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ORIGINAL RESEARCH

MicroRNA-143-3p Inhibits Wilms' Tumor Cell Growth By Targeting the Ras/Raf/MEK/ERK Pathway

Lianhai Zhang, MD; Wei Shao, MD

ABSTRACT

Background • A previous study found that microRNA-143-3p (miR-143-3p) is a tumor suppressor in various types of human cancer. However, the roles and molecular mechanisms of miR-143-3p in the progression of Wilms' tumor (WT) remain to be clarified. The aim of the present study was to determine the expression and biological functions of miR-143-3p in WT.

Material and Methods • The expression levels of miR-143-3p in primary WT tissues and adjacent tissues were determined using quantitative-reverse transcription polymerase chain reaction (qRT-PCR), and the association of the miR-143-3p expression level with various clinicopathological features of WT was investigated. Western blotting was used to evaluate the protein expression of the related signaling pathway.

Results • The expression of miR-143-3p was significantly downregulated in WT tissues and its expression levels were closely associated with tumor stage and lymph node metastasis. Overexpression of miR-143-3p in SK-NEP-1 and G401 cell lines inhibited cell proliferation by G0/G1 cell cycle phase arrest and induction of apoptosis. Moreover, k-Ras, a unique oncogene, was confirmed as a direct target of miR-143-3p, and k-Ras messenger RNA (mRNA) expression was increased in WT tissues and inversely correlated with miR-143-3p. Knockdown of k-Ras by si-k-Ras could *inhibit*, whereas overexpression of k-Ras could *promote*. cell proliferation in WT cells. Meanwhile, overexpression of k-Ras reversed the inhibitory effects on WT cells induced by miR-143-3p mimics.

Conclusion • Our findings indicate that miR-143-3p may be a potential novel prognostic biomarker and therapeutic target for WT. (*Altern Ther Health Med.* 2023;29(2):140-147)

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INTRODUCTION

Wilms' tumor (WT) is one of the most common renal malignancies in children.[1] Despite great improvements in treatment for children and adolescents with WT, patients usually have a poor clinical prognosis.[2] The development of WT involves gene and chromosome abnormalities and changes in the corresponding signal transduction pathways.[3-5] Therefore, it is of great importance to clarify the molecular mechanisms of WT progression, which may facilitate the diagnosis, prognosis and management of this disease.

MicroRNAs (miRNAs) is a group of short noncoding RNAs that inhibit or destabilize translation of the transcripts by binding to the 3′-untranslated region (3′-UTR) of target gene mRNA.[6,7] Emerging data have suggested that miRNAs are involved in a wide range of biological processes, such as cellular proliferation, apoptosis, cell cycle control and differentiation.[8] In addition, types of miRNAs have been confirmed to participate in the initiation and progression of WT. For example, Hong, et al. found that miR-590 induce G401 cell proliferation via targeting Wilms' tumor 1 (WT1).[9] Wang, et al. found that miR-613 inhibit the proliferation and metastasis of WT via targeting FRS2.[10] However, to date there have only been preliminary studies on the biological role of miRNAs in WT.

MicroRNA-143-3p (miR-143-3p), a previously identified tumor suppressor, has been found to be implicated in various kinds of cancers. For example, Li, et al. found that miR-143-3p suppressed the progression of triple-negative breast cancer cells by targeting LIM domain kinase 1.[11] Wang, et al. showed that miR-143-3p suppressed tumor growth by directly targeting AKT2 in gastric cancer (GC).[12] He, et al. demonstrated that miR-143-3p exhibited a strong tumor-suppressor benefit by inhibiting the expression of QKI-5 in

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esophageal squamous cell carcinoma (ESCC).[13] However, the expression and functional role of miR-143-3p in WT have never been examined before.

In this study, we aimed to investigate the expression and function of miR-143-3p in the development of WT. We show that miR-143-3p was downregulated in WT tissues, and its expression was associated with clinical features. Moreover, miR-143-3p acted as a tumor suppressor by targeting the k-Ras oncogene. Our findings indicate that the miR-143-3p/Ras/Raf/MEK/ERK axis is an important regulator of the development and progression of WT and provides a potential diagnostic and therapeutic target in patients with WT.

MATERIALS AND METHODS

Tissue Collection

A total of 53 pairs of WT tissues and adjacent kidney tissues were collected from patients who received routine surgery between January 2015 and May 2016 at Liaocheng People's Hospital, China. All surgical specimens were immediately snap-frozen in liquid nitrogen and stored at -80°C until use. The diagnoses of these tumor tissue samples were confirmed by pathological examination. Written informed consent was signed by all patients prior to enrolment in the study and the study protocol was approved by the Ethics Committee of Liaocheng People's Hospital.

Cell Culture

HEK 293T cell and human kidney (WT) cell lines SK-NEP-1 and G401 were purchased from the American Type Culture Collection (Manassas, Virginia, USA) and grown in Dulbecco's Modifed Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) in an incubator at 37°C with 5% CO_2.

Cell Transfection

The miR-143-3p mimics, mimics negative control (mimics NC), miR-143-3p inhibitor and inhibitor NC were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). In addition, K-Ras targeted small interfering RNA (si-K-Ras) was also constructed by GenePharma Co., Ltd. (Shanghai, China) to inhibit its expression. The coding domain sequence of human K-Ras mRNA was amplified by PCR, and inserted into pcDNA 3.0 vector (Invitrogen, Grand Island, New York, USA), called pcDNA-K-Ras. Transfection was performed using Lipofectamine™ RNAiMAX according to the manufacturer's instructions. All the transfections were independently repeated more than 3 times. All the assays were performed 48 h after transfection.

Quantitative Real-time PCR (qRT-PCR)

MiRNA was prepared using an miRNAeasy mini kit (Qiagen, Hilden, Germany) and total RNA was prepared using Trizol Reagent (Invitrogen, Waltham, Massachusetts, USA) according to the manufacturer's protocol. For miRNA reverse transcription, cDNA was synthesized using a TaqMan® microRNA Reverse Transcription kit (Applied Biosystems, Waltham, Massachusetts, USA). For mRNA reverse transcription, cDNA was synthesized using the Reverse Transcription System Kit (Promega Bio Systems, Sunnyvale, California, USA). Real-time PCR for miRNA and RNA were performed using the iTaqTM Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, California, USA) on a 7300HT thermocycler (Applied Biosystems, USA). Relative quantification was determined by normalization to U6 or GAPDH. Real-time PCR primers used for miR-143-3p are

Forward: 5′-GCGGCGGTGAGATGAAGC-3′,
Reversed: 5′-CAGTGCAGGGTCCGAGGTAT-3′);
U6 (forward: 5′-CTCGCTTCGGCAGCACA-3′,
reverse: 5′-AACGCTTCACGAATTTGCGT-3′);
k-Ras are (Forward: 5′-TCAAAGAATGGTCCTGGACC-3′,
Reverse: 5′-TTTACCTCTATTGTTGGATC-3'; GAPDH
(Forward: 5′-AAGGTGAAGGTCGGAGTCAAC-3′,
Reverse: 5′-CATGAGTCCTTCCACGATACC-3′).

All samples were run in duplicate and the change in expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell Viability

The experimental process can be seen in previous studies.[14] Triplicate experiments were performed independently.

Detection of Apoptosis by Flow Cytometry

The experimental process can be seen in previous studies.[15]

Cell Cycle Assay

The cell cycle assay was detected by flow cytometric analysis. Briefly, the cells were harvested after 48 h, washed 3 times with cold phosphate buffered saline (PBS) and fixed in 70% ethanol in PBS at -20°C for at least 1 h. Subsequently, the cells were labelled with 100 µl PBS containing 50 µg/mL PI (Bio-Science, Co. Ltd, Shanghai, China) and 50 µg/mL RNaseA (Boehringer Mannheim, Indianapolis, Indiana, USA) at 37°C for 30 min in the dark. The results were analyzed by FACScan (Bechman Coulter, Pasadena, California, USA). Experiments were performed in triplicate.

Luciferase Reporter Assay

3′-UTR of K-ras and the mutated sequence were inserted into the pGL3 control vector (Promega Corporation, Madison, Wisconsin, USA) to construct wt K-ras-3′-UTR vector and mutant K-ras-3′-UTR vector, respectively. For luciferase reporter assay, HEK293 cells were transfected with the corresponding vectors; 48 h after transfection, the dual-luciferase reporter assay system (Promega, Shanghai, the People's Republic of China) were used to measure the luciferase activity. All experiments were performed in triplicate.

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Figure 1. miR-143-3p was downregulated in WT tissues and correlated with disease progression. **(A)** miR-143-3p expression levels were calculated by qRT-PCR in Wilms' tumor tissues and adjacent tissues; n = 53. $P < .01$ vs normal tissues. **(B)** Relationship between miR-143-3p expression level and NWTS-5 grade of WT; $P = .0410$. **(C)** Relationship between miR-143-3p expression level and lymph node metastasis of WT; $P = .0195$.

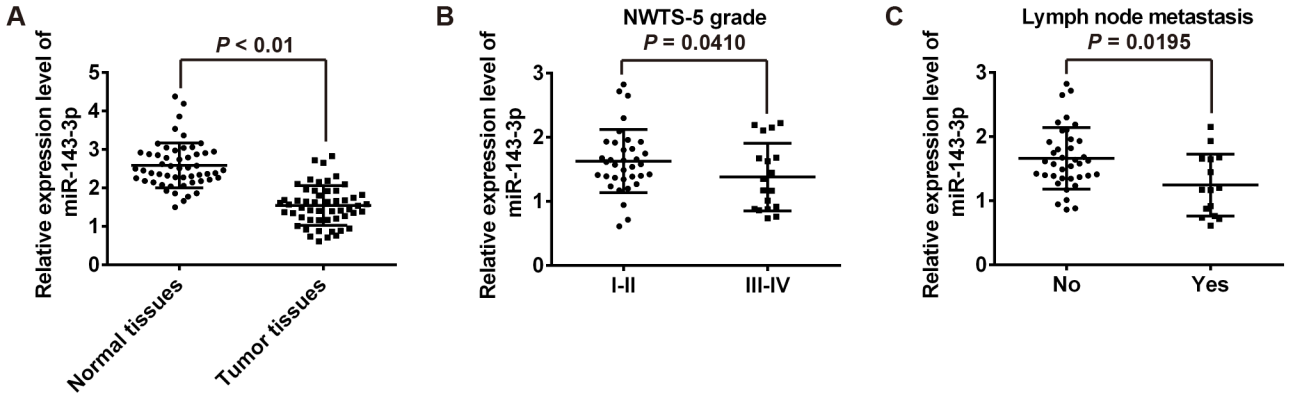
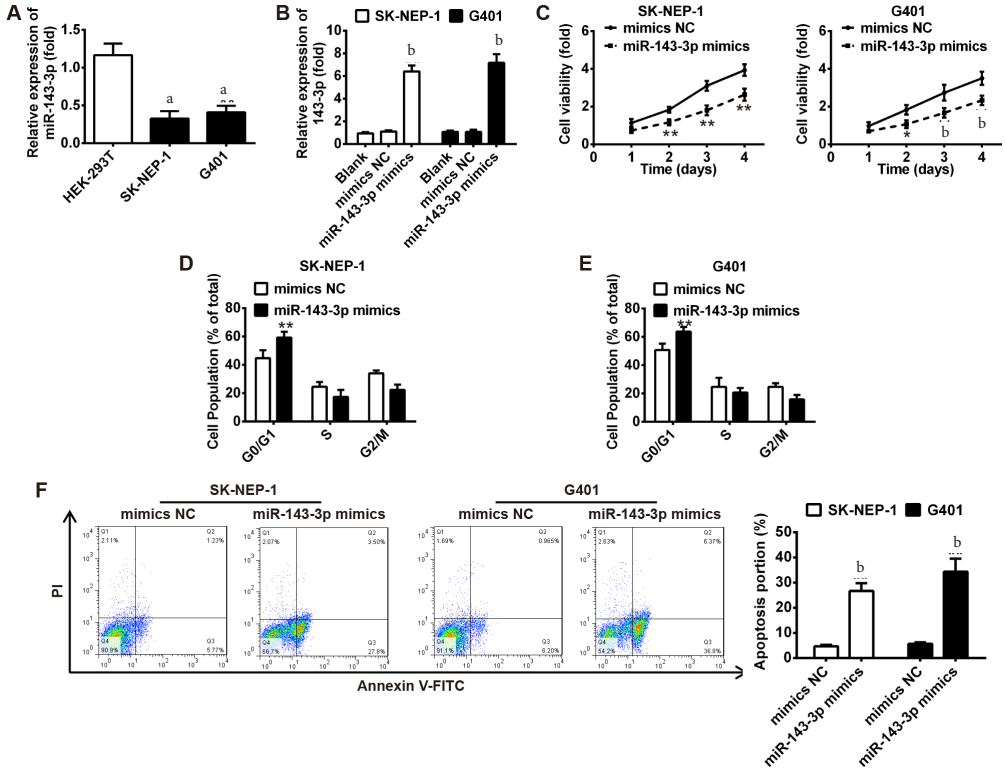


Figure 2. Overexpression of miR-143-3p inhibited cell proliferation, blocked the cell cycle and induced cell apoptosis. miR-143-3p mimics were transfected into SK-NEP-1 and G401 cells for 24 h, then cell proliferation, cell cycle and cell apoptosis were evaluated. **(A)** miR-143-3p expression levels were calculated by qRT-PCR in WT cell lines, SK-NEP-1 and G401. Data are expressed as mean ± SD. **(B)** miR-143-3p expression levels were calculated by qRT-PCR after transfection of miR-143-3p mimics in SK-NEP-1 and G401 cells. **(C)** Cell viability was determined by CCK-8 assay. **(D, E)** Cell cycle was detected by flow cytometric analysis. **(F)** Cell apoptosis was measured by flow cytometric analysis. Data are expressed as mean ± SD.



[a]$P < .01$ vs HEK 293T
[b]$P < .01$ vs mimics NC.

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Western Blot

Total protein was extracted using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China). Concentrations of total cellular protein were determined using a BCA assay kit (Pierce, Rockford, Illinois, USA). Total protein samples (40 μg) were analyzed by 8% SDS-PAGE gel and transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Freiburg, Delaware, USA) by electroblotting. Primary antibodies against K-ras (1:1000), Raf/1 (1:1000), phospho-Raf/1 (1:1000), MEK1/2 (1:1000), phosphor-MEK1/2 (1:1000), ERK1/2 (1:1000), phospho-ERK1/2 (1:1000) and β-actin (1:2000) (all from Santa Cruz Biotechnology, California, USA) were probed with proteins on the membrane at 4°C overnight. After incubating with secondary antibodies (1:10000, Cell Signaling Technology, Danvers, Massachusetts, USA), Bands were detected by enhanced chemiluminescence (ECL) kit (GE Healthcare, Freiburg, Delaware, USA). The intensity of the bands of interest was analyzed by ImageJ software (Rawak Software, Inc. Munich, Germany).

Statistical Analyses

Statistical analyses were performed with IBM® SPSS 13.0 software (SPSS, Chicago, Illinois, USA). Data are expressed as mean ± standard deviation of 3 independent experiments. One-way analysis of variance (ANOVA) or two-tailed Student's *t* test was used for comparisons between groups. $P < .05$ was considered to indicate a statistically significant difference.

RESULTS

miR-143-3p Was Low Expressed in WT Tissues and Correlated with Disease Progression

To determine the role of miR-143-3p in the pathogenesis of WT, the expression levels of miR-143-3p in 53 paired WT and adjacent tissues were measured by qRT-PCR. As shown in Figure 1A, miR-143-3p was significantly downregulated in WT tissues compared with adjacent tissues. After confirming the aberrant expression of miR-143-3p in WT tissues, the relationship between miR-143-3p expression and clinicopathological features was determined. The associations of miR-143-3p expression with various clinicopathological parameters of WT. Using the median miR-143-3p expression in all 53 WT patients as a cutoff, patients with WT were divided into a high or low miR-143-3p group. The results confirmed that low expression of miR-143-3p was related to advanced NWTS-5 grade (III/IV) ($P = .0410$) and lymph node metastasis in patients with WT ($P = .0195$) (Figures 1B, C). There was no correlation between miR-143-3p expression and other clinicopathological factors such as gender, age, histological type or recurrence. Collectively, these data indicate that miR-143-3p may serve as an effective biomarker for the prognosis of patients with WT.

Overexpression of miR-143-3p Inhibited WT Tumor Cell Growth

Given the downregulation of miR-143-3p in WT tissues, we predicted that miR-143-3p may function as a tumor suppressor. To verify our hypothesis, we first examined the expression level of miR-143-3p in WT cell lines, SK-NEP-1 and G401 by qRT-PCR. Consistent with the result in WT tissues, the expression of miR-143-3p was also downregulated in SK-NEP-1 and G401 cells compared with HEK-293T cells (Figure 2A). To assess the role of miR-143-3p in cell viability, cell cycle and apoptosis, SK-NEP-1 and G401 cells were transfected with miR-143-3p mimics. Results showed that the expression level of miR-143-3p was significantly upregulated by miR-143-3p mimics (Figure 2B). Then, we examined the alteration of the cell viability of SK-NEP-1 and G401 cells after miR-143-3p overexpression. The results of CCK-8 assay showed that overexpression of miR-143-3p markedly reduced the cell viability of WT cells (Figure 2C). Because overexpression of miR-143-3p inhibited WT cell proliferation, we investigated whether overexpression of miR-143-3p affects the cell cycle and/or apoptosis. Flow cytometry results showed that overexpression of miR-143-3p modulated the cell cycle by inducing G0/G1 arrest and promoted cell apoptosis when compared with mimics NC group (Figures 2D, E). These results suggest that overexpression of miR-143-3p can inhibit WT cell growth by blocking cell cycle progression and inducing cell apoptosis (Figure 2F).

K-ras Was a Target of miR-143-3p

To explore the molecular mechanism by which miR-143-3p modulates cell proliferation, miRBase (http://www.mirbase. org) and TargetScan (http://www.targetscan.org) were used to predict the potential targets of miR-143-3p. Among the candidate target genes, k-Ras was found to have an miR-143-3p binding site in its 3′-UTR (Figure 3A, B). Previous studies showed that several miRNAs show a tumor suppressive effect on cancer cell growth by targeting k-Ras oncogene.[16,17] More recently, Gao, et al. demonstrated that miR-143-3p functioned as a tumor suppressor via regulation of the k-Ras oncogene in colon cancer.[18] Thus, we assumed that there might be an association between miR-143-3p and k-Ras in WT. To experimentally validate whether k-Ras was a direct target of miR-143-3p, a luciferase assay was conducted in HEK 293T cells. Dual-luciferase reporter assays revealed that enforced miR-143-3p substantially inhibited the luciferase reporter activity of wild-type 3′-UTR, whereas knockdown of miR-143-3p increased the relative luciferase activities (Figure 3C). Likewise, the luciferase activity did not change significantly when the targeted sequence of k-Ras was mutated in the miR-143-3p-binding site. To further confirm that k-Ras is negatively regulated by miR-143-3p, the k-Ras protein expression level was analyzed by western blot analysis. We found that k-Ras levels were significantly downregulated after transfection with miR-143-3p mimics, while transfection with the miR-143-3p inhibitor enhanced k-Ras expression (Figure 3D).

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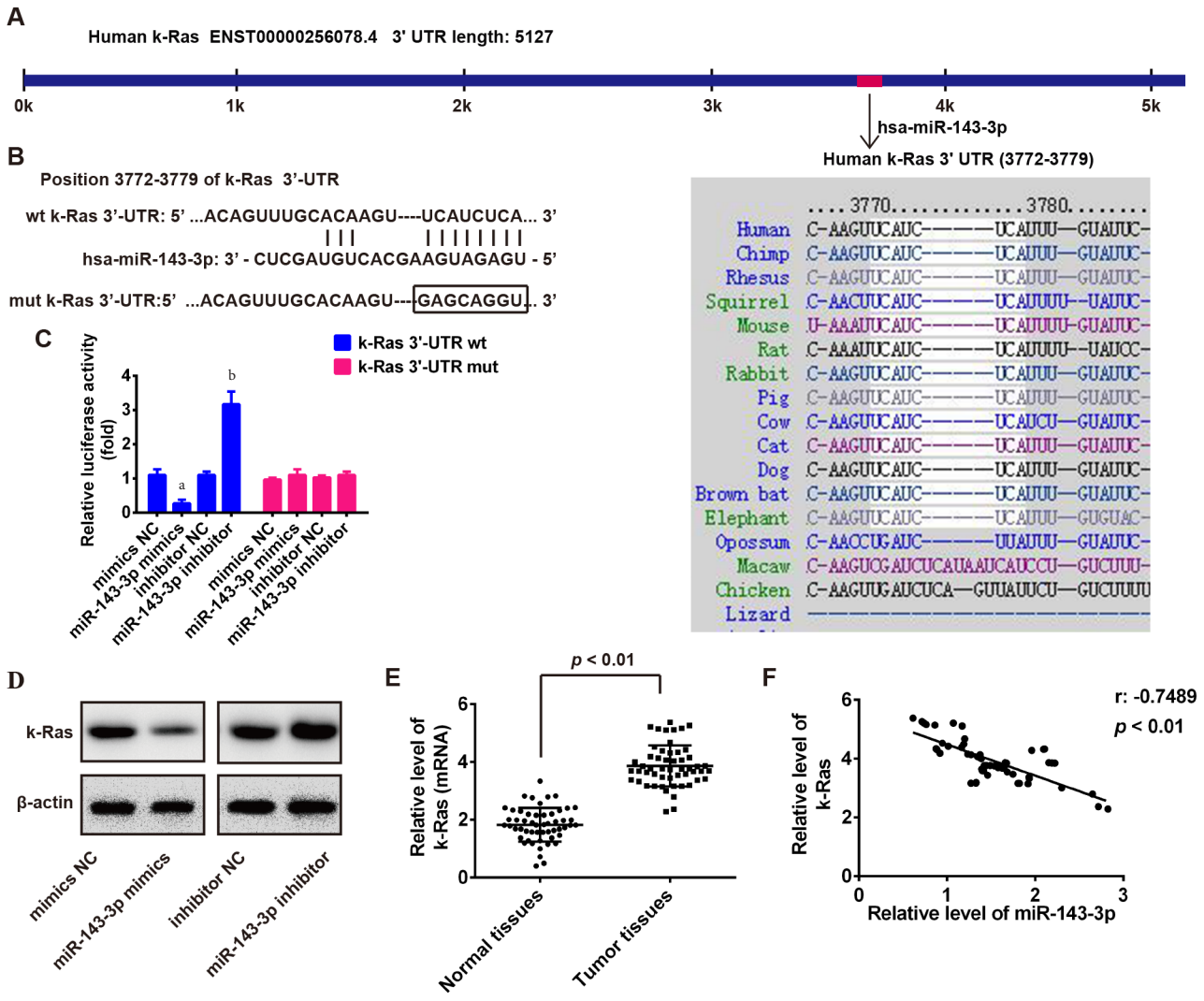
Figure 3. k-Ras was a direct target of miR-143-3p. **(A)** Prediction of k-Ras as a target of miR-143-3p in different species. **(B)** Schematic view of miR-143-3p putative targeting site in the wild type (wt) and mutant (mut) 3′-UTR of k-Ras. **(C)** Luciferase activity assay in HEK 293T cells transfected with luciferase report plasmids containing k-Ras 3′UTR (wt or mut) with control miRNA, miR-143-3p mimics or miR-143-3p inhibitor. Data are expressed as mean ± SD; **(D)** Relative protein levels of k-Ras in SK-NEP-1 cells transfected with mimics NC, inhibitor NC, miR-148a-3p mimics or miR-148a-3p inhibitor was determined by western blot analysis. **(E)** The expression of k-Ras mRNA was measured by qRT-PCR in Wilms' tumor tissues and adjacent tissues, n = 53; $P < .01$ vs normal tissues. **(F)** The inverse correlation of k-Ras protein levels with miR-143-3p expression was examined by Pearson's correlation analysis (r = -0.7489; $P < .01$).



[a]$P < .01$ vs mimics NC
[b]$P < .01$ vs inhibitor NC

In addition, we also measured the expression of k-Ras in 53 paired WT tissues and adjacent tissues by qRT-PCR. The results of qRT-PCR showed that the expression of k-Ras was significantly upregulated in WT tissues (Figure 3E), consistent with prior reports.[19] Furthermore, an inverse correlation was observed between K-ras expression and the miR-143-3p expression level (Figure 3F). All of these data indicate that k-Ras may be involved in miR-143-3p mediated regulation in WT.

miR-143-3p Suppressed WT Cell Proliferation by Directly Targeting k-Ras

It has been reported that k-Ras acts as an oncogene in various human tumors, such as lung cancer[20,21] and colorectal cancer.[22] However, the roles of k-Ras in WT remain unknown. In order to verify whether k-Ras also exerts an oncogenic role in WT, the expression of k-Ras was knockdown via small interfering RNA (siRNA) in WT cells. The efficacy of siRNA is shown in Figure 4A; more than 80% of the mRNA was efficiently silenced in SK-NEP-1 cells by siRNA-k-Ras-1. We

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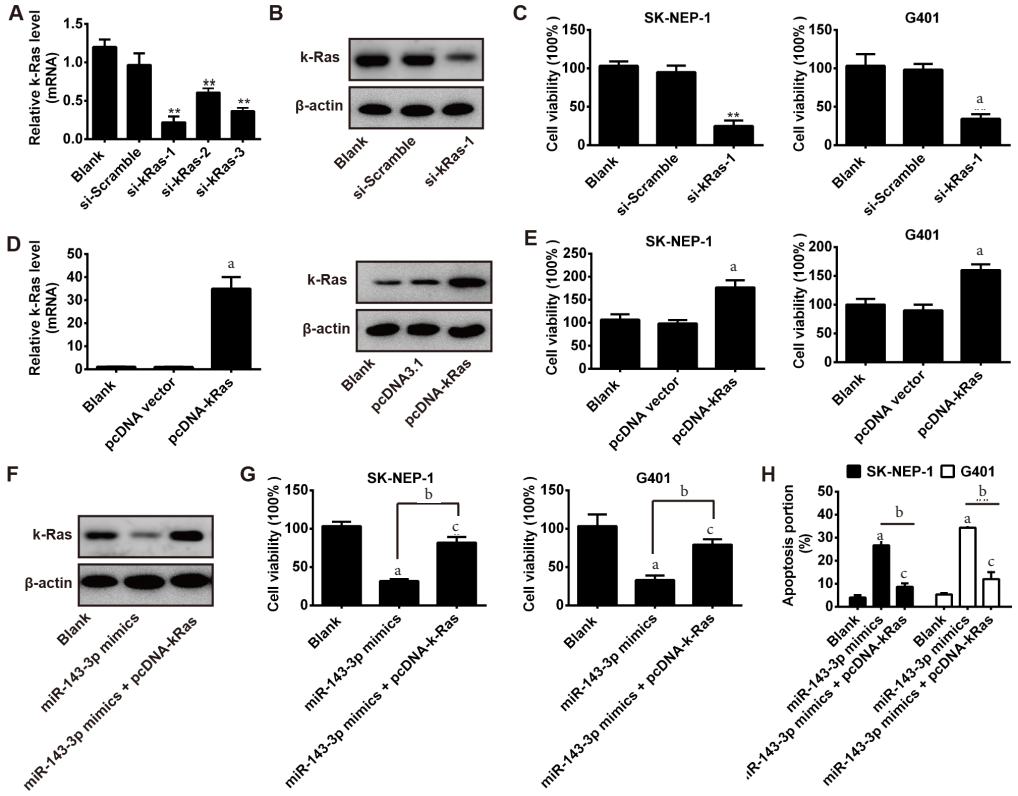
Figure 4. miR-143-3p suppressed WT cell proliferation by directly targeting k-Ras. **(A)** Silencing efficiency of siRNAs was verified using qRT-PCR. **(B)** Silencing efficiency of si-k-Ras-1 was verified using western blot assay (protein level). **(C)** Cell viability in k-Ras knockdown cells was measured using CCK-8 kit. Data are expressed as mean ± SD. **(D)** Overexpression efficiency of pcDNA-k-Ras vector was verified using qRT-PCR (mRNA level) and western blot assay (protein level). **(E)** Cell viability in k-Ras overexpressing cells was measured using CCK-8 kit. Data are expressed as mean ± SD. Then, miR-143-3p mimics and pcDNA-k-Ras vector were co-transfected into SK-NEP-1 and G401 cells, then relative protein levels of k-Ras were measured by western blot **(F)**, cell viability was determined by CCK-8 **(G)** and cell apoptosis was detected by flow cytometric analysis **(H)**. Data are expressed as mean ± SD.



[a]$P < .01$ vs Blank group
[c]$P < .05$
[b]$P < .01$ vs miR-143-3p mimics group

also detected the protein expression of k-Ras in siRNA-k-Ras-1 transfected cells and found that the protein expression of k-Ras was significantly reduced compared with in the Blank or si-Scramble groups (Figure 4B). As expected, k-Ras knockdown dramatically inhibited cell growth in both SK-NEP-1 and G401 cells (Figure 4C). On the other hand, pcDNA-k-Ras expression vector was transfected into WT cells to overexpress k-Ras. qRT-PCR and western blot results showed that pcDNA-k-Ras led to significant enhancement of k-Ras expression at the mRNA and protein levels (Figure 4D). Overexpression of K-ras significantly promoted cell proliferation in both SK-NEP-1 and G401 cells (Figure 4E).

Based on these findings, we sought to further explore whether k-Ras mediates the functional effects of miR-143-3p on WT cells. First, miR-143-3p mimics and pcDNA-k-Ras vector were co-transfected into both SK-NEP-1 and G401 cells, and then the cell proliferation and apoptosis were determined. The results showed that pcDNA-k-Ras significantly enhanced the expression of k-Ras in miR-143-3p mimics + pcDNA-k-Ras group compared with the Blank or miR-143-3p mimics group (Figure 4F). It was also found that overexpression of k-Ras blocked the inhibitory effects of miR-143-3p mimics on cell proliferation and inhibited the enhancing effects of miR-143-3p mimics on cell apoptosis (Figures 4G, H). All data suggest that miR-143-3p exerts its effects on the proliferation of WT cells by targeting k-Ras.

Overexpression of miR-143-3p Inactivated the Ras/Raf/MEK/ERK Pathway

Considering that the Raf/MEK/ERK pathway is one of the most important pathways downstream of Ras[23] and the latter was confirmed to be a target of miR-143-3p, we speculated whether the Ras/Raf/MEK/ERK pathway was involved in the anti-cancer effect of miR-143-3p in WT cells. To test this, we performed western blot analysis to determine the expression level of kinase proteins in Ras/Raf/MEK/ERK

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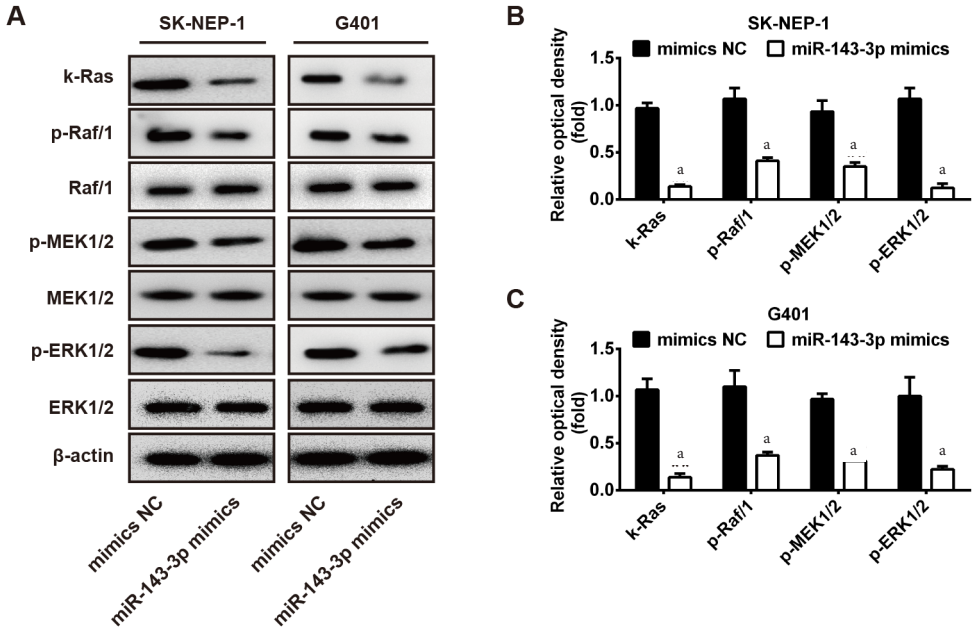
Figure 5. Overexpression of miR-143-3p inactivated the Ras/Raf/MEK/ERK pathway. **(A)** SK-NEP-1 and G401 cells were incubated with miR-143-3p mimics or mimics NC, respectively. Cell lysates were subjected to western blot analysis for total K-Ras, phosphorylated (p) and total Raf/1, phosphorylated (p) and total MEK 1/2, phosphorylated (p) and total ERK 1/2. **(B, C)** The optical densities of the bands were measured using ImageJ software. Data are expressed as mean ±vSD



[a]$P < .01$ vs mimics NC.

pathway including K-ras, Raf/1, phosphorylated-Raf/1 (p-Raf/1), MEK1/2, phosphorylated-MEK1/2 (p-MEK1/2), ERK1/2 and phosphorylated-ERK1/2 (p-ERK1/2) in both SK-NEP-1 and G401 cells. Western blot analyses showed that the protein expression of k-Ras, p-Raf/1, p-MEK1/2 and p-ERK1/2 was decreased after miR-143-3p mimics transfection (Figure 5A-C). These data suggest that overexpression of miR-143-3p inhibited cell growth by blocking the Ras/Raf/MEK/ERK pathway.

DISCUSSION

In this study, we demonstrated that miR-143-3p is downregulated in WT tissues compared with normal controls, as well as in WT cell lines, ie, SK-NEP-1 and G401. Furthermore, overexpression of miR-143-3p inhibited cell proliferation, arrested cells at the G0/G1 phase and induced cell apoptosis. We identified k-Ras as a direct target of miR-143-3p and showed that miR-143-3p functions as a tumor suppressor by blocking the Ras/Raf/MEK/ERK pathway, providing a potential diagnostic and therapeutic target for the treatment of WT.

Previous studies showed that miR-143-3p acted as a tumor suppressor in various types of human cancers. For example, Xu, et al. found that overexpression of miR-143-3p suppressed cell proliferation via the EGFR/RAS/MAPK pathway in prostate cancer cells.[24] Liu, et al also showed that overexpression of miR-143-3p inhibited human cervical cancer cell growth *in vivo* and *in vitro* by regulation of Bcl-2.[24] A 2011 study performed by Song, et al. demonstrated that miR-143-3p functioned as a tumor suppressor by targeting cyclooxygenase-2 in human bladder carcinoma.[25] However, to our knowledge, this study was the first to investigate the expression and function of miR-143-3p in WT. miR-143-3p was found to be significantly decreased in WT tissues and cell lines. In addition, clinical association analysis showed that decreased expression of miR-143-3p was related to advanced tumor stage and lymph node metastasis, indicating that miR-143-3p may serve as a promising biomarker for the diagnosis of patients with WT. Then, we investigated the biological role of miR-143-3p in WT cells and found that overexpression of miR-143-3p inhibited cell growth, blocked the cell cycle and induced cell apoptosis. These results reveal the anti-tumor role of miR-143-3p in WT.

k-Ras is one of the three members of the Ras oncogene family, which has been implicated in the carcinogenesis of many human cancers, such as oral squamous cell carcinoma (OSCC),[26] colon cancer[27] and pancreatic cancer.[28] However, the functional role of k-Ras in WT was still elusive. Our study showed that overexpression of k-Ras promoted, whereas knockdown of k-Ras inhibited, cell growth in both SK-NEP-1 and G401 cells, suggesting that k-Ras also serves an oncogenic function in WT. A previous study reported that miR-143-3p suppressed k-Ras expression in colon cancer cell lines and primary tumor tissue.[18] Hence, we hypothesized that miR-143-3p might regulate the expression of k-Ras in WT. In this study, we confirmed that k-Ras was a downstream

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target of miR-143-3p and an inverse relationship existed between miR-143-3p and k-Ras in WT tissues. Of more importance, overexpression of k-Ras could partly reverse the inhibitory effect of miR-143-3p on cell growth and inhibit miR-143-3p induced cell apoptosis. These data indicate that miR-143-3p regulates k-Ras abundance and thus exerts its anti-tumor effects on WT.

The Ras/Raf/MEK/ERK pathway is reported to be one major hyper-activated pathway, which promotes cell growth and metastasis in human cancer.[29,30] For example, Lin, et al. showed that knockdown of cadherin-17 (CDH17) inhibited cell proliferation by blocking the Ras/Raf/MEK/ERK signaling pathway in gastric cancer.[31] Zhou, et al found that miR-30a inhibited cell growth and induced cell apoptosis of hepatocellular carcinoma (HCC) cells, partially by regulating the Ras/Raf/MEK/ERK signaling pathway.[32] As mentioned above, k-Ras was proved to be a target of miR-143-3p. Therefore, we evaluated whether the MEK/ERK pathway participated in the miR-143-3p antitumor effect by regulating k-Ras. Our study demonstrated that overexpression of miR-143-3p decreased the phosphorylation of Raf/1, MEK1/2, and ERK1/2, indicating that miR-143-3p inhibited cell growth via inactivation of the k-Ras/Raf/MEK/ERK pathway.

CONCLUSION

Our data demonstrate for the first time that the expression of miR-143-3p is significantly decreased in WT tissues and cells. Furthermore, miR-143-3p can inhibited cell growth through inactivation of the k-Ras/Raf/MEK/ERK pathway. These findings indicate that miR-143-3p may act as a tumor suppressor in WT and could serve as a novel therapeutic target in the treatment of WT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Lianhai Zhang; formal analysis, Lianhai Zhang; writing andoriginal draft preparation, Lianhai Zhang; writing the review and editing, All authors; supervision, Wei Shao; funding acquisition, Wei Shao; Final approval of manuscript: All authors

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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Not applicable.

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