p in GHINK-1 cells. (**A**) miR-483-5p target sequences of the MKNK1 3'-UTR. (**B**) Relative luciferase activity of wild-type and mutant MKNK1 3'-UTR cotransfected with control or miR-483-5p mimics. (**C**) Immunofluorescent staining of MKNK1 in GHINK-1 transfected with scrambled control or miR-483-5p mimics. ** P<0.01 *vs.* control, n=3. miR – micro RNA; 3'UTR – 3' untranslated region; MKNK1 – MAP kinase-interacting serine/threonine-protein kinase 1; WT – wild-type; MT – mutated.

based on the median score. In Table 1, we summarized the association of miR-483-5p expression with certain clinicopathological parameters, including age, sex, lymphatic metastasis, histopathological tumor type, and clinical stage. Patients exhibiting low expression of miR-483-5p demonstrated a significant correlation with unfavorable histology subtype, lymphatic metastasis, and late clinical stage (stages III, IV). No statistical association was identified between miR-483-5p expression and sex or age. These results suggest that downregulation of miR-483-5p expression was related with the development of WT.

miR-483-5p inhibited the proliferation and colony formation in GHINK-1 cells

As miR-483-5p expression is inhibited in WT tissues, we evaluated whether high expression of miR-483-5p affects the proliferation and viability of GHINK-1 cells using CCK-8 and colony formation assays. Compared with the control group, cells treated with miR-483-5p mimics exhibited a significantly decreased survival rate at 48 h (P<0.05; Figure 1B) and 72 h (P<0.01; Figure 1B). The proliferation rate was not significantly different between with cells transfected with scrambled control and the DMSO control group. The influence of miR-483-5p mimics on cell survival rate was demonstrated to be time-dependent. Furthermore, in the colony formation assay, miR-483-5p mimics significantly reduced the number of colonies (P<0.01; Figure 1C, 1D). These data demonstrated that miR-483-5p obstructed GHINK-1 cell proliferation and colony formation, and may be used as a potential WT suppressor.

The apoptosis of GHINK-1 cells was promoted by miR-483-5p mimics

To investigate the impact of miR-483-5p on apoptosis, GHINK-1 cells were transfected with control, miR-483-5p mimics, or scrambled control and analyzed by flow cytometry for apoptotic rate. The results indicated that, after 48-h transfection, the early and late apoptotic rates of the miR-483-5p mimics group (32.15±3.30) were significantly higher than in the control group (4.33±0.78) and scramble group (7.40±2.24) (Figure 2A, 2B, P<0.01). There was no significant difference

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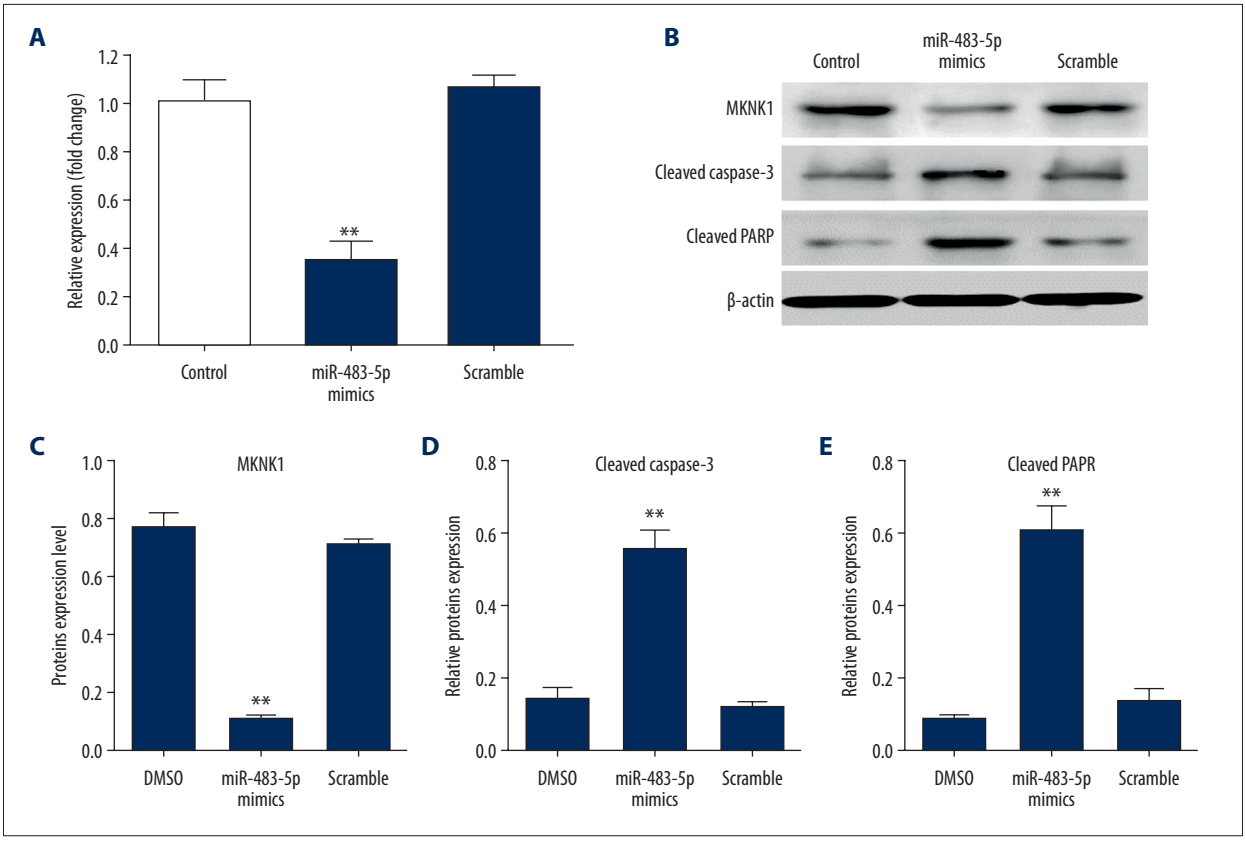

Figure 4. miR-483-5p suppressed MKNK1 expression. GHINK-1 cells were transfected with control, miR-483-5p mimics, or scrambled control for 72 h. (**A**) Relative mRNA expression levels of MKNK1 was detected with qRT-PCR. (**B**) Protein expression levels of MKNK1, cleaved caspase 3, and cleaved PARP in cells. (**C**) Protein expression level of MKNK1 normalized to that of β-actin. (**D**) Protein expression level of cleaved caspase-3 normalized to that of β-actin. (**E**) Protein expression level of cleaved PARP normalized to that of β-actin. ** P<0.01 *vs.* control group, n=3. MKNK1 – MAP kinase-interacting serine/threonine-protein kinase 1; miR – microRNA; DMSO – dimethyl sulphoxide.

detected between the control and scrambled control groups. Therefore, miR-483-5p overexpression was demonstrated to induce apoptosis in GHINK-1 cells.

miR-483-5p directly targeted MKNK1 gene in GHINK-1 cells

To find the potential target gene of miR-483-5p, 3 independent online databases were used: TargetScan, PicTar, and miRBase. MKNK1 was identified as a candidate gene, and it carries a presumed miR-483-5p binding site within its 3'UTR, located at 662-668 bp (Figure 3A). MKNK1 has been demonstrated to promote tumor cell proliferation [15]. To prove that miR-483-5p binds to the predicted region and leads to translational inhibition, a luciferase reporter plasmid, with either mutant MKNK1 3'-UTR or wild-type, was cotransfected into GHINK-1 cells with scrambled control or miR-483-5p mimics. In the wild-type group, relative luciferase activity was significantly inhibited by miR-483-5p mimics compared with the control group (*P*<0.01; Figure 3B), while this inhibition was not observed in the MKNK1 mutant group. In addition, the data from immunofluorescent staining indicated miR-483-5p mimics markedly suppressed the expression of MKNK1 in GHINK-1 cells (Figure 3C). All these results indicate MKNK1 was repressed by miR-483-5p due to direct binding of the 3'-UTR.

miR-483-5p suppressed MKNK1 expression in a caspase 3-dependent manner

The mRNA and protein expression levels of MKNK1 after treatment with DMSO, miR-483-5p mimics, or scrambled control were detected with RT-pPCR and Western blotting. The results demonstrated that in GHINK-1 cells, miR-483-5p overexpression obviously inhibited the mRNA level and protein expression levels of MKNK1 when compared with the control group (P<0.01; Figure 4A–4C). There was no significant difference between the scrambled control and untreated control groups. It has been previously demonstrated that miR-483-5p promoted the protein expression level of cleaved caspase-3 and cleaved PARP in cells undergoing apoptosis [17]. These results show

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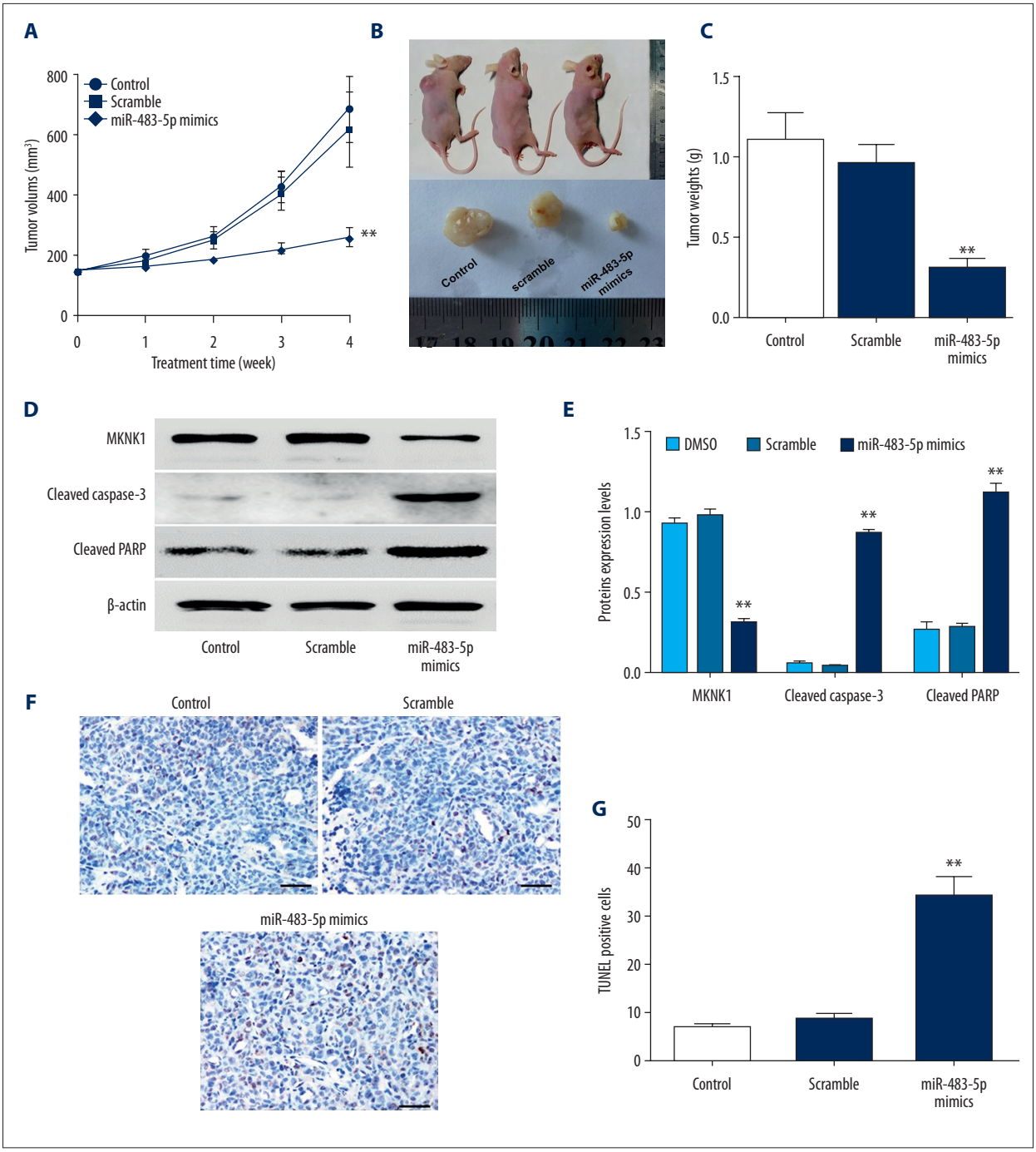

Figure 5. miR-483-5p inhibited tumor growth in GHINK-1 xenograft mice. (**A**) Tumor volumes were measured weekly following treatment with miR-483-5p mimics. Values are expressed as the mean ± standard deviation (n=6). (**B**) The tumor-bearing mice were sacrificed and photographed at the end of the experiment. (**C**) The tumors were excised from the mice and weighed. (**D**) The protein levels of MKNK1, cleaved caspase 3, and cleaved PARP in tumor tissues were detected with Western blot. (**E**) Normalized protein expression level of MKNK1 cleaved caspase 3 and cleaved PARP to that of β-actin. (**F**) Apoptotic cells were measured with TUNEL assay. (**G**) The quantification of TUNEL-positive cells. Scale bar: 50 μM. ** P<0.01 *vs.* control. miR – microRNA.

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that miR-483-5p suppressed MKNK1 expression, both at the mRNA level and protein level, resulting in caspase 3-dependent apoptosis.

miR-483-5p inhibited tumor growth in GHINK-1 xenografts *in vivo*

The anti-tumor effect of miR-483-5p was detected using the xenograft nude mouse model. The results indicated the tumor volumes of mice were significantly inhibited by miR-483-5p mimics treatment compared with the control group (Figure 5A). Tumor weight was radically reduced after treatment (Figure 5B, 5C). In addition, miR-483-5p mimics markedly induced tumor cell apoptosis via downregulation of MKNK1 and upregulation of cleaved caspase 3 and cleaved PARP levels *in vivo* (Figure 5D–5G). All these results further confirmed *in vitro* data and validated the pro-apoptotic function of miR-483-5p in Wilms' tumor.

Discussion

WT is one of the leading causes of cancer-related deaths in children [19,20]. miRNAs are important in tumor development, and evidence suggests that miRNAs are dysregulated in WT [19,20]. Thus, improved understanding of the mechanisms of miRNA involvement in WT progression and development is essential for improving prognosis. In this study, miR-483-5p expression was found to be decreased in WT tissue, and it targeted MKNK1 to inhibit GHINK-1 cell proliferation via caspase-dependent cell apoptosis.

In the present study, miRNA-483-5p was found to be a candidate novel therapeutic target for WT. Using RT-qPCR, miR-483-5p downregulation was demonstrated in tumor tissues but not in adjacent tissues (Figure 1A). In contrast, former studies have indicated that miR-483-5p was upregulated in WT [20]. The research focused solely on tissues may contribute to this inconsistency, due to variation in sample source. Our results also disclosed that low expression of miR-483-5p was significantly associated with unfavorable histology subtype, lymphatic metastasis, and late clinical stage (Table 1; $P<0.01$). Human miR-483-5p is inserted within the second intron of insulin-like growth factor 2 (IGF2), which has been demonstrated to increase cell proliferation and promote tumor development [21,22]. The role of miR-483-5p and its correlation with carcinogenesis remains unclear. In the present study, the proliferation and colony formation rates of GHINK-1 cells transfected with miR-483-5p were significantly decreased compared with the control group (Figure 1B–1D). The same results were observed in the apoptosis assay (Figure 2A, 2B). Wang et al. [23] revealed that miR-483-5p was remarkably downregulated in human primary glioma as well as in 3 glioma cell lines, and overexpression of miR-483-5p constrained cell proliferation. Larsen et al. [24] identified miR-483-5p as a suppressor of liver cell colonization and metastasis. The present results are consistent with these data, indicating that miR-483-5p could be a growth suppressor for WT.

Eukaryotic initiation factor 4E (eIF4E) is an important translation factor during protein synthesis, but it induces tumorigenesis when deregulated and overexpressed [25]. MKNK1/2 specifically phosphorylate serine 209 of eIF4E and regulate the initiation of translation. MKNK1/2-knockout mice exhibited no eIF4E phosphorylation and significantly attenuated tumor growth [26,27], and therefore present MKNK1/2 an attractive therapeutic strategy for cancer [28]. Grzmil et al. [29] reported that MKNK1 inhibition suppressed eIF4E phosphorylation, proliferation, and colony formation in GBM cells. Santag et al. [30] reported that a novel MKNK1 selective inhibitor suppressed the expression levels of MKNK1 downstream targets in a number of cancer cell lines *in vitro* and *in vivo*. These previous studies all used small molecular compounds. In the present study, MKNK1 was verified as a direct target of miR-483-5p. GHINK-1 cells transfected with miR-483-5p inhibited the expression of MKNK1 at the RNA and protein levels. To the best of our knowledge, this is the first study to show that MKNK1 is a target of miRNA in WT cells.

Conclusions

In conclusion, our study proves that miR-483-5p, which acts as a tumor suppressor, was decreased in WT tissues. Overexpression of miR-483-5p inhibited proliferation of WT cells, colony formation, and apoptosis through targeting MKNK1. Our findings illustrated that decreased expression of miR-483-5p in WT is responsible for the development and progression of WT. Subsequently, miR-483-5p may be regarded as a probable therapeutic target for WT patients. These findings may provide novel insights into the mechanisms underlying WT progression, and show it is a potential therapeutic candidate for the treatment of WT.

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Conflicts of interest

None.

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