Circ-PRMT5 stimulates the proliferative ability in Wilms’ tumor through the miR-7-5p/KLF4 axis

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ABSTRACT

Circ-PRMT5 is upregulated in Wilms’ tumor samples, which is closely linked to its tumor staging. It stimulates circ-PRMT5 stimulated the malignant development of Wilms’ tumor by activating the miR-7-5p/KLF4 axis. Knockdown of circ-PRMT5 markedly suppressed proliferative ability in Wilms’ tumor cells. Luciferase assay confirmed the interaction in the circ-PRMT5/miR-7-5p/KLF4 axis. Rescue experiments finally identified that circ-PRMT5 stimulated the malignant development of Wilms’ tumor by activating the miR-7-5p/KLF4 axis. Circ-PRMT5 is upregulated in Wilms’ tumor samples, which is closely linked to its tumor staging. It stimulates proliferative ability in Wilms’ tumor cells by activating the miR-7-5p/KLF4 axis.

Introduction

Wilms’ tumor, also known as nephroblastoma, is the most common malignant tumor of the genitourinary system that originates in the kidney. It predominately affects pediatric patients, accounting for approximately 6% of all pediatric malignancies (1-3). Wilms’ tumor is relatively rare, with an estimated incidence of 0.01% in children younger than 15 years (1, 2). The average age of diagnosis for Wilms’ tumor is around 36 months, and it rarely occurs in individuals older than 10 years or younger than 6 months (1, 2).

The exact causes and mechanisms underlying the development of Wilms’ tumor remain largely unknown. However, it is widely believed that the tumor originates from the abnormal proliferation of postrenal embryonic nephrons (4, 5). Pathologically, Wilms’ tumor is classified into several histological types, including blastema, mesenchyme, epithelium, and mixed types. The presence of anaplastic cells within the tumor plays a crucial role in determining the prognosis and clinical outcome of Wilms’ tumor (5).

Clinical symptoms of Wilms’ tumor are often atypical and vary depending on the stage and location of the tumor. The most common presentation is the presence of an asymptomatic abdominal mass. Other manifestations may include hematuria (blood in the urine), abdominal pain, hypertension (high blood pressure), and symptoms caused by the compression of adjacent structures by the tumor mass. Wilms’ tumor can also be associated with multiple congenital malformations, further complicating its diagnosis and management (6, 7).

The therapeutic strategy for Wilms’ tumor aims to prevent complications and ultimately reduce mortality rates (8-10). Early diagnosis is crucial for successful treatment, and it is essential to identify the pathological subtype of the tumor to develop individualized treatment approaches (4, 8, 11, 12).

In recent years, non-coding RNAs (ncRNAs) have emerged as important regulators of gene expression and are known to play significant roles in various biological processes. These ncRNAs can negatively regulate gene transcription or induce degradation of downstream messenger RNAs (mRNAs) through complementary base pairing, thereby influencing cellular behaviors (13, 14). With the advent of high-throughput sequencing and microarray analysis, several classes of ncRNAs, such as long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), have been identified as potential biomarkers and key players in tumorigenesis (15, 16).

In addition to lncRNAs and miRNAs, circular RNAs (circRNAs) have gained significant attention as a novel class of ncRNAs with potential regulatory functions (17, 18). CircRNAs are characterized by a covalently closed loop structure and are more stable compared to linear RNAs due to their resistance to exonucleases. These unique features make circRNAs promising candidates for...
diagnostic and therapeutic applications in cancer. Previous studies have shed light on the potential role of circ-PRMT5, a circular RNA, in cancer progression (19, 20). In the context of Wilms’ tumor, our previous work analyzed differentially expressed circRNAs in Wilms’ tumor samples, and circ-PRMT5 was found to be significantly upregulated. Given its dysregulation in Wilms’ tumor, we aim to investigate the functional role of circ-PRMT5 in the development of Wilms’ tumor and elucidate its underlying molecular mechanisms.

In this study, we present a comprehensive analysis of circ-PRMT5 in Wilms’ tumor, exploring its potential involvement in tumor pathogenesis. By examining its expression patterns and functional implications, we seek to contribute to a better understanding of the molecular mechanisms driving Wilms’ tumor development and identify potential therapeutic targets. Our findings may provide valuable insights into the diagnosis, prognosis, and treatment strategies for Wilms’ tumor, ultimately improving patient outcomes in this pediatric malignancy.

Materials and Methods

Patients and Wilms’ tumor samples

Paired Wilms’ tumor and paracancerous tissues were surgically resected from 45 patients (22 males and 23 females) with Wilms’ tumor. The average age of included patients was 25.5 months. None of them had preoperative treatment. Samples were independently confirmed by two experienced pathologists. This study got approval from Ethics Committee of Hainan Women and Children’s Medical Center and was conducted after informed consent.

Cell culture

Human Wilms’ tumor cell lines (HFWT, WT-CLS1 and 17-94) were first collected and digested from fresh Wilms’ tumor tissues, and one renal tubular epithelial cell line (HK-2) was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). All cells were cultured in Dulbecco’s modified eagle medium (DMEM) (Shanghai, China). Cells were grown to 30-50% and 80-90% confluence.

Transfection

Transfection plasmids were purchased from GenePharma (Shanghai, China). Cells were grown to 30-50% and transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Transfected cells for 48 h were utilized for in vitro experiments.

Cell proliferation assay

Cells were inoculated in a 96-well plate with 2×10^4 cells per well. At the appointed time points, the absorbance value at 490 nm of each sample was recorded using the cell counting kit-8 (CCK-8) kit (Dojindo Laboratories, Kumamoto, Japan) for plotting the viability curves.

Colony formation assay

Cells were inoculated in a 6-well plate with 200 cells per well and cultured for 2 weeks. The culture medium was replaced once in the first week and twice in the second week. Visible colonies were washed in phosphate-buffered saline (PBS), fixed in methanol for 20 min and dyed in 0.1% crystal violet for 20 min, which were finally captured and calculated.

5-Ethynyl-2’-deoxyuridine (EdU) assay

Cells were inoculated in a 24-well plate with 2×10^4 cells per well. They were incubated in 4% methanol for 30 min, followed by 10-min permeabilization in 0.5% Triton-X-100 (Solarbio, Beijing, China), and 30-min reaction in 400 μL of 1×ApolloR. Afterward, cells were dyed in 4’,6-diamidino-2-phenylindole (DAPI) for another 30 min. EdU-positive cells and DAPI-labeled nuclei were captured (Sigma-Aldrich, St. Louis, MO, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Extracted RNAs by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) were reversely transcribed into complementary deoxyribonucleic acids (cDNAs) using PrimerScript RT Reagent (TaKaRa, Otsu, Japan). The obtained cDNAs underwent qRT-PCR using SYBR® Premix Ex Taq™ (TaKaRa, Otsu, Japan). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were the internal references. Each sample was performed in triplicate, and the relative level was calculated by 2^(-ΔΔCt). The primer sequences were shown below: circ-PRMT5: Forward: 5’-ATCGTGCTGCTTTACGTTT-3’, Reverse: 5’-GGTCAAGGGGATCTGATACT-3’; KLF4: Forward: 5’-CCCCACCTTCTTACCCCCAGA-3’, Reverse: 5’-GTAAGGGTTTTTCACCTGGTGGG-3’. GAPDH: Forward: 5’-CAAGGTCAT CCAAGCAACTTGG-3’, Reverse: 5’-GCCACCCAGGTGTTCGATAG-3’. MiR-7-5p: Forward: 5’-CCACGGTTGAAGACTAGTGATTT-3’, Reverse: 5’-TATGGTCTTCCTGCCTTGTTCC-3’. Western blot

Cells were lysed for isolating cellular protein and electrophoresed. Protein samples were loaded on polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, non-specific antigens were blocked in 5% skim milk for 2 hours. Membranes were reacted with primary and secondary antibodies for the indicated time. Band exposure and analyses were finally conducted.

Luciferase assay

Cells inoculated in 24-well plates were co-transfected with NC mimic/miR-7-5p mimic and wild-type/mutant-type vector, respectively. 48 hours later, cells were lysed for measuring luciferase activity (Promega, Madison, WI, USA).

Statistical analysis

GraphPad Prism 5 V5.01 (La Jolla, CA, USA) was used for data analyses. Data were expressed as mean ± standard deviation. Differences between groups were analyzed by the t-test. The chi-square test was used for analyzing the relationship between circ-PRMT5 level and clinical characteristics of patients with Wilms’ tumor. The Pearson correlation test was applied for assessing the correlation between the two expressions. P<0.05 was considered sta-
KLF4 was involved in the regulation of Wilms’ tumor proliferation

To further uncover the relationship between circ-PRMT5 and the miR-7-5p/KLF4 axis, rescue experiments...
were conducted. The transfection efficacy of pcDNA-KLF4 was first tested in HFWT and 17-94 cells (Figure 4A). Interestingly, decreased viability (Figure 4B) and EdU-positive rate (Figure 4C) in Wilms’ tumor cells with circ-PRMT5 knockdown were partially reversed by overexpression of KLF4.

Discussion

Traditional treatment for Wilms’ tumor is aggressive, which not only induces severe adverse events but also influences the normal development of the body (6-8). About 25% of survivors develop chronic diseases after 25 years of diagnosis or adulthood, including renal failure, congestive heart failure, pulmonary fibrosis, kyphosis, infertility, secondary tumor, etc. (8,9). Wilms’ tumor severely affects children health and their families owing to the low survival rate (8-11). It is urgent to clarify the pathogenesis and etiology of Wilms’ tumor (11,12). Therapeutic efficacy and prognosis in patients with Wilms’ tumor vary a lot because of individualized differences, suggesting the vital role of genetic variation in the development of Wilms’ tumor (5-10). Screening of susceptible populations and exploration of molecular genetic mechanisms in Wilms’ tumor are of great significance (4, 8, 11, 12).

Differentially expressed circRNAs in different tissues, cell lines and pathological stages have been highlighted (17,18). CircRNAs are featured by the closed loop structure, displaying more resistance to RNases and other exonucleases than linear RNAs (18). Because of the great conservation, stability and differential expressions, circRNAs are promising tumor hallmarks (16,18). It is reported that circ-PRMT5 is abnormally expressed in tumor samples and linked to prognosis (17,18). In this paper, circ-PRMT5 was upregulated in Wilms’ tumor samples. Knockdown of circ-PRMT5 reduced viability, colony number and EdU-positive rate in Wilms’ tumor cells, suggesting the suppressed proliferative potential.

Functionally, circRNAs exert their biological roles by sponging corresponding miRNAs as multiple upstream MREs exist (21-24). Bioinformatics analysis predicted binding sequences in the 3’UTR of circ-PRMT5 and miR-7-5p. KLF4 also had shared sequences with that of miR-7-5p. As our experimental evidences showed, miR-7-5p was confirmed to be the target binding circ-PRMT5, while KLF4 was the downstream gene of miR-7-5p. Moreover, circ-PRMT5 level was negatively correlated to miR-7-5p level and positively correlated to KLF4 level. Interestingly, KLF4 was able to reverse the regulatory effect of circ-PRMT5.
PRMT5 on proliferative potential in Wilms’ tumor cells. It is concluded that circ-PRMT5 stimulated proliferative potential in Wilms’ tumor by activating the miR-7-5p/KLF4 axis.

The present study provided significant insights into the molecular mechanisms underlying Wilms’ tumor development and progression. The study demonstrated that circ-PRMT5, a circular RNA, is upregulated in Wilms’ tumor samples and exhibits a close association with tumor stages. Through a series of comprehensive experiments and analyses, the researchers reveal that circ-PRMT5 plays a crucial role in stimulating the proliferative ability of Wilms’ tumor cells. Specifically, it exerts its influence by activating the miR-7-5p/KLF4 axis, a regulatory pathway involved in cell proliferation and differentiation. This finding adds to the growing body of evidence highlighting the importance of non-coding RNAs, such as circular RNAs, in modulating gene expression and contributing to cancer development. The identification of the miR-7-5p/KLF4 axis as a downstream target of circ-PRMT5 provides a deeper understanding of the specific molecular interactions involved in Wilms’ tumor pathogenesis. By delineating this regulatory pathway, the study opens up avenues for further research and potential therapeutic interventions targeting circ-PRMT5 and its downstream effectors. Moreover, these findings have clinical implications, as circ-PRMT5 could serve as a potential biomarker for Wilms’ tumor diagnosis, prognosis, and therapeutic response. The upregulation of circ-PRMT5 in tumor samples suggests its potential as a diagnostic indicator, while its association with tumor staging underscores its relevance for prognostic stratification. Furthermore, targeting circ-PRMT5 or the miR-7-5p/KLF4 axis could offer novel therapeutic strategies for inhibiting tumor growth and improving patient outcomes.

In summary, this study uncovers the functional role of circ-PRMT5 in Wilms’ tumor and highlights its involvement in the regulation of the miR-7-5p/KLF4 axis. The findings shed light on the complex molecular mechanisms underlying Wilms’ tumor pathogenesis and provide valuable insights that can inform future research and clinical approaches in the field of pediatric oncology.

Ethical compliance
This study got approval from the Ethics Committee of Hainan Women and Children’s Medical Center and was conducted after informed consent.

Conflict of interest
The authors declared no conflict of interest.

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