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Long noncoding RNA MIAT acts as an oncogene in Wilms' tumor through regulation of DGCR8

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Abstract. – OBJECTIVE: Recent researches have proved that long noncoding RNAs (lncRNAs) cover an important role in malignant tumors. Our study showed how lncRNA myocardial infarction-associated transcript (MIAT) functions in the development of Wilms' tumor.

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was utilized to detect the MIAT expression in Wilms' tumor patients. The MIAT expression level and the patients' overall survival time were analyzed. Then, we conducted functional experiments to identify the changes in the bio behaviors of Wilms' tumor cells due to of MIAT. Moreover, further experiments re performed to explore the potential mecha

RESULTS: By comparing with MIAT exp sion in adjacent tissues, the MIAT expressi level was significantly higher s' tumo samples. Moreover, the cel bility of Wilms' tumor cells was i ted to the loss of MIAT. The migrat nd inva bility of the Wilms' tumor ce inh the loss of MIAT. Fu her expression of DGCR8 was dow ulated the loss of MIAT. In addition as found DGCR8 expression was ively correla MIAT expression in or tissues.

CONCLUS : The results suggested that MIAT und promot cell proliferation and the stasis of Wilm or by upregulating D 8, which indicated hat MIAT might be a tial ta for the diagnosis and therapy ilms' or.

Key W
Long ing R MIAT, Wilms' tumor, DG-

Introduction

Wilms' tumor accounts for the most com- diatric renal cancer. 10,000 children are diag ed with Wilms' tumor annually, with an overall su of more tha 0%[1]. Wilms' tumor h ens w e embryonic nephrogenic cells fail to under minal differentiation. Alt he combinati erapy was available f ms' tumor, almost 10% of patients with ms' tumors s ents distant metastasis and urrence[2,3]. Th it is crucial to realize the unying molecu mechanism and find a new t nt strat or Wilms' tumors.

Long noncoding RNAs (lncRNAs) are one of the newly discovered subgroups of noncoding RNAs (ncRNAs) which are longer than 200 s. Numerous studies showed that lncRNAs are a new frontier in the research of the biological processes in the malignant cancers. For instance, lncRNA FAL1 is found to be a potential oncogene for colon cancer by promoting proliferation and inhibiting apoptosis of colon cancer cells[4]. LncRNA LINC01133 promotes colorectal cancer metastasis by inducing the epithelial-mesenchymal transition[5]. LncRNA ZNFX1 is significantly increased in the hepatocellular carcinoma and gastric cancer, which contributes to the development and progression of cancer[6]. Recent researches have revealed that lncRNA myocardial infarction-associated transcript (MIAT) functions as a novel oncogene in tumorigenesis. However, the function of lncRNA MIAT in Wilms' tumor and the potential molecular mechanism remain unknown so far. In this study, we discovered that the expression of MIAT was remarkably higher in the Wilms' tumor tissues. Moreover, the proliferation and metastasis of Wilms' tumor cells were inhibited due to the loss of MIAT. Previous studies have identified that DGCR8 is a fundamental regulator in tumor development. Our further experiment also found that MIAT functioned in the Wilms' tumor development by regulating DGCR8.

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Patients and Methods

Tissue Samples

A total of 50 Wilms' tumor patients were gathered from Wilms' tumor patients at The First Hospital of Jilin University from March 2016 to December 2018. The written informed consent was taken before the operation. No radiotherapy or chemotherapy was performed before the surgery. All the fresh tissues obtained from the surgery were preserved immediately at -80°C. This study was approved by the Ethics Committee of The First Hospital of Jilin University. The signed written informed consents were obtained from all participants before the study.

Cell Culture

The human Wilms' tumor cells were collected and digested from the fresh Wilms' tumor tissues. The culture medium consisted of 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA), Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA), as well as penicillin. The cells were cultured in an incubator containing 5% CO_2 at 37°C.

Cell Transfection

The cDNA oligonucleotides specifically ... geting MIAT (sh-MIAT) and the negative con... (NC) were synthesized by GenePharma (Shangha... China) and were cloned into ...VH1/GF-... P+Puro vector (GenePharm... ...hang... China). Then, sh-MIAT or NC w... ...ed for ...sfection in Wilms' tumor cells, an... ...which ... tive cells were selected ...r fu... ...riments.

RNA Extraction ...d Real Tim... Quantitative ...rase Chain ...ction (RT-qPCR)

The tota... RNA was se... ...d by using TRIzol reagent (I... ...rogen, Carlsbad, ...USA). Through reverse ...scription Kit (TaKa... Biotechnology Co., ... Dalia... ...hina), the total RNA was re-ver... ...ascri... ...to com... ...mentary deoxyribose nucle... ...cDNAs... ...he primers using for RT-qPCR ... foll... MIAT primers forward: 5′-... ...ACGT... ...CCACACTG-3′, reverse: 5′-T... ...CACTT... ...CATTCTAGG-3′; glyceraldehy... ...3-phosphate dehydrogenase (GAPDH) pr... ... 5′-CCAAAATCAGATGGGG-...TGCTGG-3′ and reverse 5′-TGATGGCAT-...ACTGTGGTCATTCA-3′. The thermal cycle w... follows: 30 sec at 95°C, 5 sec for 40 cycles at 9... 35 sec at 60°C.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide)

2×10^3 transfected cells were see... ...l 96-w... plates. Following the manufact... ...s protocol, the cell proliferation was ass... ...by the Cell Proliferation Reagent Kit I (MT... ... Basel, Switzerland) every 24 h. T... absorb... ...t 490 nm was assessed using ... ...LISA reade... (Multiskan Ascent, L... ...stems, Helsinki, ...land).

Colony Forma...n A...

The transf... ...cells w... ...ce... ...a 6-well plate for 2 w... The colonie... ...eated with methanol ... Then, 0.5... ...crystal violet was use... stain... ...5 min. The colonies containing more than 50... ...were counted, and the mea... ...y numbers w... ...lculated. The analy... ...as conducted with Image-Pro Plus 6.0 (Sil... Springs, MD, USA).

...ynyl Deox... ...dine (EdU) ...oratio... ...say

A... the manufacturer's manual, an EdU Kit (Roche, Mannheim, Germany) was uti-...ed to monitor the cell proliferation of transfect-... Zeiss Axiophot Photomicroscope (Carl ...iss, ...berkochen, Germany) was performed to take the representative images.

Transwell Assay

For detecting the invading ability of these treated cells, 5×10^4 cells in serum-free DMEM were replanted in the top chamber (Corning, Inc., Corning, NY, USA) of 24-well plates pre-coated with or without Matrigel Matrix dilution (BD Biosciences, San Jose, CA, USA). In addition, in the bottom chamber DMEM and FBS were added. The top surface of the chambers was wiped by cotton swab 48 h later and immersed for 10 min with precooling methanol. Then, they were stained in crystal violet for 30 min. Three fields were randomly chosen to count the data for the invasion membrane under a Leica DMI4000B microscope (Leica Microsystems, Heidelberg, Germany).

Western Blot Analysis

The protein was extracted from cells by Reagent radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China). The bicinchoninic acid (BCA) protein assay kit was used for quantifying the protein concentrations (TaKaRa Ltd., Dalian, China). The sodium dodecyl sulphate-polyacrylamide gel

electrophoresis (SDS-PAGE) was utilized to separate the target proteins. Then the gels were transferred to the polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) and further incubated with antibodies. The rabbit anti-GAPDH and rabbit anti-DGCR8 were provided by Cell Signaling Technology (CST, Danvers, MA, USA), as well as the goat anti-rabbit secondary antibody. The Image J software was applied for the assessment of the protein expression.

Statistical Analysis

The GraphPad Prism 5.0 (La Jolla, CA, USA) was adopted to conduct the statistical analysis. The data were expressed as mean ± SD (standard deviation). The Student's *t*-test and the Kaplan-Meier method were utilized. A *p*-value of less than 0.05 was considered statistically significant.

Results

MIAT Expression Level in Wilms' Tumor Tissues and Cells

Firstly, the MIAT expression was dete RT-qPCR in 50 Wilms' tumor patients' t s. The results showed that MIAT was signific upregulated in tumor tissue samples (Figure We divided 50 patients into two gro accordi to the median expression of M nts with high MIAT expression leve d po overall survival than those with MIAT ression level (Figure 1B).

Cell Proliferation of Wilms' Tumor Cells Was Inhibited Due to Loss of MI

Wilms' tumor cells were tra ed w MIAT shRNA or NC, and the t ection effi- ciency was detected by RT-q Figure 2A). The results of the MTT assay sho the cell growth ability of Wilms' t r cells hibit ed due to the loss of MI Figure 2B). ony formation assay al owed t the nu of colonies was rem bly de ed due to the loss of MIAT in W m lls (Fi 2C). Moreover, the r ts dU in oration assay also reve that the ta the EdU positive cell ced due to t f MIAT in Wilms' tu (Figure 2D).

Cell Invasion of W Tumor Cells Was Inh Due to Los MIAT

detect the function of MIAT in Wilms' or metastasis the transwell assay was performed. The resu howed that the cell migration ty of Wilms' mor cells was inhibited due to t of MIA igure 3A). The outcome of the trans so revealed that the cell invaded ability of Wilms' tumor cells was remarkably decreased due to the loss of MIAT (Figure 3B).

The Interaction Between DGCR8 and MIAT in Wilms' Tumor

DGCR8 was predicted as the target proteins of MIAT through StarBase v2.0 (http://starbase.sysu.edu.cn/starbase2/rbpLncRNA.php). RT-qPCR results showed that the expression level of DGCR8 in Wilms' tumor cells was remarkably lower in the

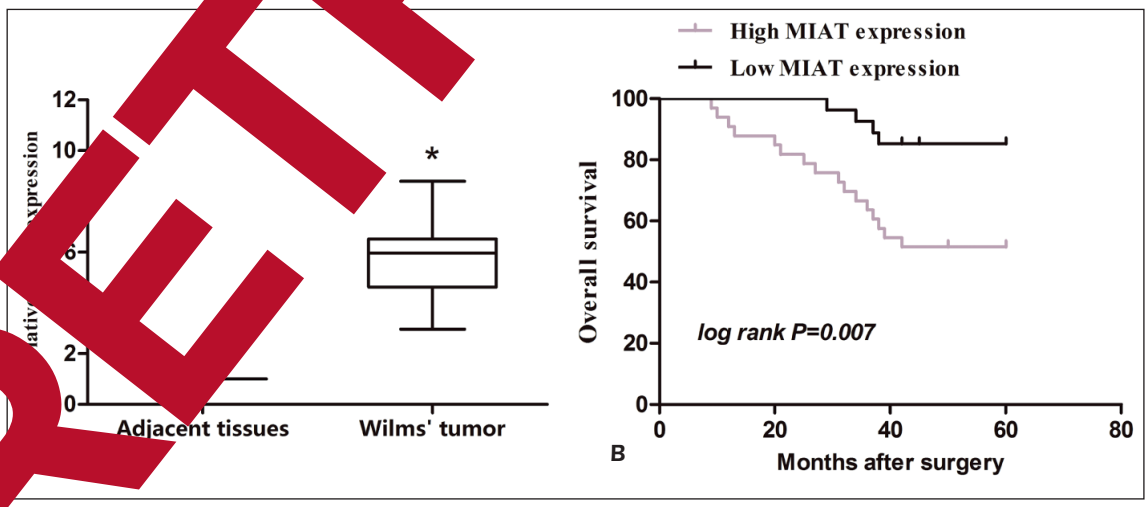

Figure 1. The expression levels of MIAT were increased in Wilms' tumor tissues. **A**, The MIAT expression was significantly in in the Wilms' tumor tissues compared with adjacent tissues. **B**, The expression levels of MIAT was negatively associated n patients' overall survival time. The data are presented as the mean ± standard error of the mean. *$p<0.05$.

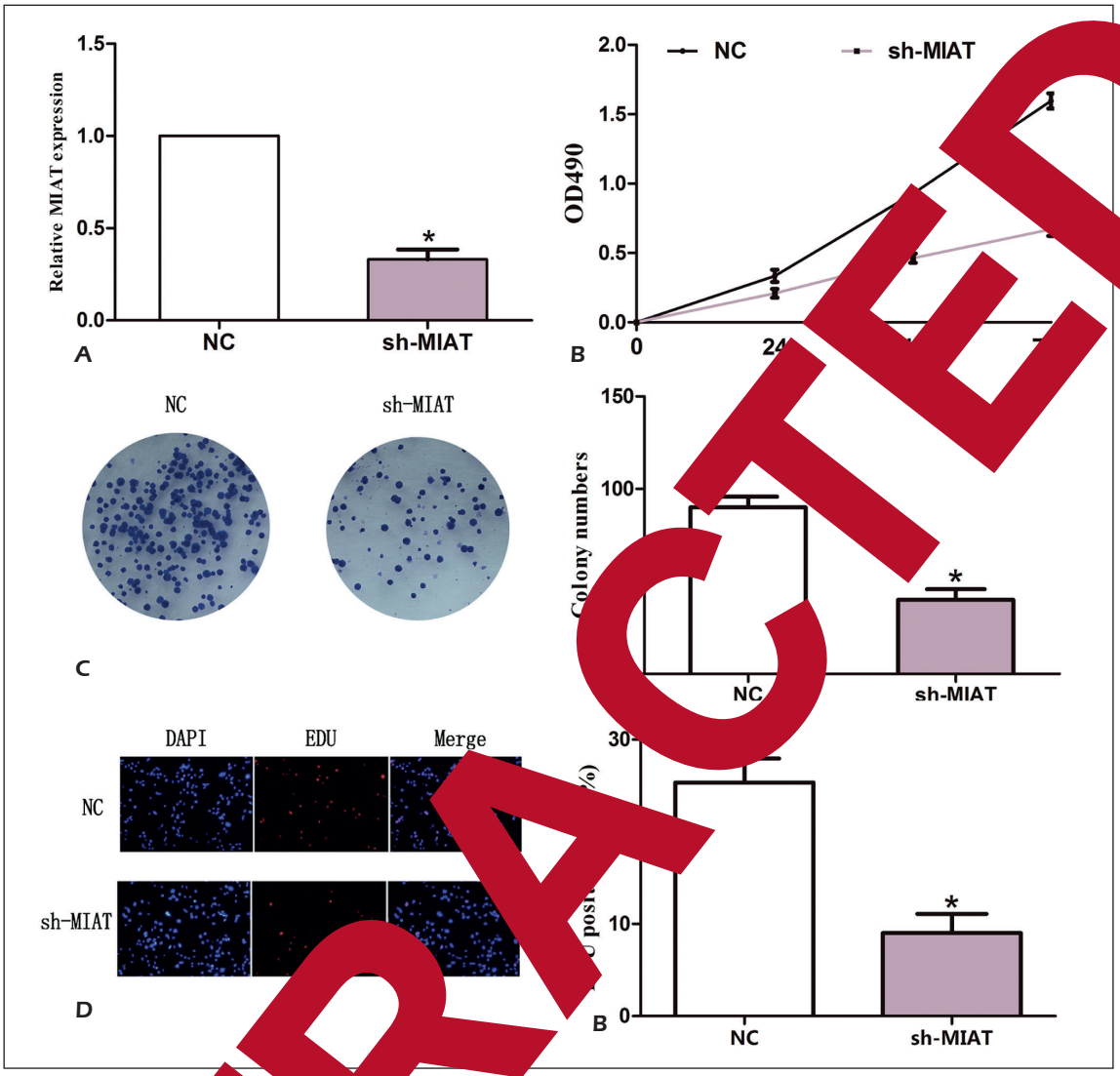

Figure 2. Wilms' tumo[r] prolife[ration] [was] inhibited due to the loss of MIAT. **A**, The MIAT expression in Wilms' tumor cells transfected with [sh-MIAT] and NC w[ere] [detec]ted by RT-qPCR. GAPDH was used as an internal control. **B**, The MTT assay showed that the kno[ckdown] of MIAT signifi[cantly] [re]pressed cell proliferation in Wilms' tumor cells. **C**, The colony formation assay showed that [the number] of colonies was [sig]nificantly decreased via knockdown of MIAT in Wilms' tumor cells (magnification: 10×). [The] Ed[U incor]poration assay showed that the number of EdU positive cells was significantly decreased via knockdow[n] of MIAT in W[ilms' tu]mor cells. The results represent the average of three independent experiments (mean ± standard er[ror] the mean). *$p<0.$ [co]mpared with the control cells.

sh-[MIAT] grou[p] [co]mpared [w]ith that in the NC group (Figu[re] [4A]). Weste[rn blo]t assay results showed that the e[xpress]ion le[vel] of DGCR8 in Wilms' tumor cells wa[s] [sig]nificantly lower in the sh-MIAT grou[p] [co]mpared [w]ith that in the NC group (Figure 4B). [Be]sides, the DGCR8 expression in Wilms' tumor [was] remarkably higher when compared [w]ith that of the adjacent tissues (Figure 4C). The [co]rrelation analysis demonstrated that the DGCR8 e[xpress]ion level positively correlated to MIAT expression in cancer tissues (Figure 4D).

Discussion

Numerous studies have proved that lncRNAs are important regulators in the initiation and progression of the Wilms' tumor. For example, lncRNA LINP1 promotes tumorigenesis of Wilms' tumor via Wnt/β-catenin signaling pathway[7]. LINC00473 suppresses miR-195 and further participates in regulating the Wilms' tumor via IKKα[8].

Located on chromosome 22q12, lncRNA MIAT has been found to be involved in numerous

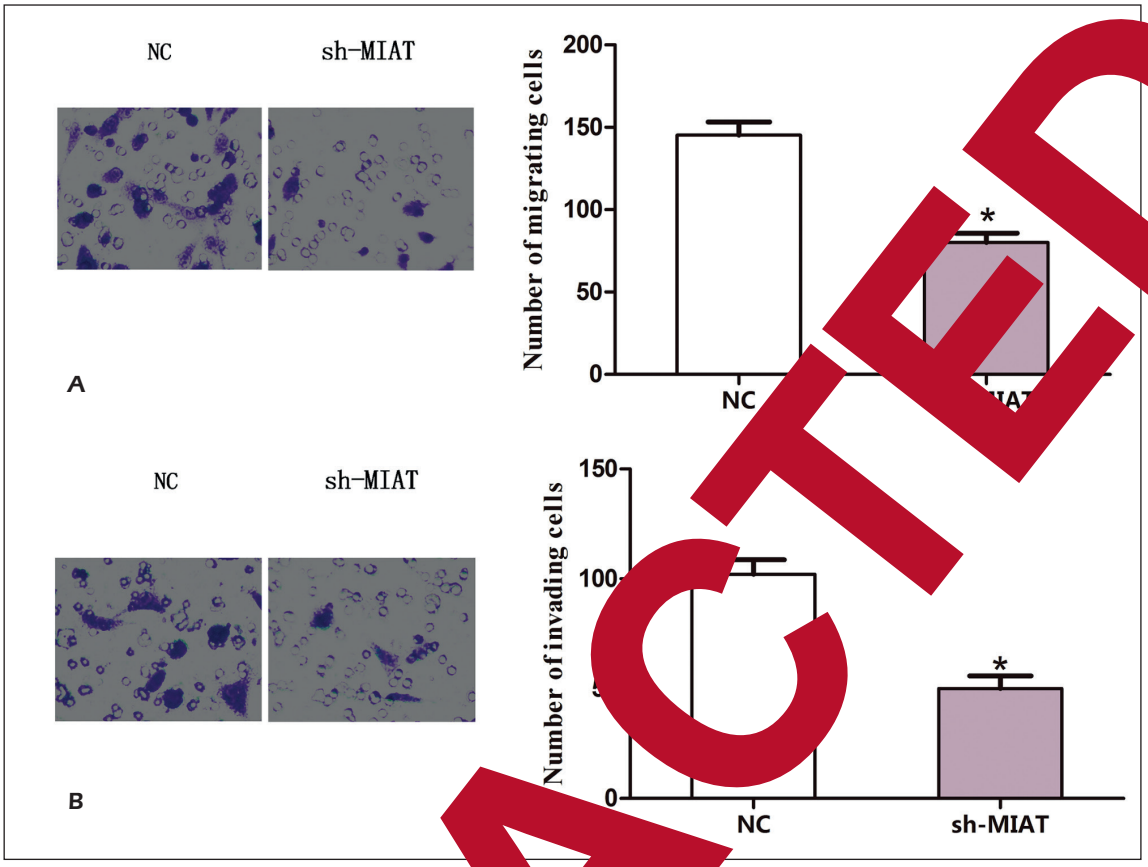

Figure 3. The transwell assay showed that the knoc[illegible]n [illegible]IAT inhibited Wilms' tumor cell migration and invasion. **A**, The transwell assay showed that number of migrated [illegible]s significantly decreased due to the knockdown of MIAT in Wilms' tumor cells (magnification: 40×). **B**, The transwe[illegible]y showed that number of invaded cells was significantly decreased due to the knockdown of MI[illegible]' tumor ce[illegible]agnification: 40×). The results represent the average of three independent experiments (mean ± [illegible]ard d[illegible]f the mean). [illegible]0.05, as compared with the control cells.

diseases, including th[illegible]dev[illegible]of cancers. MIAT enhances c[illegible]ctal ca[illegible]rowth and tumor metastasis [illegible]gulating mi[illegible]/Derlin-1 pathway[9]. MIA[illegible]ces the prog[illegible]sion of breast cancer [illegible]ervi[illegible]ceRNA for miR-155-5p[10]. By r[illegible]dulating mi[illegible]/DDX5 signaling pathway, [illegible]T facilitates tu[illegible]owth and metastasis [illegible]gastric cancer[11]. In this study, we found that M[illegible]T was h[illegible]y expressed in Wilms' tumor sa[illegible]. Besi[illegible]the cell [illegible]oliferation and metastasis [illegible]tumor [illegible] were inhibited due to th[illegible]loss o[illegible]T. Th[illegible]ove results indicated that [illegible]promi[illegible]rigenesis of Wilms' tumor and [illegible]ht act as [illegible]ncogene.

T[illegible]rther identify the underlying mechanism by[illegible] affects Wilms' tumor, we used [illegible]Base v2.0 to predict the target proteins of [illegible]T and picked DGCR8 as the potential target o[illegible]T. DGCR8 gene has been reported to participa[illegible]n the progression of cancers. For example, cell proliferation and invasion were inhibited due to the knockdown of DGCR8 in ovarian cancer[12]. DGCR8 inhibits tumor progression of prostate cancer[13]. Some researches[14,15] revealed that DGCR8 has a role in the development and metastasis of Wilms' tumor. In the present study, we first discovered the interaction between DGCR8 and MIAT. DGCR8 could be downregulated after the knockdown of MIAT in Wilms' tumor tissues. Besides, the DGCR8 expression was also higher in Wilms' tumor tissues and was positively related to MIAT expression.

Conclusions

In summary, MIAT could facilitate cell proliferation and metastasis in Wilms' tumor by upregulating DGCR8, which might serve as a candidate target for Wilms' tumor diagnosis and therapy.

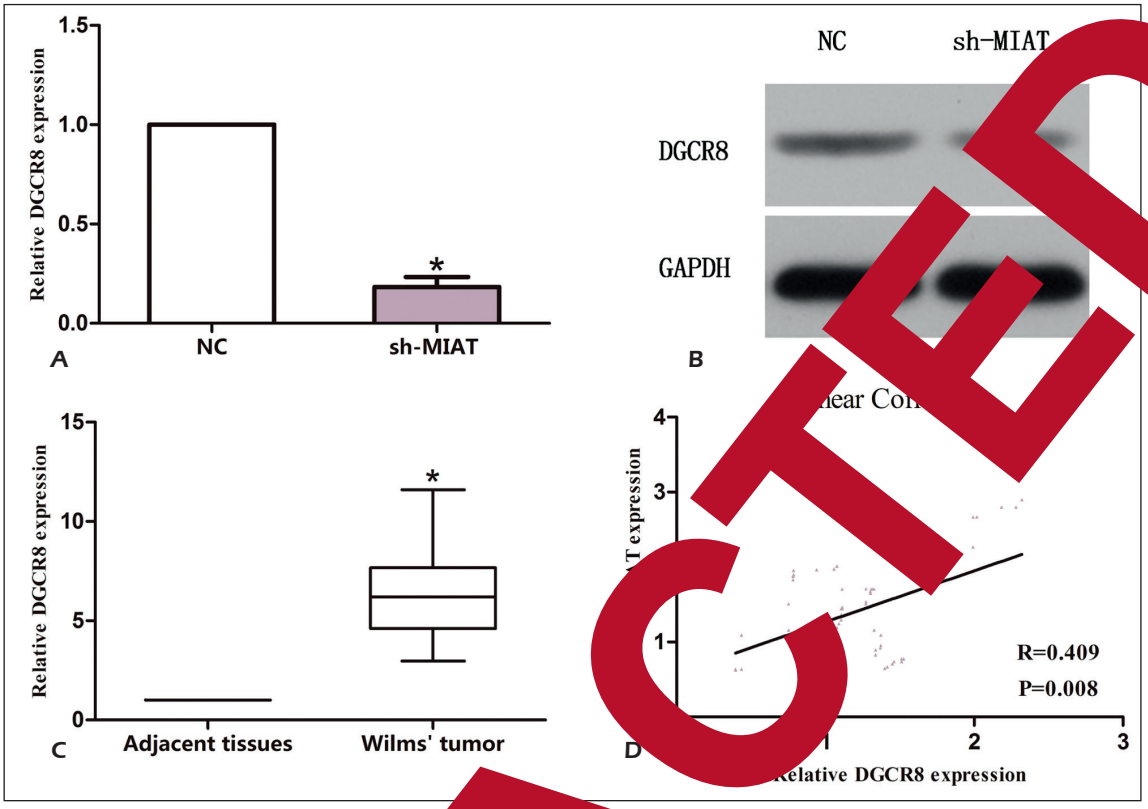

Figure 4. The interaction between MIAT and D[illegible]. **A**, [illegible]R results showed that DGCR8 expression was decreased in sh-MIAT compared with the NC group. [illegible]e We[illegible]ay results showed that DGCR8 expression was decreased in sh-MIAT compared with the the NC gr[illegible] [illegible]R8 was significantly upregulated in Wilms' tumor tissues compared with adjacent tissues. **D**, The linear correlatio[illegible] the expression level of DGCR8 and MIAT in Wilms' tumor tissues. The results represent the avera[illegible] three indepe[illegible] experiments. The data are presented as the mean ± standard error of the mean. *p<0.05.

Conflict of Interests

The Authors declare th[illegible]y have n[illegible]t of interests.

Re[illegible]ces

1) Charlt[illegible] Pavasovic V, Pi[illegible] Jones K. Biomarkers t[illegible]ect Wilms tumors [illegible]diatric patients: wh[illegible]re we [illegible]ow? Future Oncol 2015; 11: 2221-
2) [illegible] EB, [illegible]on SS, [illegible]an Noord M, Tracy ET, [illegible]h JC. B[illegible]rkers for Wilms tumor: a [illegible]ic revi[illegible] Urol 2016; 196: 1530-1535.
[illegible] CL, W[illegible]n YL, Zhuang HW, Xu M, Chen [illegible] Liu JX. [illegible]44-3p inhibits the proliferation [illegible] metastasis of pediatric Wilms' tumor cells by [illegible]din. Eur Rev Med Pharmacol Sci [illegible]71-7678.
Wu K, Zhang N, Ma J, Huang J, Chen J, Wang L, Zhang J. Long noncoding RNA FAL1 promotes [illegible]liferation and inhibits apoptosis of human co-[illegible]ancer cells. IUBMB Life 2018; 70: 1093-1100.
5) Kong J, Sun W, Li C, Wan L, Wang S, Wu Y, Xu E, Zhang H, Lai M. Long non-coding RNA LINC01133 inhibits epithelial-mesenchymal transition and metastasis in colorectal cancer by interacting with SRSF6. Cancer Lett 2016; 380: 476-484.
6) He A, He S, Li X, Zhou L. ZFAS1: a novel vital oncogenic lncRNA in multiple human cancers. Cell Prolif 2019; 52: e12513.
7) Zhu KR, Sun QF, Zhang YQ. Long non-coding RNA LINP1 induces tumorigenesis of Wilms' tumor by affecting Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2019; 23: 5691-5698.
8) Zhu S, Fu W, Zhang L, Fu K, Hu J, Jia W, Liu G. LINC00473 antagonizes the tumour suppressor miR-195 to mediate the pathogenesis of Wilms tumour via IKKα. Cell Prolif 2018; 51. doi: 10.1111/cpr.12416.
9) Liu Z, Wang H, Cai H, Hong Y, Li Y, Su D, Fan Z. Long non-coding RNA MIAT promotes growth and metastasis of colorectal cancer cells through regulation of miR-132/Derlin-1 pathway. Cancer Cell Int 2018; 18: 59.
10) Luan T, Zhang X, Wang S, Song Y, Zhou S, Lin J, An W, Yuan W, Yang Y, Cai H, Zhang Q, Wang L. Long

non-coding RNA MIAT promotes breast cancer progression and functions as ceRNA to regulate DUSP7 expression by sponging miR-155-5p. Oncotarget 2017; 8: 76153-76164.
11) Sha M, Lin M, Wang J, Ye J, Xu J, Xu N, Huang J. Long non-coding RNA MIAT promotes gastric cancer growth and metastasis through regulation of miR-141/DDX5 pathway. J Exp Clin Cancer Res 2018; 37: 58.
12) Guo Y, Tian P, Yang C, Liang Z, Li M, Sims M, Lu L, Zhang Z, Li H, Pfeffer LM, Yue J. Silencing the double-stranded RNA binding protein DGCR8 inhibits ovarian cancer cell proliferation, migration, and invasion. Pharm Res 2015; 32: 769-778.
13) Belair CD, Paikari A, Moltzahn F, Shenoy A, Yau C, Dall'Era M, Simko J, Benz C, Blelloch R. DGCR8 is essential for tumor progression following PTEN loss in the prostate. EMBO Rep 2015; 16: 1219-1232.
14) Walz AL, Ooms A, Gadd S, Gerhard DS, Smith MA, Guidry AJ, Meerzaman D, Chen QR, Hsu CH, Yan C, Nguyen C, Hu Y, Bowlby R, Brooks D, Ma Y, Mungall AJ, Moore RA, Schein J, Marra [illegible] V, Dome JS, Chi YY, Mullighan CG, [illegible] DA, Hampton OA, Jafari N, Ross [illegible]stier-Foster JM, Perlman EJ. Recurrent DG[illegible]S, DROSHA, and SIX homeodomain mut[illegible]in favorable histology Wilms tumors. Canc[illegible]2015; 27: 286-297.
15) Wegert J, Ishaque N, Va[illegible]ur R, Geo[illegible] Bieg M, Ziegler B, Bau[illegible]n S, Nourkami N, [illegible] N, Keller A, Grimm C[illegible]tz S, W[illegible]ms RD, Ch[illegible] T, Pritchard-Jones [illegible] Sluis P[illegible]kmann R, Koster J, Versteeg R, Ach[illegible]J, Bode[illegible]Niggli F, Tytgat GA, [illegible] Ti[illegible]n den H[illegible]-Eibrink MM, Meese [illegible]kuhl C[illegible]hner I[illegible]f N, Eils R, Pfister [illegible]kool M, Ges[illegible]M[illegible]ions in the SIX1/2 [illegible]y and the DR[illegible]GCR8 miRNA m[illegible]or complex [illegible]erlie high-risk blas[illegible]l type[illegible]c tumors. Cancer Cell 2015; 27: 29[illegible]-311.