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Original Article

Declined circular RNA mitofusin 2 constrains the deterioration of Wilms tumor via modulating microRNA-372-3p/transforming growth factor-β receptor type 2 axis

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Abstract

To explore the action and mechanism in which circular RNA (circRNA) mitofusin 2 (MFN2) repressed the malignant proliferation of Wilms tumor (WT) via modulating microRNA (miR)-372-3p/transforming growth factor-β receptor type 2 (TGFBR2) axis. CircRNA MFN2 was distinctly elevated in the tissues and cells of WT patients, while miR-372-3p was silenced in the tissues and cells of WT. Test of TGFBR2, PCNA and Bax was implemented. Transfection with si-circRNA MFN2 or miR-372-3p-mimic restrained cancer cell advancement and the number of PCNA content was declined, while transfection with miR-372-3p-inhibitor was opposite, and PCNA content was augmented. MiR-372-3p-inhibitor turned around si-circRNA MFN2's therapeutic action after co-transfection with si-circRNA MFN2 + miR-372-3p-inhibitor. Ultimately, it was verified that circRNA MFN2 was negatively associated with miR-372-3p, which was negatively linked with TGFBR2, and circRNA MFN2 was positively associated with TGFBR2. To sum up, the results of this research illuminated circRNA MFN2 repressed WT's malignant proliferation via modulating miR-372-3p/TGFBR2 axis.

Keywords: Wilms tumor, Circular RNA MFN2, MicroRNA-372-3p, Transforming growth factor-β receptor type 2.

1. Introduction

Wilms tumor (WT) is a tumor nearly associated with early kidney development, frequently taking place in human infants and young children [1]. WT stemming from the kidney's aberrant development, is crucially associated with heredity, leading to this disease's associated genes' alteration and implicating in fetal kidney development [2]. The annual incidence of this cancer is approximately 9 cases in per million people, and the survival rate ranges from 9% to 17% [3]. This illness takes up 7% of all childhood cancers and 90% of childhood kidney diseases [4]. WT's prognosis is linked with age, histological type and tumor size, while the critical prognostic factors are the tumor's associated invasion and metastasis [5]. WT development's associated mechanisms have not been completely illuminated, so it is critical to hunt for early biomarkers and therapeutic targets.

Circular RNA (circRNA), a novel member of non-coding RNA, is characterized via jarless sequence and structure, elevated abundance and specificity, and is available to express in diversified tissues and cells [6]. Owing to the constant development and progress of sequencing technology, numerous reports have illuminated that circRNA presents aberrant expression patterns in cancer and influences the disease's development [7]. For instance, circ-ACACA is available to accelerate the advancement with glycolysis of cervical cancer cells [8], circ-0001686 is available to boost the growth of prostate cancer cells LNCaP and CWR22RV1 [9], and circ-0039569 is available to accelerate the kidney cancer cells' growth and metabolism [10], etc. CircRNA mitofusin 2 (MFN2), as a type of circRNA, has been discovered to accelerate cancers' advancement, like colon cancer (CC) and pancreatic cancer [11], but its action and regulatory mechanism in WT remains unknown.

MicroRNAs (miRNAs) are small non-coding RNAs, prevalently composed of 20-24 nucleotides. After being transcribed and modified into mature miRNAs, they are available to target protein mRNAs, leading to over-expression, degradation or under-expression of proteins [12]. MiRNAs' mal-regulation leads to aberrant protein and ultimately gives rise to diseases' occurrence like cancer. Studies have elucidated that miR-485-5p is available to boost glioblastoma's growth and metabolism via modulating aberrant CSF-1 [13]. MiR-142-5p is available to make cancer-associated fibroblast exosomes accelerate lung cancer's advancement via targeting PD-L1 [14]. MiR-372-3p, as a member of a large family, is also available to cause aberrant target proteins, for instance, declined miR-372-3p renders elevated mitogen-activated protein kinase 7 to expedite osteosarcoma (OS)'s growth [15] and enables

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elevated Rab23 to boost hepatocellular carcinoma's development [16], etc. Nevertheless, the action and regulatory mechanism of miR-372-3p in WT remains uncertain.

In this study, the selection of circRNA MFN2 was as the research object to explore the role and regulatory mechanism of circRNA MFN2/miR-372-3p/transforming growth factor-β receptor type 2 (TGFBR2) molecular axis in WT, offering a brand-new curative target for WT.

2. Materials and methods

2.1. Experimental subjects

From April 2018 to April 2020, the enrollment of 40 patients who were initially pathologically diagnosed with WT in Chongqing General Hospital was performed in this study. None of the patients received any associated treatment. WT samples and adjacent healthy tissues (≥ 3 cm) were collected and immediately frozen with liquid nitrogen, and storing was implemented. All patients signed written informed consent, and the review and authorization of the study was via the Ethics Committee of Chongqing General Hospital.

2.2. Cell culture

Culture of human WT WiT49 cells (Thermo Scientific, USA) and human HK-2 cells (Gerd Chemical, China) was in 1: 1 high glucose and Dulbecco phosphate buffer modified Eagle medium/nutrient mixture F-12. Addition of 10% Fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin was implemented and placing was in an incubator. The selection of cells in the logarithmic phase was for experiments.

2.3. Cell transfection

Purchase of small interfering RNA of targeting circRNA MFN2 and negative control (NC) (si-circMFN2, siRNA), miR-372-3p-mimic, miR-372-3p-inhibitor and NC (miR-372-3p--NC) was implemented (Shanghai GeneBiogist Technology Co., Ltd). Transfection of the above plasmids or oligonucleotides was into the cells adopting Lipofectamine 2000 (ThermoFisher, USA) on the grounds of the manufacturer's instructions. After 48 h, the cells were harvested for the following experiments. Verification of the transfection efficiency was via Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

2.4. Test of circRNA stability

Treatment of WIT49 cells was with actinomycin D (2 μg/mL, Sigma) for 0, 4, 8, 12 and 24 h. Then, after collecting cells, the examination of circRNA MFN2 and linear MFN2's stability was via RT-qPCR.

To examine RNase R resistance, incubation of total RNA of circRNA MFN2 and RNF111 (2 μg) was with 3 U/μg RNase-R (07250, Epicentre Technologies, USA). Then, the detection of circRNA MFN2 and linear MFN2 was implemented by adopting RT-qPCR. Adoption of untreated RNA as a control (Mock).

2.5. Cell Counting Kit-8 (CCK-8) assay and colony formation assay

Measurement of WiT49 cell proliferation was implemented by adopting a CCK-8 kit (Abmole, USA) in line with the manufacturer's instructions. Seeding of the cells was on a 96-well plate with a density of 3×10^3 per well. After transfecting for 24, 48 and 72 h, addition of 10 μL CCK-8 solution was to each well and incubation was implemented. Examination of the optical density at 450 nm was done with a microplate reader.

After obtaining the above transfected WT cells, re-seeding was on a 6-well plate at a density of 5000 cells/well. After 2 weeks of culture, fixation of the surviving colonies was with paraformaldehyde and staining was with 0.5% crystal violet solution. Counting of the surviving cells was implemented.

2.6. Transwell experiment

After trypsin treatment, the seeding of 2×10^4 WiT49 cells was into the upper chamber of Transwell plate and addition was with serum-free Dulbecco's Modified Eagle Medium (DMEM). Addition of 800 μL DMEM and 10% FBS was in the lower chamber, and incubation was implemented. Subsequently, fixation was with 95% ethanol, and staining was with 0.1% crystal violet. Random selection of the number of cells in 5 fields was implemented and counting was under an optical microscope to detect WT cell migration ability. In the invasive ability test, a tile of 30 μL Matrigel was on the upper chamber of each Transwell plate and placing was performed. After the gel was solidified, hydration of the basement membrane was with serum-free DMEM. The subsequent experimental steps were identical to above mentioned.

2.7. Flow cytometry

Examination of the number of apoptotic cells was performed by adopting flow cytometry. Staining of WiT49 cells was with Annexin V/fluorescein isothiocyanate kit (BD Biosciences, USA) and test was on flow cytometer in line with manufacturer's instructions [17].

2.8. RT-qPCR detection

Extraction of total RNA was from tissues and cells adopting RNA extraction kit (Sigma Aldrich Trading, China) and reverse transcription was into a complementary DNA. Taking of 2 μL reverse transcription product was for qPCR test with β-actin or U6 as the standard reference. Primer sequences are presented in Table 1. Examination of qPCR was implemented on the grounds of SYBR Primix Ex TaqTM instructions (Bao Bioengineering, China), and calculation of circRNA MFN2, miR-372-3p and TGFBR2 in WT cells was performed adopting $2^{-\Delta\Delta Ct}$ method.

2.9. Western blot test

The addition of lysates covering protease inhibitors was to extract total proteins of tissues and cells, and examina-

Table 1. Primer sequences.

Genes		Primer sequences
MiR-372-3p	F:	CCTGGAAGATGGTGATGGGAT
	R:	CAGTGCGTGTCGTGGAG
U6	F:	GCTTCGGCAGCACATATACTAAAAT
	R:	CGCTTCACGAATTTGCGTGTCAT
CircRNA MFN2	F:	CTCTCGATGCAACTCTATCGTC
	R:	TCCTGTACGTGTCTTCAAGGAA
TGFBR2	F:	GGAATGTCTTGGGCAAATCT
	R:	ACCTGAATGCTTGTGCTTTTATT
β-actin	F:	CCACATCGCTCAGACACCAT
	R:	ACCAGGCGCCCAATACG

tion of the protein concentration was implemented in line with the requirements of the bicinchoninic acid kit (Boster Biological Technology Co., Ltd., Hubei, China). Separation of the protein was implemented by adopting sodium dodecyl sulfate-polyacrylamide gel electrophoresis (30 μg), and electroblot was onto polyvinylidene fluoride membrane. Incubation of the membrane was with the following primary antibodies: Anti-TGFBR2 (1: 1000), anti-Bax antibodies (1: 1000), anti-PCNA antibodies (1: 2000) and anti-glyceraldehyde-3-phosphate dehydrogenase antibodies (1: 1000); Addition of horseradish peroxidase-labeled goat anti-human secondary antibodies (1: 1000) was implemented. Purchase of the antibodies was performed (Boster Biological Technology Co., Ltd., Hubei, China). Visualization of the signal was implemented by adopting the electrogenerated chemiluminescence kit (34080, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the adoption of ImageJ software was for density analysis.

2.10. The luciferase activity assay

Seeding of WiT49 cells was in 24-well plates until 60% confluence was reached. Synthesis of the wild-type (WT) and mutant-type (MUT) sequences of circRNA MFN2 or TGFBR2 3' untranslated region (UTR) of was implemented (Shanghai Gene Pharmaceutical Co., Ltd., Shanghai, China), and cloning was into empty plasmid vectors (Promega Corporation, Madison, WI, USA) for luciferase activity assay. In line with the manufacturer's protocol, co-transfection of the luciferase reporter plasmid with miR-372-3p mimics and their NCs was into cells adopting Lipofectamine 2000 (ThermoFisher). After 48 h of transfection, measurement of luciferase activity was performed adopting the luciferase reporter gene assay (Promega Shanghai, China).

2.11. Statistical analysis

The manifestation of data was in mean ± standard deviation (SD), and analysis was implemented by adopting PSS 24.0 software. All functional tests in this study were repeated three times. The adoption of GraphPad Prism 7.0 software was to plot the resulting images, and the adoption of linear correlation coefficient R was to assess the cells' relevance, $P < 0.05$ was accepted as indicative of distinct differences.

3. Results

3.1. CircRNA MFN2 is elevated in WT tissues and cells

Detection of circRNA MFN2 in WT and adjacent normal tissues was implemented. CircRNA MFN2 was overtly elevated in WT tissues vs. the normal tissues (Fig. 1A). CircRNA MFN2 was distinctly strengthened in WT cells vs. the HK-2 cells (Fig. 1B). Actinomycin D attenuated circRNA MFN2 and exerted minor influence on mRNA (Fig. 1C). After RNase R treatment, circRNA MFN2 in cells was distinctly weakened and exerted little impact on mRNA (Fig. 1D). To sum up, circRNA MFN2 might be elevated in WT and implicate in the disease's process.

3.2. Declined circRNA MFN2 blocks WT cell advancement

After transfection of si-circrNA MFN2 into WiT49 cells, measurement of circRNA MFN2 was implemented, clarifying that circRNA MFN2 in cells was overtly declined (Fig. 2A). Silenced circRNA MFN2 distinctly constrained WT cell advancement (Fig. 2 B/C/E/F/H). Additionally, quantitative analysis of PCNA protein was implemented. Data elucidated the content of PCNA protein after transfection was declined (Fig. 2D). Then quantitative analysis of the Bax protein was implemented, illuminating that the amount of Bax protein after transfection was elevated (Fig. 2G). All in all, declined circRNA MFN2 was available to constrain WT cell activity in the body.

3.3. CircRNA MFN2 targets miR-372-3p

Forecast of circRNA MFN2's target gene was performed in line with the biological information database on https://starbase.sysu.edu.cn/, elucidating that miR-372-3p was circRNA MFN2's candidate target genes (Fig. 3A).

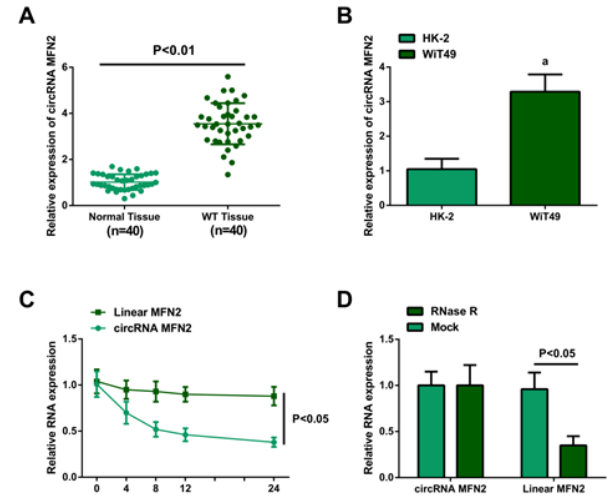

Fig. 1. CircRNA MFN2 is elevated in WT tissues and cells. A: CircRNA MFN2 in WT tissues; B: CircRNA MFN2 in WT cells; C-D: Actinomycin D and RNase R assay evaluation of circRNA MFN2's stability in WT cells; Manifestation of measurement data is in mean ± SD; a vs. the HK-2 cells, $P < 0.05$.

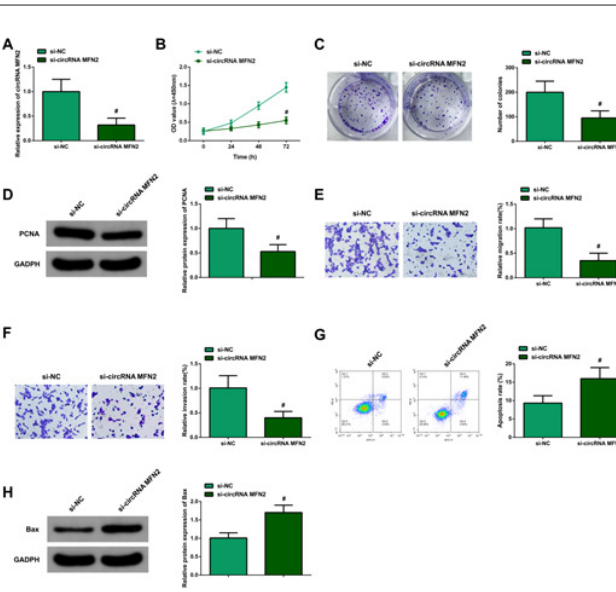

Fig. 2. Declined circRNA MFN2 blocks WT cell advancement. A: RT-qPCR test of circRNA MFN2; B-C: CCK-8 assay and cell colony formation evaluation of WT cells; D: The number of PCNA protein; E-F: Transwell assessment of WT cells; G: Flow cytometry detection of WT cells; H: the number of Bax protein after silencing circRNA MFN2; B-G. After silencing circRNA MFN2. Manifestation of measurement data is in mean ± SD; # Vs. si-NC, $P < 0.05$.

The luciferase activity was declined after co-transfection with miR-372-3p mimic and circRNA MFN2 WT, while luciferase activity was not impacted after co-transfection with miR-372-3p mimic and circRNA MFN2 MUT (Fig. 3B). After declining circRNA MFN2, miR-372-3p was overtly strengthened (Fig. 3C). Additionally, miR-372-3p in WT clinical tissues and cell samples was distinctly declined vs. the adjacent normal tissues and HK-2 cells (Fig. 3D), and miR-372-3p was negatively linked with circRNA MFN2 (Fig. 3E). In general, miR-372-3p was the target gene of circRNA MFN2, and miR-372-3p and miR-372-3p were opposite.

3.4. Elevated miR-372-3p constrains WT cell advancement

After transfecting miR-372-3p-NC/inhibitor/mimic into WiT49 cells, examination of miR-372-3p in cells was implemented, elucidating that transfection with miR-372-3p-mimic/inhibitor augmented or declined mir-372-3p, separately (Fig. 4A). Functional experiments clarified elevated miR-372-3p restrained WiT49 cell advancement (Fig. 4B/C/E/F/H) and declined the PCNA (Fig. 4D). Nevertheless, declined miR-372-3p was opposite. In short, elevated miR-372-3p was available to repress WT cell activity in vivo.

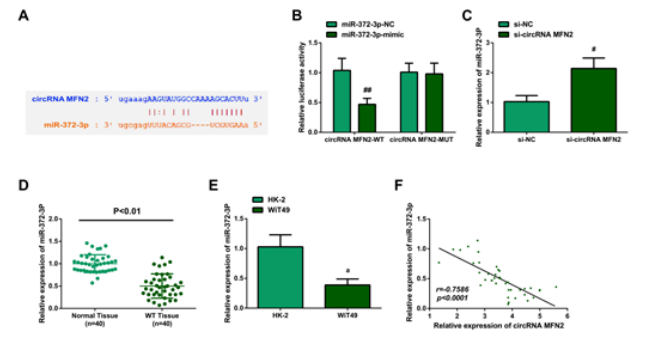

Fig. 3. CircRNA MFN2 targets miR-372-3p. A: Binding region of miR-372-3p and circRNA MFN2; B: The luciferase activity assay detection of the targeting of circRNA MFN2 with miR-372-3p; C: MiR-372-3p after silencing circRNA MFN2; D: MiR-372-3p in WT tissues and cells; E: Association of miR-372-3p with circRNA MFN2; Manifestation of measurement data is in mean ± SD; ## Vs. the miR-372-3p-NC, P < 0.05, # Vs. si-NC, P < 0.05, a Vs. HK-2, P < 0.05.

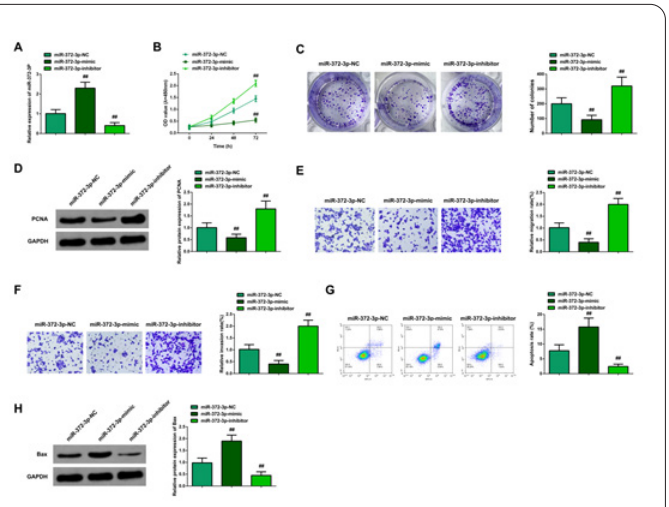

Fig. 4. Elevated miR-372-3p constrains WT cell advancement. Note: MiR-372-3p in WT cells was modulated via transfection with miR-372-3p-mimic/inhibitor. A: RT-qPCR test of miR-372-3p; B-C: CCK-8 assay and cell colony formation assay detection of cell proliferation; D: Western blot examination of PCNA protein; E-F: Transwell assay test of cell migration and invasion; G: Flow cytometry detection of cell apoptosis; H: Western blot test of Bax protein; Manifestation of measurement data is in mean ± SD; ## Vs. miR-372-3p-NC, P < 0.05.

3.5. Declined miR-372-3p turns around the therapeutic action of knockdown of circRNA FMN2 on WT cells

Co-transfection of si-circRNA FMN2 and miR-372-3P-inhibitor was into WiT49 cells to explore their regulatory effects on malignant behaviors of WT cells. After transfecting si-circRNA MFN2, miR-372-3p was overtly elevated in WT cells, while miR-372-3p was distinctly declined after co-transfection with si-circRNA MFN2 and miR-372-3p-inhibitor (Fig. 5A). Functional experiments elucidated silenced circRNA MFN2 declined advancement with repressed absorbance (Fig. 5B-H). Nevertheless, these actions were turned around via transfection with miR-372-3p-inhibitor. In short, declined miR-372-3p blocked silenced circRNA MFN2's therapeutic action on WT cells.

3.6. MiR-372-3p targets TGFBR2 in WT cells

Forecast of miR-372-3p's target genes was implemented on the grounds of the biological information database (https://starbase.sysu.edu.cn/), discovering that TGFBR2 was miR-372-3p's candidate target genes (Fig. 6A). MiR-372-3p was available to bind to the 3'UTR region of TGFBR2 and negatively modulated TGFBR2 (Fig. 6B). Additionally, after transfecting miR-372-3p-mimic, TGFBR2 in WT cells was weakened, while after transfecting miR-372-3p-inhibitor, TGFBR2 in cells was enhanced (Fig. 6C-D). This elucidated miR-372-3p was available to target TGFBR2. Vs. the adjacent normal tissues and HK-2 cells, TGFBR2 was elevated in WT tissues (Fig. 6E-F) and cells (Fig. 6G-H). TGFBR2 was positively associated with circRNA MFN2 (Fig. 6I) and miR-372-3p (Fig. 6J). All in

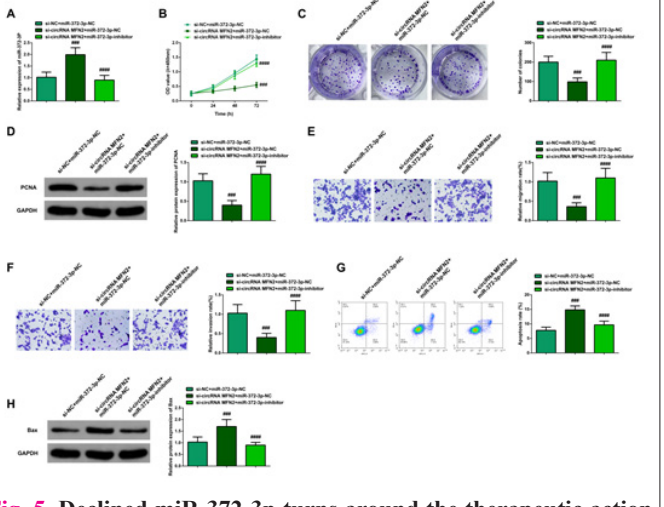

Fig. 5. Declined miR-372-3p turns around the therapeutic action of repressive circRNA FMN2 on WT cells. Note: The si-circRNA MFN2 was turned around via transfection with miR-372-3p-inhibitor. A: RT-qPCR test of miR-372-3p; B-C: CCK-8 assay and cell colony formation assay detection of cell proliferation; D: Western blot examination of PCNA protein; E-F: Transwell assay test of cell migration and invasion; G: Western blot examination of Bax protein; H: Flow cytometry detection of cell apoptosis. ### Vs. the si-NC + miR-372-3p-NC, P < 0.05; #### Vs. si-circRNA FMN2 + miR-372-3p-NC, P < 0.05.

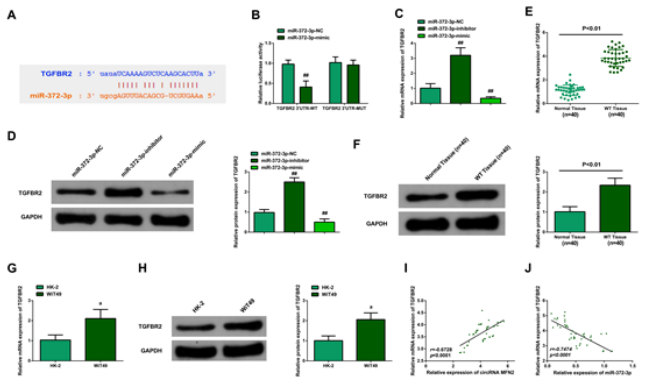

Fig. 5. MiR-372-3p modulates TGFBR2 in WT cells. A: MiR-372-3p combines with the 3'UTR region of TGFBR2; B: RT-qPCR test of relevance of miR-372-3p with TGFBR2; C-D: RT-qPCR and Western blot detection of the influence of declined miR-372-3p or circRNA MFN2 on TGFBR2; E-F: RT-qPCR or Western blot examination of TGFBR2 in WT tissues; G-H: RT-qPCR or Western blot test of TGFBR2 protein in WT cells; I: Pearson correlation analysis evaluation of the association of TGFBR2 with circRNA MFN2 and miR-372-3p. a vs. miR-372-3P-NC, P <0.05, b vs. normal tissue, P < 0.05, c vs. HK-2, P < 0.05.

all, TGFBR2 was miR-372-3p's target gene, and circRNA MFN2 was available to modulate TGFBR2 via miR-372-3p.

4. Discussion

The incidence of WT is not elevated, but its survival rate is inferior, and WT survivors have an overall augmented risk of hospitalization few years later, covering all critical organ system diseases, among which the urinary and endocrine systems are provided with extremely elevated risk [18]. Consequently, it is crucial to hunt for brand-new therapeutic targets. Antecedent studies have elucidated that circRNA is available to elevate or silence WT, for instance, circCDYL is available to silence WT and restrain tumor growth and metastasis [19], and circ-0017247 is available to elevate WT and guide tumor metabolism [20], etc.

Mitochondria are dynamic organelles of constant fission and fuse, controlling numerous critical cellular functions. The balance of mitochondrial division with fusion impacts mitochondria's morphology and function [21]. MFN2 gene was initially discovered in vascular smooth muscle cells of spontaneously hypertensive rats, encoding a mitochondrial GTPase on the outer membrane of mitochondria and exerting a critical action in mitochondrial fusion, thereby modulating mitochondrial morphology and function [22]. Mitochondrial dysfunction has been testified to expedite multiple human malignant tumors' development [23]. Recently, MFN2, as a biomarker, has been extensively explored for its action in tumor genesis and advancement, and it varies with types of cancer. For instance, elevated circRNA MFN2 is a marker of negative prognosis of human gastric cancer, and suppression of its expression is provided with anticancer effects [24]. Suppressive circRNA MFN2 is available to constrain the melanoma's occurrence and metastasis [25]. Declined circRNA MFN2 is available to repress the advancement with radiation resistance of colorectal cancer (CRC) cells. This clarified circRNA MFN2 is provided with a carcinogenic action in CRC [26]. MFN2 has been extensively explored in numerous cancers, but it has been poorly figured out in associated cancers of the kidney. The research initially testified that circRNA MFN2 was distinctly elevated in WT tissues and Wit 49 cells. In line with the experimental results, declined circRNA MFN2 was available to block WT cell advancement, illuminating that circRNA MFN2 exerted a carcinogenic action in WT.

Recently, several studies have discovered that miRNA's disorder is a critical factor for the of diversified illnesses' occurrence and development [27]. For WT, miRNA has also been gradually testified to be crucial in modulating WT. For instance, miR-483-3p is available to expedite WT cell advancement and stimulate WT cells to produce drug resistance [28]. MiR-483-5p restrains WT cell advancement in vivo via targeting MKNK1 [29]. MiR-372-3p also modulates cancer via elevating or silencing multiple downstream target genes. Studies have discovered elevated miR-372-3p is available to stimulate OS's development and expedite lung squamous cell carcinoma cell growth and metabolism [30, 31], while declined miR-372-3p represses associated signaling pathways in CRC [32], etc. ENCOPI discovered the binding sites were presented of circRNA MFN2 with miR-372-3p, and meanwhile, miR-372-3p was available to combine with circRNA MFN2. The research initially verified that circRNA MFN2 was available to target miR-372-3p. The results elucidated elevated miR-372-3p was available to repress WT cell advancement, and suppressive miR-372-3p mimics were available to turn around the curative action of restraining circRNA MFN2. These results elaborated that miR-372-3p repressed WT cell advancement.

Glycosylation prevalently modifies membrane-associated and secreted proteins. Owing to its specific position on the cell surface and extracellular, polysaccharides exert a critical action in controlling intercellular communication, receptor activation and signal transduction. Cell-surface polysaccharides are critical to cells in receiving external signals. Critical growth factors like EGF, hepatocyte growth factor, transforming growth factorβ (TGF-β) and vascular endothelial growth factor exert a vital action in receptors' glycosylation, thereby modulating receptor to ligands' sensitivity, tumor progression and signal transduction's effectiveness [33]. Furthermore, the TGF-β pathway is considered as extremely crucial pathway leading to carcinogenic stem cell transformation in different tumor entities [34]. Several critical glycosylated proteins were presented in the TGF-β pathway, like TGF-β, Smad and TGFBR. Foregoing studies have elucidated several glycosyltransferases and glycosidases are associated with TGF-β receptors' phosphorylation in TGF-β pathway, like fucose transferase 8 in lung cancer [35] and salivary acidification in CC [36]. TGF-β signal implicates in the of diversified types of cancer's occurrence and development, while TGFBR2 is a crucial mediator of TGF-β growth repression signal. TGFBR2 receptor, a serine/threonine kinase receptor, catalyzes type I receptors' phosphorylation [37]. The research has discovered modulation of TGFBR2's glycosylation strengthens breast cancer cells' stem cell properties and radiation resistance of [38]. Declined molecular axis covering TGFBR2 is available to restrain glioma cell advancement [39]. Silenced TGFBR2 and repressive tumor suppressive activity of TGF-β pathway is available to render prostate tumor to exert a carcinoge-

nic action [40]. TGFBR2 has been explored in multiple cancers, but it is rarely figured out in kidney's associated cancers. The study initially explored the association of TGFBR2 with WT. The prediction of TGFBR2 was to be a latent target gene of miR-372-3p based on bioinformatics tools. The results of the experiment and forecast verified that TGFBR2 was miR-372-3p's target gene, and they were provided with binding sites, and miR-372-3p negatively modulated TGFBR2, which was also elevated in WT tissues and cells in the meantime. Additionally, elevated circRNA MFN2 was available to expedite TGFBR2. The above experimental results elucidated TGFBR2 implicated in WT's modulation and elevated the disease.

In brief, the results of this research illuminated circRNA MFN2 accelerated the growth of WT tissues and Wit 49 cells via silencing miR-372-3p and elevating TGFBR2. CircRNA MFN2/miR-372-3p/TGFBR2 axis exerted a crucial action in the development of WT and was expected to offer brand-new experimental result support for circRNA MFN2 as a marker for WT's diagnosis and treatment. The research discovered circRNA MFN2 was available to restrain the WT's malignant proliferation via modulating miR-372-3p/TGFBR2 axis, but this pathway's specific curative mechanism remained to be explored. Additionally, clinical trials were not conducted in this study, so it was impossible to assess the therapeutic action on patients. Consequently, the more complete experimental analysis should be implemented for verification later.

Informed consent

The authors report no conflict of interest.

Availability of data and material

We declared that we embedded all data in the manuscript.

Authors' contributions

XM conducted the experiments and wrote the paper; XX and TW analyzed and organized the data; PX conceived, designed the study and revised the manuscript.

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References

1. Spreafico F, Fernandez CV, Brok J, Nakata K, Vujanic G, Geller JI, Gessler M, Maschietto M, Behjati S, Polanco A, Paintsil V, Luna-Fineman S, Pritchard-Jones K (2021) Wilms tumour. Nat Rev Dis Primers. 7(1):75. doi: 10.1038/s41572-021-00308-8.
2. Treger TD, Chowdhury T, Pritchard-Jones K, Behjati S (2019) The genetic changes of Wilms tumour. Nat Rev Nephrol 15(4):240-251. doi: 10.1038/s41581-019-0112-0.
3. Tonning Olsson I, Brinkman TM, Hyun G, Banerjee P, Mulrooney DA, Huang IC, Green DM, Srivastava D, Robison LL, Hudson MM, Krull KR (2019) Neurocognitive outcomes in long-term survivors of Wilms tumor: a report from the St. Jude Lifetime Cohort. J Cancer Surviv 13(4):570-579. doi: 10.1007/s11764-019-00776-8.
4. Breslow N, Olshan A, Beckwith JB, Green DM (1993) Epidemiology of Wilms tumor. Med Pediatr Oncol 21(3):172-81. doi: 10.1002/mpo.2950210305.
5. Wang X, Du G, Wu Y, Zhang Y, Guo F, Liu W, Wu R. Association between different levels of lipid metabolism-related enzymes and fatty acid synthase in Wilms' tumor. Int J Oncol. 2020 Feb;56(2):568-580. doi: 10.3892/ijo.2019.4948.
6. Huang W, Lu Y, Wang F, Huang X, Yu Z (2020) Circular RNA circRNA_103809 Accelerates Bladder Cancer Progression and Enhances Chemo-Resistance by Activation of miR-516a-5p/FBXL18 Axis. Cancer Manag Res 12:7561-7568. doi: 10.2147/CMAR.S263083.
7. Zhan W, Liao X, Chen Z, Li L, Tian T, Yu L, Wang W, Hu Q (2020) Circular RNA hsa_circRNA_103809 promoted hepatocellular carcinoma development by regulating miR-377-3p/FGFR1/ERK axis. J Cell Physiol 235(2):1733-1745. doi: 10.1002/jcp.29092.
8. Huang D, Li C (2021) circ-ACACA promotes proliferation, invasion, migration and glycolysis of cervical cancer cells by targeting the miR-582-5p/ERO1A signaling axis. Oncol Lett 22(5):795. doi: 10.3892/ol.2021.13056.
9. Pan J, Liu Z, Yang Z, Liang E, Fang C, Zhang D, Zhou X, Niu Y, Xin Z, Chen Y, Cai Q (2022) Circ_0001686 Promotes Prostate Cancer Progression by Up-Regulating SMAD3/TGFBR2 via miR-411-5p. World J Mens Health 40(1):149-161. doi: 10.5534/wjmh.200204.
10. in C, Shi L, Li Z, Liu W, Zhao B, Qiu Y, Zhao Y, Li K, Li Y, Zhu Q (2019) Circ_0039569 promotes renal cell carcinoma growth and metastasis by regulating miR-34a-5p/CCL22. Am J Transl Res 11(8):4935-4945.
11. Nguyen ND, Yu M, Reddy VY, Acevedo-Diaz AC, Mesarick EC, Abi Jaoude J, Yuan M, Asara JM, Taniguchi CM (2021) Comparative Untargeted Metabolomic Profiling of Induced Mitochondrial Fusion in Pancreatic Cancer. Metabolites 11(9):627. doi: 10.3390/metabo11090627.
12. Tan PY, Wen LJ, Li HN, Chai SW (2020) MiR-548c-3p inhibits the proliferation, migration and invasion of human breast cancer cell by targeting E2F3. Cytotechnology 72(5):751-761. doi: 10.1007/s10616-020-00418-3.
13. Liu R, Dai W, Wu A, Li Y (2021) CircCDC45 promotes the malignant progression of glioblastoma by modulating the miR-485-5p/CSF-1 axis. BMC Cancer 21(1):1090. doi: 10.1186/s12885-021-08803-7.
14. Jiang Y, Wang K, Lu X, Wang Y, Chen J (2021) Cancer-associated fibroblasts-derived exosomes promote lung cancer progression by OIP5-AS1/ miR-142-5p/ PD-L1 axis. Mol Immunol 140:47-58. doi: 10.1016/j.molimm.2021.10.002.
15. Gao Y, Ma H, Gao Y, Tao K, Fu L, Ren R, Hu X, Kou M, Chen B, Shi J, Wen Y (2020) CircRNA Circ_0001721 Promotes the Progression of Osteosarcoma Through miR-372-3p/MAPK7 Axis. Cancer Manag Res 12:8287-8302. doi: 10.2147/CMAR.S244527.
16. Fan J, Zhang J, Huang S, Li P (2020) lncRNA OSER1-AS1 acts as a ceRNA to promote tumorigenesis in hepatocellular carcinoma by regulating miR-372-3p/Rab23 axis. Biochem Biophys Res Commun 521(1):196-203. doi: 10.1016/j.bbrc.2019.10.105.
17. Yin Y, Lu X. Yang M. et al. (2021) Inhibition of lncRNA MALAT1 reduces myocardial ischemia-reperfusion injury of rat cardiomyocytes through regulating the miR-135a-5p/HIF1AN axis. 25(4):e3392. doi: 10.1002/jgm.3392.
18. Høgsholt S, Asdahl PH, Bonnesen TG, Holmqvist AS, Madanat-Harjuoja L, Tryggvadottir L, Bautz A, Albieri V, Green D, Winther JF, Hasle H. Disease-specific hospitalizations among 5-year survivors of Wilms tumor: A Nordic population-based cohort study. Pediatr Blood Cancer. 2021 May;68(5):e28905. doi: 10.1002/pbc.28905.
19. Zhou R, Jia W, Gao X, Deng F, Fu K, Zhao T, Li Z, Fu W, Liu G

(2021) CircCDYL Acts as a Tumor Suppressor in Wilms' Tumor by Targeting miR-145-5p. Front Cell Dev Biol 9:668947. doi: 10.3389/fcell.2021.668947.

20. DU A (2021) Circ_0017247 induces Wilms' tumor metastasis through targeting miR-145. Minerva Med 112(6):826-828. doi: 10.23736/S0026-4806.20.06845-7.
21. Bereiter-Hahn J, Vöth M (1994) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc Res Tech 27(3):198-219. doi: 10.1002/jemt.1070270303.
22. Chen G, Liu N, Zhou A, Tang C, Ma D, Tang J (2001) The role of hypertension-related gene in aortic vascular smooth muscle cells from mice and rats. Chin Med J (Engl) 114(8):833-6.
23. Kozakiewicz P, Grzybowska-Szatkowska L, Ciesielka M, Rzymowska J (2021) The Role of Mitochondria in Carcinogenesis. Int J Mol Sci 22(10):5100. doi: 10.3390/ijms22105100.
24. Fang CL, Sun DP, Chen HK, Lin CC, Hung ST, Uen YH, Lin KY (2017) Overexpression of Mitochondrial GTPase MFN2 Represents a Negative Prognostic Marker in Human Gastric Cancer and Its Inhibition Exerts Anti-Cancer Effects. J Cancer 8(7):1153-1161. doi: 10.7150/jca.17986.
25. Lee YS, Jung YY, Park MH, Yeo IJ, Im HS, Nam KT, Kim HD, Kang SK, Song JK, Kim YR, Choi DY, Park PH, Han SB, Yun JS, Hong JT (2018) Deficiency of parkin suppresses melanoma tumor development and metastasis through inhibition of MFN2 ubiquitination. Cancer Lett 433:156-164. doi: 10.1016/j.canlet.2018.07.007.
26. Liu D, Peng S, Li Y, Guo T (2021) Circ-MFN2 Positively Regulates the Proliferation, Metastasis, and Radioresistance of Colorectal Cancer by Regulating the miR-574-3p/IGF1R Signaling Axis. Front Genet 12:671337. doi: 10.3389/fgene.2021.671337.
27. Moradi Marjaneh R, Khazaei M, Ferns GA, Avan A, Aghaee-Bakhtiari SH (2019) MicroRNAs as potential therapeutic targets to predict responses to oxaliplatin in colorectal cancer: From basic evidence to therapeutic implication. IUBMB Life 71(10):1428-1441. doi: 10.1002/iub.2108.
28. Che G, Gao H, Tian J, Hu Q, Xie H, Zhang Y (2020) MicroRNA-483-3p Promotes Proliferation, Migration, and Invasion and Induces Chemoresistance of Wilms' Tumor Cells. Pediatr Dev Pathol 23(2):144-151. doi: 10.1177/1093526619873491.
29. Liu K, He B, Xu J, Li Y, Guo C, Cai Q, Wang S (2019) miR-483-5p Targets MKNK1 to Suppress Wilms' Tumor Cell Proliferation and Apoptosis In Vitro and In Vivo. Med Sci Monit 25:1459-1468. doi: 10.12659/MSM.913005.
30. Li Y, Liu JJ, Zhou JH, Chen R, Cen CQ (2020) LncRNA HULC induces the progression of osteosarcoma by regulating the miR-372-3p/HMGB1 signalling axis. Mol Med 26(1):26. doi: 10.1186/s10020-020-00155-5.
31. Wang Q, Liu S, Zhao X, Wang Y, Tian D, Jiang W (2017) MiR-372-3p promotes cell growth and metastasis by targeting FGF9 in lung squamous cell carcinoma. Cancer Med 6(6):1323-1330. doi: 10.1002/cam4.1026.
32. Peng H, Pan X, Su Q, Zhu LS, Ma GD (2019) MiR-372-3p promotes tumor progression by targeting LATS2 in colorectal cancer. Eur Rev Med Pharmacol Sci O23(19):8332-8344. doi: 10.26355/eurrev_201910_19144.
33. Ferreira IG, Pucci M, Venturi G, Malagolini N, Chiricolo M, Dall'Olio F (2018) Glycosylation as a Main Regulator of Growth and Death Factor Receptors Signaling. Int J Mol Sci 19(2):580. doi: 10.3390/ijms19020580.
34. Zuo ZQ, Chen KG, Yu XY, Zhao G, Shen S, Cao ZT, Luo YL, Wang YC, Wang J (2016) Promoting tumor penetration of nanoparticles for cancer stem cell therapy by TGF-β signaling pathway inhibition. Biomaterials; 82:48-59. doi: 10.1016/j.biomaterials.2015.12.014. Epub 2015 Dec 21. PMID: 26751819.
35. Wang X, Inoue S, Gu J, Miyoshi E, Noda K, Li W, Mizuno-Horikawa Y, Nakano M, Asahi M, Takahashi M, Uozumi N, Ihara S, Lee SH, Ikeda Y, Yamaguchi Y, Aze Y, Tomiyama Y, Fujii J, Suzuki K, Kondo A, Shapiro SD, Lopez-Otin C, Kuwaki T, Okabe M, Honke K, Taniguchi N (2005) Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. Proc Natl Acad Sci U S A. 102(44):15791-6. doi: 10.1073/pnas.0507375102.
36. Lee J, Warnken U, Schnölzer M, Gebert J, Kopitz J (2015) A new method for detection of tumor driver-dependent changes of protein sialylation in a colon cancer cell line reveals nectin-3 as TGFBR2 target. Protein Sci 24(10):1686-94. doi: 10.1002/pro.2741.
37. Huang YS, Zhong Y, Yu L, Wang L. Association between the TGFBR2 G-875A polymorphism and cancer risk: evidence from a meta-analysis. Asian Pac J Cancer Prev. 2014;15(20):8705-8. doi: 10.7314/apjcp.2014.15.20.8705.
38. Sun X, He Z, Guo L, Wang C, Lin C, Ye L, Wang X, Li Y, Yang M, Liu S, Hua X, Wen W, Lin C, Long Z, Zhang W, Li H, Jian Y, Zhu Z, Wu X, Lin H (2021) ALG3 contributes to stemness and radioresistance through regulating glycosylation of TGF-β receptor II in breast cancer J Exp Clin Cancer Res 40(1):149. doi: 10.1186/s13046-021-01932-8.
39. Li F, Li F, Chen W (2020) Propofol Inhibits Cell Proliferation, Migration, and Invasion via mir-410-3p/Transforming Growth Factor-β Receptor Type 2 (TGFBR2) Axis in Glioma. Med Sci Monit 26:e919523. doi: 10.12659/MSM.919523.
40. Mishra S, Deng JJ, Gowda PS, Rao MK, Lin CL, Chen CL, Huang T, Sun LZ (2014) Androgen receptor and microRNA-21 axis downregulates transforming growth factor beta receptor II (TGFBR2) expression in prostate cancer. Oncogene. 33(31):4097-106. doi: 10.1038/onc.2013.374.