Genome-wide screening of m6A-modified transcript in the Wilms' tumor

Zhuo Liu
Capital Institute of Pediatrics
Feng He
Capital Institute of Pediatrics
Jing Liu
Children's Hospital of Capital Institute of Pediatrics
Shengrong OuYang
Capital Institute of Pediatrics
Zexi Li
Children's Hospital of Capital Institute of Pediatrics
Yuanyuan Li
Capital Institute of Pediatrics
Feifei Ma
Capital Institute of Pediatrics
Huibo Chang
Capital Institute of Pediatrics
Xiaolin Ma
Children's Hospital of Capital Institute of Pediatrics
Dingding Cao
Capital Institute of Pediatrics
Yurui Wu
Children's Hospital of Capital Institute of Pediatrics
Jianxin Wu (jianxinwu_2000@163.com)
Beijing Tongren Hospital

Research Article

Keywords: Wilms' tumor, Epigenetics, N6-methyladenosine

Posted Date: February 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-197376/v1

Abstract

Background

Wilms' tumor, also called nephroblastoma, is the most common pediatric renal malignancy. The pathogenesis of Wilms' tumor has been attributed to several genetic and epigenetic factors. However, the most pervasive internal mRNA modification that affects almost every process of RNA metabolism, RNA N6-Methyladenosine (m6A) methylation, has not been characterized in Wilms' tumor.

Methods

Wilms' tumor (WT) and adjacent non-cancerous (NC) tissue samples were obtained from 23 children with nephroblastoma, and the global m6A levels were measured by mass spectrometry. Analyses by m6A-mRNA epitranscriptomic microarray and mRNA microarray were performed, and m6A-related mRNAs were validated by quantitative real-time PCR for input and m6A-immunoprecipitated RNA samples from WT and NC tissues. Gene ontology analysis and KEGG pathway analysis were performed for differentially expressed genes, and expression of RNA methylation-related factors was measured by quantitative real-time PCR.

Results

The total m6A methylation levels in total RNA of WT samples and NC samples were (0.21 ± 0.01)% and (0.22 ± 0.01)%, respectively, with no statistically significant difference. Fifty-nine transcripts were differentially m6A-methylated between the WT and NC groups, which showed distinct m6A modification patterns. Gene ontology analysis indicated that m6A-modified genes were enriched in cancer-associated pathways, including the mTOR pathway, and conjoint analysis of the unique methylation and gene expression patterns in WT samples suggested an association with metabolic pathways.The mRNA levels of the m6A-related "reader" genes, *YTHDF1*, *YTHDF2* and *IGF2BP3*, were statistically higher in WT samples than in NC samples.

Conclusion

This is the first study to determine the m6A modification profiles in Wilms' tumor. Our data provide novel information regarding patterns of m6A modification that correlate with carcinogenesis in Wilms' tumor.

Background

Wilms' tumor, also called nephroblastoma,, is one of the most common malignant, solid tumors in children, with an annual incidence rate of 1 case per 100,000 children. Nephroblastoma has a complex etiology that is poorly understood. Although the molecular mechanisms of nephroblastoma remain

undiscovered, increasing evidence suggests that genetic and epigenetic characteristics play an important role in the disease process[1].

Recently, RNA modification has increasingly been evaluated as a novel mechanism for regulating gene expression. Over 100 forms of modification of RNA have been discovered, and N6-methyladenosine (*m6A*) has emerged as one of the most prevalent epitranscriptomic markers in eukaryotes. In mammals, this modification influences different aspects of RNA metabolism, resulting in mRNA stability and splicing [2, 3] and translation efficiency [4], as well as effects on noncoding RNA such as microRNA processing [5]. Evidence suggests that m6A modification affects biological processes, including cell apoptosis, inflammation, cell autophagy and tumorigenesis. Research has demonstrated that m6A participates in colorectal cancer[6], hepatocellular carcinoma[7], and breast cancer[8]. However, the relationship between m6A and nephroblastoma has not been studied, and the m6A-methylation profile of nephroblastoma remains elusive. In the present study, we provide the first transcriptome-wide m6A profile of nephroblastoma. Our results characterize a novel mechanism underlying nephroblastoma that will facilitate further understanding of disease pathogenesis.

Methods

Clinical tissue specimens

Wilms' tumor (WT) and adjacent non-cancerous (NC) tissue samples were obtained from children with nephroblastoma undergoing tumor resection at the Department of Surgery in the Affiliated Children's Hospital of the Capital Institute of Pediatrics. The 23 patients were recruited from September 2017 to April 2019 and did not receive chemotherapy prior to surgical resection. The tissue samples were immediately frozen in liquid nitrogen and stored at − 80°C until further analysis.

Written informed consent was obtained from all guardians of study participants prior to sample collection.
The study was approved by the Ethics Committee for clinical research of the Capital Institute of Pediatrics, the methods in our work were also approved by the Ethics Committee for clinical research of the Capital Institute of Pediatrics (NO.KSDWLL2017024).
All methods were carried out in accordance with relevant guidelines and regulations.

Histological analysis

The WT and NC tissues were fixed in formaldehyde overnight and embedded in paraffin. Five-micrometer-thick sections were cut, stained with hematoxylin-eosin, and examined for infiltrating cells and inflammation using a light microscope (Nikon Instruments, Japan).

LC-MS/MS analysis of m6A levels

The m6A levels in WT and NC samples were evaluated by the CloudSeq company (CloudSeq Biotech, Shanghai, China). Briefly, Total RNA was isolated and mRNA was purified using the NEBNext® Poly(A)

mRNA Magnetic Isolation Module (New England Biolabs Biotech, USA). The mRNA was then digested and centrifuged. The supernatant after centrifugation was analyzed by liquid chromatography and mass spectrometry/mass spectrometry (LC-MS/MS), with the nucleosides separated by reverse phase high-performance liquid chromatography. The quantitation of modifications was based on nucleoside-to-base ion mass transitions. The m6A levels were calculated as the ratio of m6A to A.

Methylated RNA immunoprecipitation (MeRIP)

Total RNA was isolated from tissue specimens using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. Total RNA (3 μg) and m6A spike-in control mixture suspended in IP buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% NP-40 and 40 U/μl RNase Inhibitor) were incubated with 2 μg anti-m6A antibody at 4°C for 2h. The reaction mixture was then incubated for an additional 2h at 4°C with Dynabeads M-280 Sheep Anti-Rabbit IgG (Thermo Scientific). The beads were rinsed 3 times with IP buffer and 2 times with wash buffer (50 mM Tris-HCl pH 7.4, 50 mM NaCl, 0.1% NP-40 and 40 U/μL RNase Inhibitor) at room temperature to remove unbound antibody. Then, the enriched RNA was eluted from the beads in elution buffer at 42°C and purified using the RNeasy Mini Kit (Qiagen).

Microarray hybridization

RNA or immunoprecipitated RNA samples from adjacent NC (n = 3) and WT (n = 5) tissues samples were labeled with Cy5 fluorescent dye using the Super RNA Labeling Kit (Arraystar) and were purified using a RNeasy Mini Kit. Cy5 labeled cRNAs were fragmented and hybridized to a mouse m6A epitranscriptomic microarray (8×60K, Arraystar) that contained 44,122 mRNA degenerate probes. The hybridized arrays were scanned using an Agilent Scanner G2505C.

RNA purification and quantitative real-time PCR (qRT-PCR)

The mRNA level of m6A-related genes, *METTL14*, *METTL3*, *WTAP*, *ALKBH5*, *FTO*, *YTHDF1*, *YTHDF2*, and *IGF2BP3*, and mTOR pathway-related genes, *STK1*, *CAB39L*, *AKT1S1*, *PDPK1*, *STRADA*, *BRAF*, *PIK3CB*, *EIF4E2*, and *PTEN*, were analyzed by quantitative real-time PCR (qRT-PCR). Briefly, total RNA was isolated from tissue specimens using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. The quality and quantity of isolated RNA were verified using a NanoDrop ND-1000 (Thermo Fisher Scientific, USA). One microgram of total RNA was reverse-transcribed to generate the first-strand cDNA, and then quantitative real-time PCR was run in triplicate with the PrimeScript™ RT Reagent kit (Takara, Dalian, China) as described by the manufacturer. RT-PCR was conducted using SYBR Premix Ex Taq™ (Takara) on a 7900HT system. β-actin was used as an internal control to normalize the data.

Statistical analysis

All data from independent experiments are presented as means ± s.d. Statistical

analyses were conducted using SPSS 22.0. The paired Student's *t*-test was performed between WT and NC samples. One-way analysis of variance (ANOVA) was used to assess differences among three or more groups. Differences with $p < 0.05$ were defined as significant.

Results

Global RNA m6A modification patterns differ in WT and NC samples

To determine the global effect on m6A RNA modification in Wilms' tumor, we collected 23 tumor (WT) and adjacent non-tumorous control (NC) tissue from patients undergoing tumor resection. Histopathologic analysis confirmed that the cancer cells are densely arranged in tubular shape with small size, deep staining of the nuclei, and sparse cytoplasm. Representative images were shown in Fig. 1A.

To determine the global m6A levels in NC and WT samples, we performed LC-MS/MS. The total m6A methylation levels in total RNA of WT samples and NC samples were (0.21 ± 0.01, n = 23)% and (0.22 ± 0.01, n = 23)%, respectively. The WT samples exhibited relatively lower total m6A levels than the NC samples (Fig. 1B). Hower, these was no statistically significant difference between the two groups. To evaluate transcript-specific m6A changes in the WT group, we performed microarray hybridization. Immunoprecipitated m6A-methylated RNAs were isolated from WT and NC group, and the methylated RNAs were profiled using microarray with probes for 41,246 mRNAs. The results showed that 59 transcripts were differentially m6A-methylated between the WT and NC groups (fold change > 1.5, $p < 0.05$). Nine of the transcripts were significantly hypermethylated and 50 of the transcripts were significantly hypomethylated in WT samples. The hierarchical clustering and the scatter plot of the microarray hybridization data are shown in Fig. 2A and B.

We further evaluated potential differences in the expression levels among all m6A-methylated genes in nephroblastoma. A total of 1335 m6A-methylated transcripts were differentially expressed between the WT and NC groups (fold change > 1.2, $p < 0.05$). These results suggest that WT samples tend to have lower levels of m6A methylation and higher expression of m6A-hypomethylated genes.

Abnormal m6A-modified genes are enriched in cancer related pathways

To determine the function of the 1335 genes that are differentially m6A-methylated in WT, we performed protein-protein interaction (PPI) network analysis using Cytoscape software. Network analysis of 117 key differentially expressed genes was carried out using STRING software (https://string-db.org/), with the confidence value set at 0.9, and the degree ≥ 5 (Fig. S1). These results suggest that the m6A-methylated genes that are highly expressed in WT may be functionally related.

To explore the pathological and physiological roles of m6A-modified genes in the WT group, we analyzed the functions of the 1335 differentially expressed m6A-methylated transcripts (fold change > 1.2, $p < 0.05$) by gene ontology (GO) and KEGG pathway analyses. The GO analysis identified "GTPase activity" and "identical protein binding" as the main biological process. "Cytosol" and "extracellular exosome" were significantly enriched GO terms in the cellular component category, and "positive regulation of gene expression" and "protein transport" were major functions (Fig. 3A). The KEGG pathway analysis revealed

that among the enriched pathways, the differently methylated genes in the WT group were enriched in the “Wnt signaling pathway”, “mTOR signaling pathway”, and “Proteoglycans in cancer”. These results suggest that differentially m6A-modified RNAs and/or m6A-modified genes with differential expression may participate in the occurrence and development of cancer developmental processes (Fig. 3B).

Among the altered m6A-modified KEGG pathways enriched in WT, the mTOR signaling pathway has been established to be associated with carcinogenesis signaling[9, 10]. Closer examination suggested that STK11, CAB39L, AKT1S1, PDPK1, STRADA, BRAF, PIK3CB, EIF4E2, and PTEN were altered m6A-modified genes within the mTOR signaling pathway. Among the 9 genes, STK11, CAB39L, AKT1S1, STRADA, PIK3CB, EIF4E2 and PTEN were hyper-methylated, PDPK1and BRAF were hypo-methylated. To validate the result of microarray data for these genes, we conducted gene-specific m6A qPCR assays for the 9 genes. The results suggest that these 9 mRNAs were uniformly hypomethylated in WT, though statistical differences were observed for PDPK1, STRADA, PIK3CB and PTEN (Fig. 4A). Then we analyzed the total mRNA expression levels of these 9 genes by qPCR (Fig. 4B). The results showed that PDPK1 and PIK3CB were significantly lowly expressed (Fold Change > 1.5, P < 0.05), and EIF4E2 was significantly highly expressed (Fold Change > 1.5, P < 0.05) in WT. Overall, these results suggest that m6A modification may function in the process of WT development by changing the regulation of the mTOR signaling pathway and other cancer-related signaling pathways.

Conjoint analysis of MeRIP and RNA in WT and NC samples

For a more comprehensive analysis of the mRNA expression profile in the WT group, we also profiled mRNA expression using microarray with probes for 41,246 mRNAs. There were 2091 mRNAs with differential expression levels between the WT and NC groups, of which 615 mRNAs were upregulated and 1167 mRNAs were downregulated (Fold Change > 1.5, P < 0.05). The hierarchical clustering and scatter plot of the microarray hybridization data are shown in Fig. 5A and 5B. The abnormally regulated genes were selected for gene ontology and KEGG pathway analyses. The results suggest that the dysregulated genes in WT were significantly enriched in “small molecular metabolic process”, “regulation of biology quality”, and “transport”. Moreover, pathway analysis showed that “proteoglycans in cancer”, “PPAR signaling pathway” and “PI3K-Akt signaling pathway” were significantly altered in WT (Fig. 5C and 5D).

To further clarify the correlation between altered mRNAs and m6A-modified mRNAs, we performed conjoint analysis of the differently expressed methylated RNAs and differently expressed RNAs (Fold Change > 1.5, P < 0.05 for mRNA and fold change > 1.2, p < 0.05 for m6A). A total of 120 m6A hyper-methylated mRNAs were identified, among which 51 transcripts were significantly downregulated (hyper-down), and 59 transcripts were significantly upregulated (hyper-up). We also detected a total of 668 hypo-methylated m6A mRNAs, with 212 transcripts obviously upregulated (hypo-up) and 456 transcripts obviously downregulated (hypo-down, Fig. 5E). The ranking of the top three genes in each quadrant of Fig. 5E is listed in Table S1.

The m6A “readers” YTHDF1, YTHDF2 and IGF2BP3 are upregulated in WT tissues

We speculated that the altered m6A abundance in the WT group could potentially be the result of altered m6A modification-related genes. To evaluate this possibility, we detected the expression of m6A methylase complex subunits and m6A demethylase by mRNA qPCR. The “writer” METTL3, METTL14 and WTAP were only slightly upregulated while the “eraser” FTO and ALKBH5 were downregulated; however, these differences were not significant ($P > 0.05$, Fig. 6). More importantly, expression of the m6A “readers” YTHDF1, YTHDF2 and IGF2BP3 was statistically greater in the WT group than in the NC group (1.5 fold, 1.2 fold and > 2.0 fold, respectively, $p < 0.05$. Figure 6). These results are consistent with the possibility that the overall levels of hypomethylation in the WT tissues are associated with higher expression of m6A “readers”.

Discussion

Nephroblastoma, also called Wilms’ tumor, is one of the most common solid tumors in children, with an annual incidence rate of 1 case per 100,000 children[11]. It is a prototypical embryonal malignancy of young children and infants[12]. The pathogenesis of nephroblastoma remains undiscovered, though increasing evidence suggests that changes of the epitranscriptome may be a key characteristic of pathogenesis. Epitranscriptomic modifications may be targeted to mRNAs, lncRNAs and miRNAs. According to MODOMIC, a comprehensive database resource for systems biology of RNA modification, 172 different RNA chemical modifications have been found to date in all living organisms, including m6A, which is the most abundant and reversible modification, as well as m5C and m7G, Am, Gm and others[13]. Recent studies have demonstrated that various types of cancers, including cervical cancer, hepatocellular carcinoma and glioblastoma, are associated with abnormal m6A abundance[14–16]. We characterized the specific m6A methylation changes and related signaling pathways in Wilms’ tumor through experimental and bioinformatics analyses. To our knowledge, this is the first study to focus on the m6A modification profile on Wilms’ tumor.

Our results demonstrate that there is a tendency toward hypomethylation of global m6A levels in WT tissues, and that differentially m6A-methylated genes are enriched in cancer-related pathways. Notably, abnormally m6A-modified genes were shown to be enriched in the mTOR pathway. The mTOR pathway is a signal regulatory system that performs a variety of important biological functions[17, 18]. Recently, the mTOR pathway has been shown to be associated with various cancer processes [19, 20], and genes in this pathway, such as PTEN and BRAF1, also function in the process of Wilms’ tumor[21, 22]. We demonstrated by MeRIP-qPCR that five m6A-modified genes in the mTOR pathway (STK11, CAB39L, AKT1S1, EIF4E2, and PTEN) were hypomethylated in the WT group compared with the NC group, and the mRNA levels of AKT1S1 and EIF4E2 were upregulated in the WT group. These results indicate complex regulatory mechanisms of m6A that involve established cancer signaling pathways.

For further mechanistic insight, we analyzed m6A-modifying enzymes in Wilms’ tumor. Ao Lin et al. has recently demonstrated that polymorphisms of the m6A methylase METTL3 are related with Wilms tumor susceptibility in Chinese children[23], and Rui-Xi Hua et al.'s work suggests that SNPs in the m6A demethylase ALKBH5 may exert a weak influence on susceptibility to Wilms’ tumor[24]. These studies

analyzed only the possible relation between the WT and m6A “writers” and “erasers”, though effects on “readers” in Wilms’ tumor remained undetermined prior to our analysis. In our study, m6A "writers", such as METTL3, METTL14 and WTAP, were not altered, and m6A "erasers", including FTO and ALKBH5, also showed comparable levels between WT and NC. In contrast, YTHDF1, YTHDF2 and IGF2BP3, which are the m6A “readers”, were significantly elevated in the WT group. The reason for differences between the results of our study and those of Ao Lin et al. and Rui-Xi Hua et al. are unknown; however, prior studies suggest that the specific role of m6A modification in the expression of particular genes largely depends on the function of downstream m6A “readers” [25]. Han D et al. demonstrated that durable neoantigen-specific immunity is regulated by mRNA m6A methylation of YTHDF1: Ythdf1-deficient mice show an elevated antigen-specific $CD8^+$ T cell antitumor response[26]. Furthermore, Shi Y et al. demonstrated that YTHDF1 is associated with poor prognosis in patients with non-small cell lung cancer progression[27]; Paris J, Morgan M et al. demonstrated that YTHDF2 promotes cancer stem cell targeting that compromises acute myeloid leukemia progression[28]; and Qiang Wang et al demonstrated that IGF2BP3 directly recognizes and binds to HDGF mRNA and enhances its stability, promoting tumor angiogenesis[29]. Finally, our results imply that elevated m6A “readers” may be associated with the progression of Wilms’ tumor, thus extending the understanding of m6A-modifying enzymes in cancer.

Conclusions

In summary, our study shows that global m6A levels in Wilms’ tumor are significantly increased. m6A modification-related enzymes were detected, and m6A "readers" were shown to be increased significantly. Bioinformatics analysis was performed to understand the possible role of m6A modification in Wilms’ tumor. Finally, our results suggest that m6A methylation may be involved in Wilms’ tumor by regulating a variety of significant signal transduction pathways, such as the mTOR signaling pathway. Collectively, these results suggest that m6A modification may be a potential target for further research.

Declarations

Ethics approval and consent to participate

Written informed consent for the use of tissue samples was obtained from all patients and healthy participants. The study was approved by the Ethics Committee for Clinical Research of Capital Institute of Pediatrics, Beijing, China. Written informed consent was obtained from all guardians of study participants prior to sample collection. The methods in our work were also approved by the Ethics Committee for clinical research of the Capital Institute of Pediatrics (NO.KSDWLL2017024). All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication Not applicable

Availability of data and materials

The datasets generated during the current study are available in the GEO repository, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE167054]. All data generated or analyzed during this study are included in this published article.**Competing interests**The authors declare that they have no competing interests.**Funding**

This work was supported by Beijing Municipal Administration of Hospitals Incubating Program (PX2021052), the CAMS Central Public Welfare Scientific Research Institute Basal Research Expenses (2016ZX310182-3) and CAMS Initiative for Innovative Medicine (CAMS-I2M) and National Natural Science Foundation of China (No. 81701591) .The funding body had no role in the design of the study and collection, analysis and interpretation of data and in writing the manuscript.

Authors' contributions

JW and YW are the overall project lead and the grant holder with responsibility for the conception and design the protocol. ZL, JL and FH contributed the histological and MeRIP analysis for the study. SOY, ZL and YL performed the data collection and bioinformatics analysis. FM and HC carried out the LC-MS/MS assay and q-PCR assay. DC and XM contributed the critical revision of the manuscript. All authors read and approved the final manuscript.

AcknowledgementsWe would like to thank all of our colleagues for their contribution to this study.**Contributor Information**Zhuo Liu, Email: liuzhuozhuo2005@163.com.Feng He, hefengfeng2001@163.com. Jing Liu, Email: jingjing11262001@sohu.com. Shengrong OuYang, Email: cipbiochem@163.com. Zexi Li, Email: jeersy1986@sina.com.Yuanyuan Li, Email: liyuanyuan075@sina.com. Feifei Ma, Email: mafei0817@163.com.Huibo Chang, Email: huibochang2013@163.com.Xiaolin Ma, Email: pediamaxl@outlook.com. Dingding Cao, Email: cddzlm@163.com.Yurui Wu, Email: wuyrr@163.com.Jianxin Wu, Email: jianxinwu_2000@163.com.

Abbreviations

NC Adjacent non-tumorous

LC-MS/MS Liquid chromatography and mass spectrometry/mass spectrometry

MeRIP Methylated RNA immunoprecipitation

mTOR Mammalian target of rapamycin

PPI Protein-protein interaction

References

1. Anvar Z, Acurzio B, Roma J, Cerrato F, Verde G: **Origins of DNA methylation defects in Wilms tumors**. *Cancer letters* 2019, **457**:119-128.

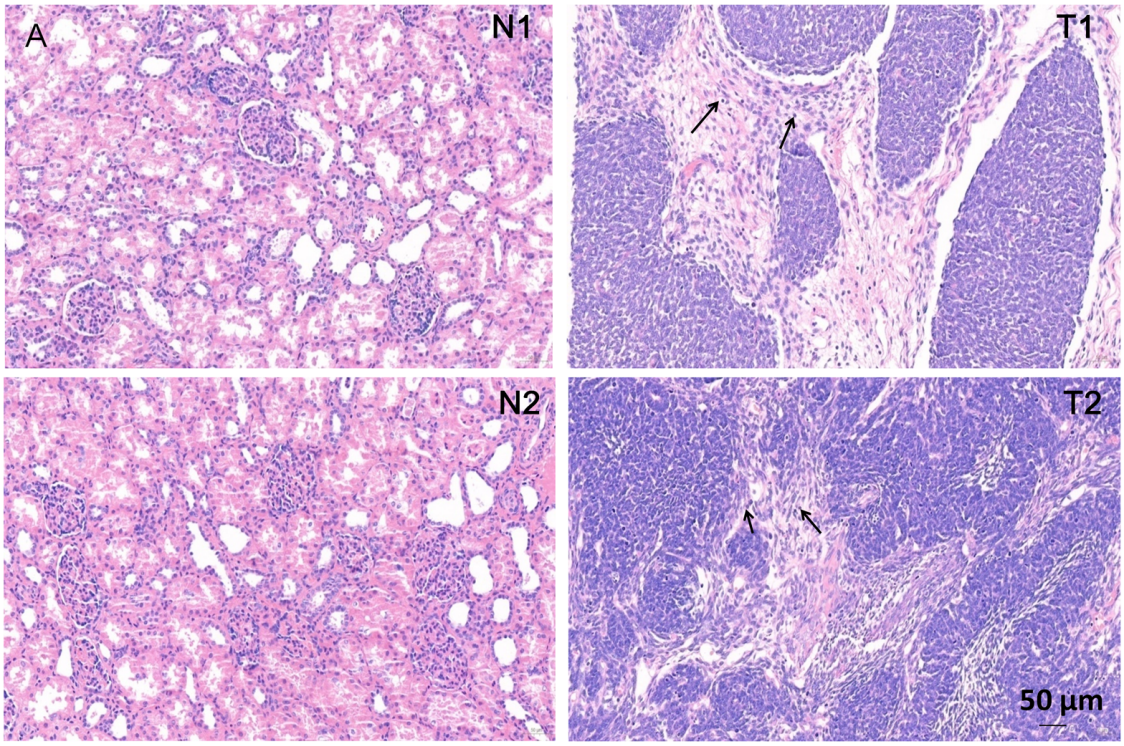
2. Zaccara S, Jaffrey SR: **A Unified Model for the Function of YTHDF Proteins in Regulating m(6)A-Modified mRNA**. *Cell* 2020, **181**(7):1582-1595 e1518.
3. Zhang Z, Luo K, Zou Z, Qiu M, Tian J, Sieh L, Shi H, Zou Y, Wang G, Morrison J *et al*: **Genetic analyses support the contribution of mRNA N(6)-methyladenosine (m(6)A) modification to human disease heritability**. *Nature genetics* 2020, **52**(9):939-949.
4. Hsu PJ, Zhu Y, Ma H, Guo Y, Shi X, Liu Y, Qi M, Lu Z, Shi H, Wang J *et al*: **Ythdc2 is an N(6)-methyladenosine binding protein that regulates mammalian spermatogenesis**. *Cell research* 2017, **27**(9):1115-1127.
5. Alarcon CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF: **N6-methyladenosine marks primary microRNAs for processing**. *Nature* 2015, **519**(7544):482-485.
6. Chen H, Gao S, Liu W, Wong CC, Wu J, Wu J, Liu D, Gou H, Kang W, Zhai J *et al*: **RNA m(6)A methyltransferase METTL3 facilitates colorectal cancer by activating m(6)A-GLUT1-mTORC1 axis and is a therapeutic target**. *Gastroenterology* 2020.
7. Xu J, Wan Z, Tang M, Lin Z, Jiang S, Ji L, Gorshkov K, Mao Q, Xia S, Cen D *et al*: **N(6)-methyladenosine-modified CircRNA-SORE sustains sorafenib resistance in hepatocellular carcinoma by regulating beta-catenin signaling**. *Molecular cancer* 2020, **19**(1):163.
8. Chang G, Shi L, Ye Y, Shi H, Zeng L, Tiwary S, Huse JT, Huo L, Ma L, Ma Y *et al*: **YTHDF3 Induces the Translation of m(6)A-Enriched Gene Transcripts to Promote Breast Cancer Brain Metastasis**. *Cancer cell* 2020.
9. Ediriweera MK, Tennekoon KH, Samarakoon SR: **Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: Biological and therapeutic significance**. *Seminars in cancer biology* 2019, **59**:147-160.
10. Ayuk SM, Abrahamse H: **mTOR Signaling Pathway in Cancer Targets Photodynamic Therapy In Vitro**. *Cells* 2019, **8**(5).
11. Apoznanski W, Sawicz-Birkowska K, Palczewski M, Szydelko T: **Extrarenal nephroblastoma**. *Central European journal of urology* 2015, **68**(2):153-156.
12. Coorens THH, Treger TD, Al-Saadi R, Moore L, Tran MGB, Mitchell TJ, Tugnait S, Thevanesan C, Young MD, Oliver TRW *et al*: **Embryonal precursors of Wilms tumor**. *Science* 2019, **366**(6470):1247-1251.
13. Boccaletto P, Machnicka MA, Purta E, Piatkowski P, Baginski B, Wirecki TK, de Crecy-Lagard V, Ross R, Limbach PA, Kotter A *et al*: **MODOMICS: a database of RNA modification pathways. 2017 update**. *Nucleic acids research* 2018, **46**(D1):D303-D307.
14. Qu N, Qin S, Zhang X, Bo X, Liu Z, Tan C, Wen G, Jiang H: **Multiple m(6)A RNA methylation modulators promote the malignant progression of hepatocellular carcinoma and affect its clinical prognosis**. *BMC cancer* 2020, **20**(1):165.
15. Dixit D, Prager BC, Gimple RC, Poh HX, Wang Y, Wu Q, Qiu Z, Kidwell RL, Kim LJY, Xie Q *et al*: **The RNA m6A reader YTHDF2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells**. *Cancer discovery* 2020.

16. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, Zhao BS, Mesquita A, Liu C, Yuan CL *et al*: **Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation**. *Nature cell biology* 2018, **20**(3):285-295.
17. Ozpolat B, Akar U, Steiner M, Zorrilla-Calancha I, Tirado-Gomez M, Colburn N, Danilenko M, Kornblau S, Berestein GL: **Programmed cell death-4 tumor suppressor protein contributes to retinoic acid-induced terminal granulocytic differentiation of human myeloid leukemia cells**. *Molecular cancer research : MCR* 2007, **5**(1):95-108.
18. Lamper AM, Fleming RH, Ladd KM, Lee ASY: **A phosphorylation-regulated eIF3d translation switch mediates cellular adaptation to metabolic stress**. *Science* 2020, **370**(6518):853-856.
19. Dong P, Wang X, Liu L, Tang W, Ma L, Zeng W, Sun S, Zhang L, Zhang N, Shen X *et al*: **Dampened VEPH1 activates mTORC1 signaling by weakening the TSC1/TSC2 association in hepatocellular carcinoma**. *Journal of hepatology* 2020, **73**(6):1446-1459.
20. Deng J, Bai X, Feng X, Ni J, Beretov J, Graham P, Li Y: **Inhibition of PI3K/Akt/mTOR signaling pathway alleviates ovarian cancer chemoresistance through reversing epithelial-mesenchymal transition and decreasing cancer stem cell marker expression**. *BMC cancer* 2019, **19**(1):618.
21. Obasaju P, Shahab S, Dunn E, Rhee DS, Jiang L, Dome JS, Friedman AD, Argani P, Pratilas CA: **BRAF V600E-mutated metastatic pediatric Wilms tumor with complete response to targeted RAF/MEK inhibition**. *Cold Spring Harbor molecular case studies* 2020, **6**(2).
22. An NN, Shawn J, Peng JP, Wu MD, Huang LG: **Up-regulation of miR-190b promoted growth, invasion, migration and inhibited apoptosis of Wilms' tumor cells by repressing the PTEN expression**. *European review for medical and pharmacological sciences* 2018, **22**(4):961-969.
23. Lin A, Zhou M, Hua RX, Zhang J, Zhou H, Li S, Cheng J, Xia H, Fu W, He J: **METTL3 polymorphisms and Wilms tumor susceptibility in Chinese children: A five-center case-control study**. *The journal of gene medicine* 2020, **22**(11):e3255.
24. Hua RX, Liu J, Fu W, Zhu J, Zhang J, Cheng J, Li S, Zhou H, Xia H, He J *et al*: **ALKBH5 gene polymorphisms and Wilms tumor risk in Chinese children: A five-center case-control study**. *Journal of clinical laboratory analysis* 2020, **34**(6):e23251.
25. Zhu T, Roundtree IA, Wang P, Wang X, Wang L, Sun C, Tian Y, Li J, He C, Xu Y: **Crystal structure of the YTH domain of YTHDF2 reveals mechanism for recognition of N6-methyladenosine**. *Cell research* 2014, **24**(12):1493-1496.
26. Han D, Liu J, Chen C, Dong L, Liu Y, Chang R, Huang X, Liu Y, Wang J, Dougherty U *et al*: **Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells**. *Nature* 2019, **566**(7743):270-274.
27. Shi Y, Fan S, Wu M, Zuo Z, Li X, Jiang L, Shen Q, Xu P, Zeng L, Zhou Y *et al*: **YTHDF1 links hypoxia adaptation and non-small cell lung cancer progression**. *Nature communications* 2019, **10**(1):4892.
28. Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, Mapperley C, Lawson H, Wotherspoon DA, Sepulveda C *et al*: **Targeting the RNA m(6)A Reader YTHDF2 Selectively**

Compromises Cancer Stem Cells in Acute Myeloid Leukemia. *Cell stem cell* 2019, **25**(1):137-148 e136.

29. Wang Q, Chen C, Ding Q, Zhao Y, Wang Z, Chen J, Jiang Z, Zhang Y, Xu G, Zhang J *et al*: **METTL3-mediated m(6)A modification of HDGF mRNA promotes gastric cancer progression and has prognostic significance**. *Gut* 2020, **69**(7):1193-1205.

Figures



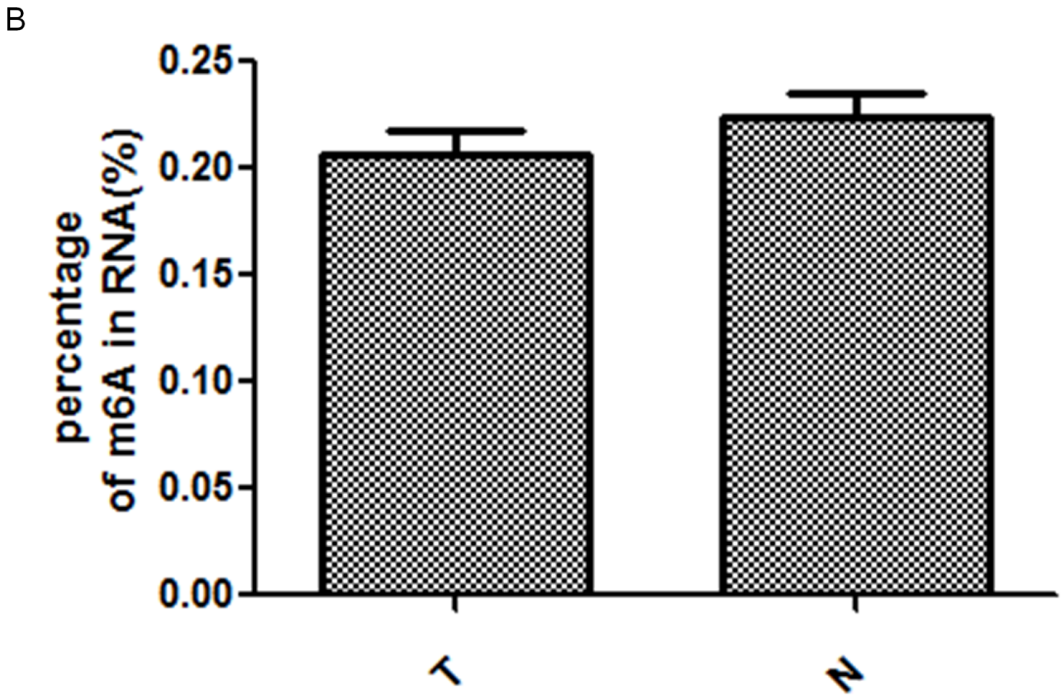


Fig.1

Figure 1

Global RNA m6A modification patterns differ in WT and NC samples. (A)Nephroblastoma tissue and adjacent non-cancerous tissue were analyzed for histopathology. (B) The total m6A methylation levels in total RNA of WT samples and NC samples were (0.21±0.01, n=23)% and (0.22±0.01, n=23)%, respectively. The WT samples exhibited relatively lower total m6A levels than the NC samples.

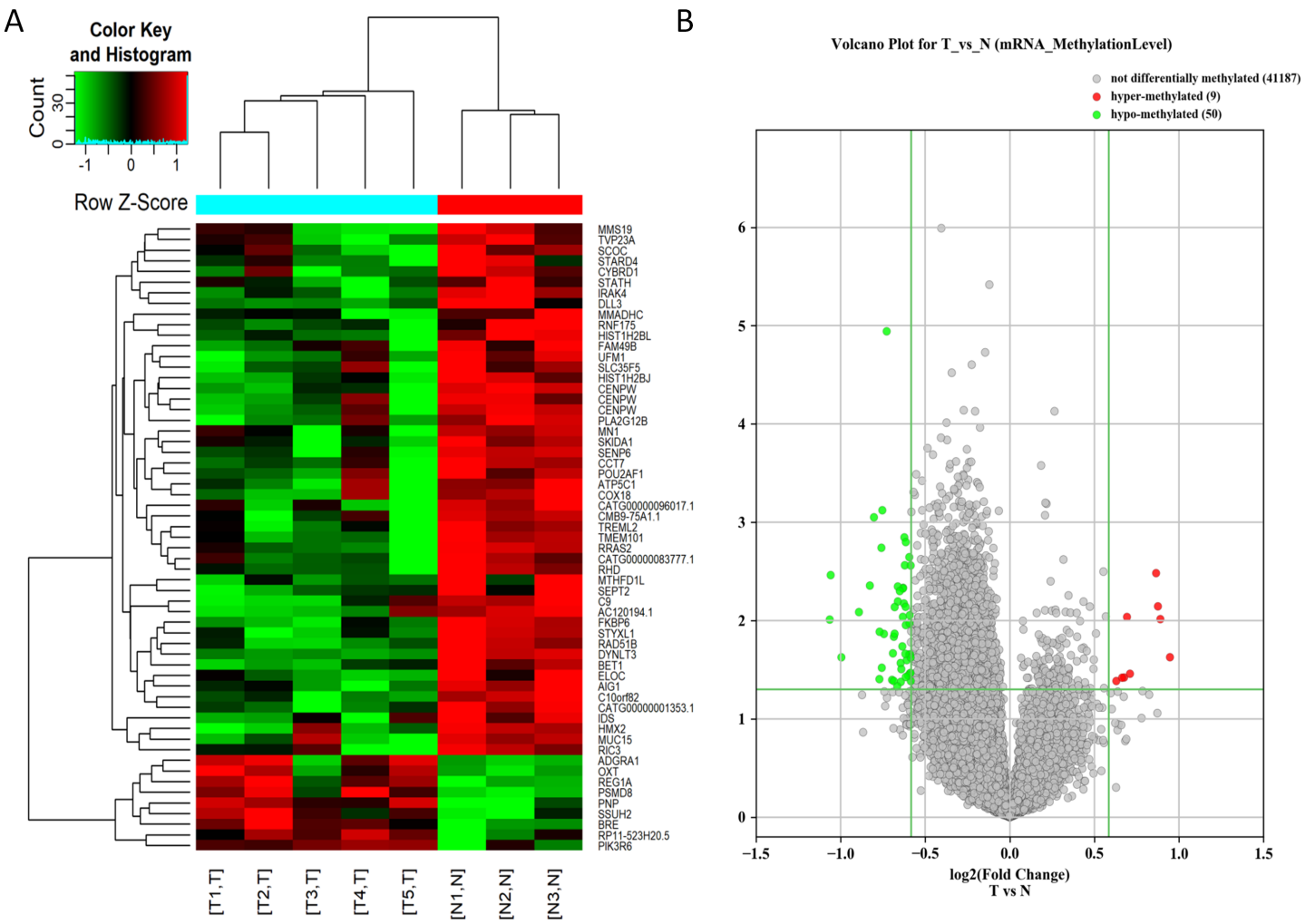


Fig.2

Figure 2

Global m6A modification changes in WT compared with NC tissues. (A) Cluster analysis of the altered m6A-methylated mRNAs (red: higher methylation level in WT, green: lower methylation level in WT). n = 3 in the control group and n =5 in WT. (B) Volcano plot: hypermethylation (red dot) and hypomethylation (green dot) in WT and NC (fold change >1.5, p<0.05).

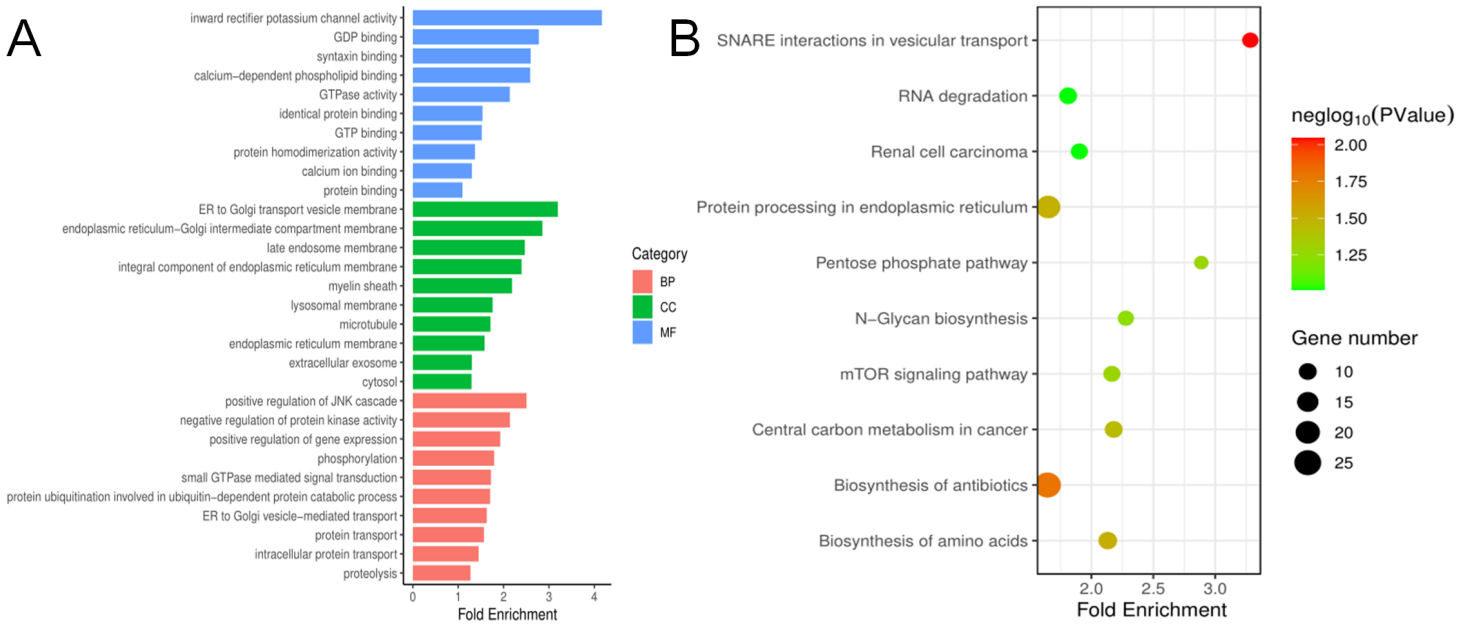


Fig.3

Figure 3

Gene ontology enrichment and pathway analyses of differentially altered m6A-modified mRNA between WT and NC. Pathway analysis was performed for the 1335 differentially expressed m6A-methylated transcripts. (A) Significantly enriched gene ontology terms for the differentially methylated genes. (B) Enrichment patterns for the top 10 significantly enriched pathways were shown.

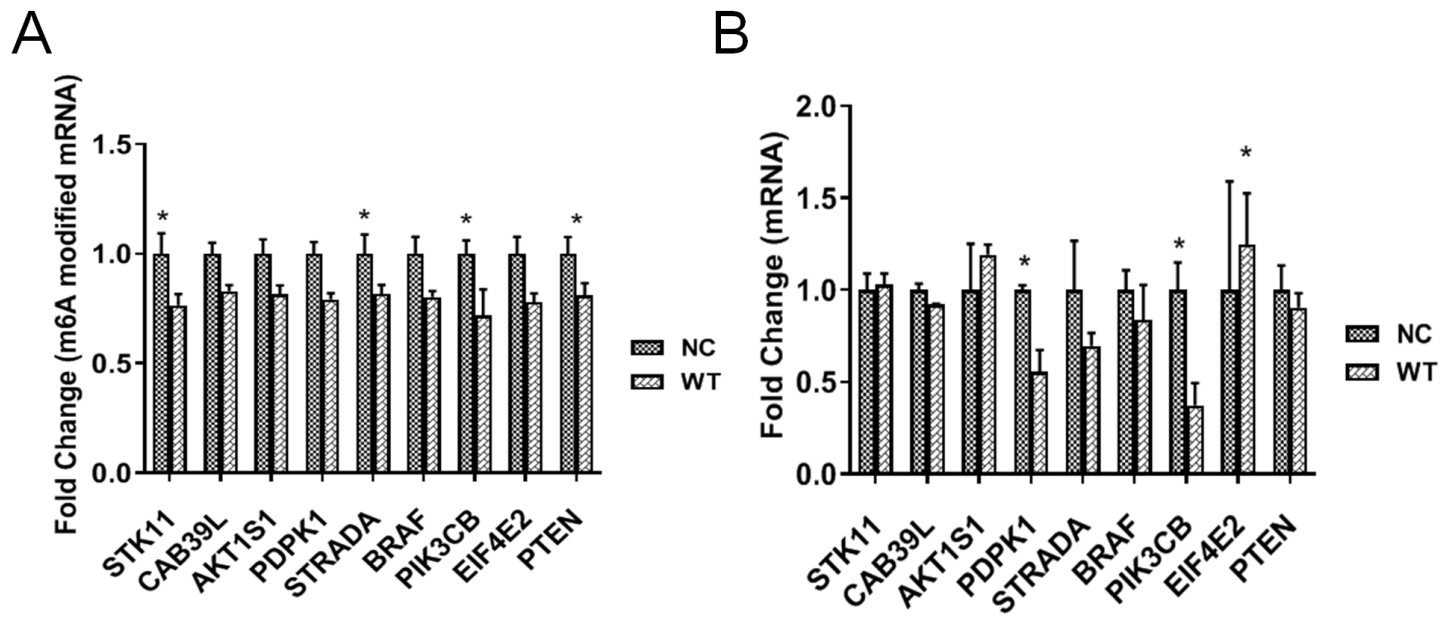


Fig.4

Figure 4

The expression of mTOR signaling pathway related genes in WT group (n=23) compared with NC group (n=23). (A) Methylated RNA immunoprecipitation was performed using a m6A antibody and was followed by qPCR for 9 specific mTOR signaling pathway-related genes. Fold change was calculated by normalizing the % (MeRIP/Input) of WT. (B) qPCR was performed to detect mRNA levels for these 9 genes. qPCR was conducted in triplicate. *P<0.05 compared with NC sample.

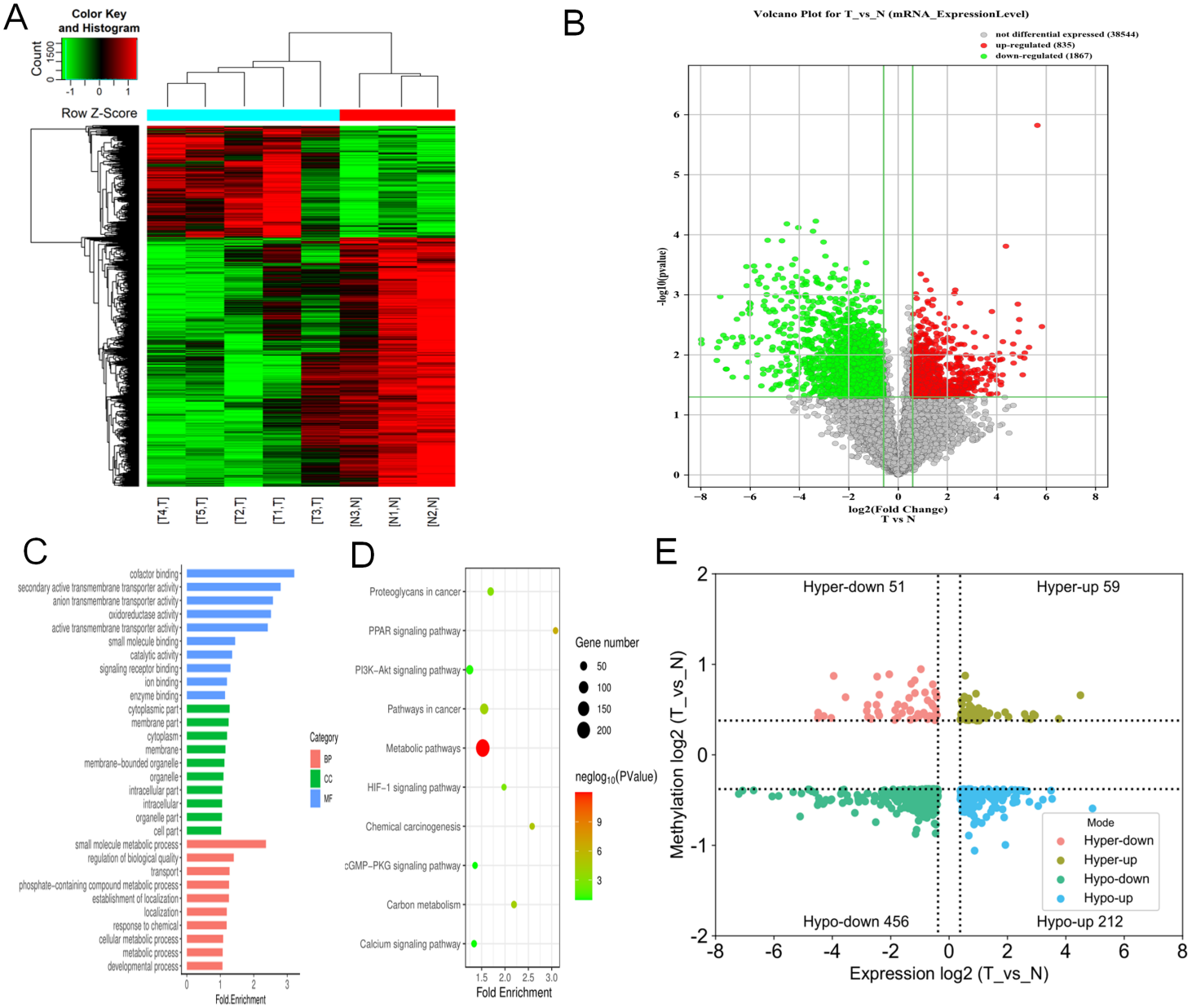


Fig.5

Figure 5

Conjoint analysis of MeRIP and RNA in WT and NC samples. (A) Microarray analysis was conducted to determin mRNA expression characteristic between WT and NC. Cluster analysis of the altered mRNAs (red: increased expression in WT, green: decreased expression in WT). n = 3 in the control group and n =5

in WT. (B) Volcano plot: upregulated (red dot) and downregulated (green dot) of WT and NC (fold change >1.5, p<0.05). Gene ontology enrichment and pathway analysis of differentially expressed mRNAs between WT and NC. (C) Significantly enriched gene ontology terms for the differently expressed genes. (D) The top 10 significantly enriched pathways are shown. (E) Conjoint analysis of the differently expressed methylated RNAs and total mRNAs. The four quadrant graph shows the distribution of transcripts with significant changes in m6A modification and corresponding mRNA expression (fold change>1.5, P<0.05 for mRNA and fold change >1.2, p<0.05 for m6A).

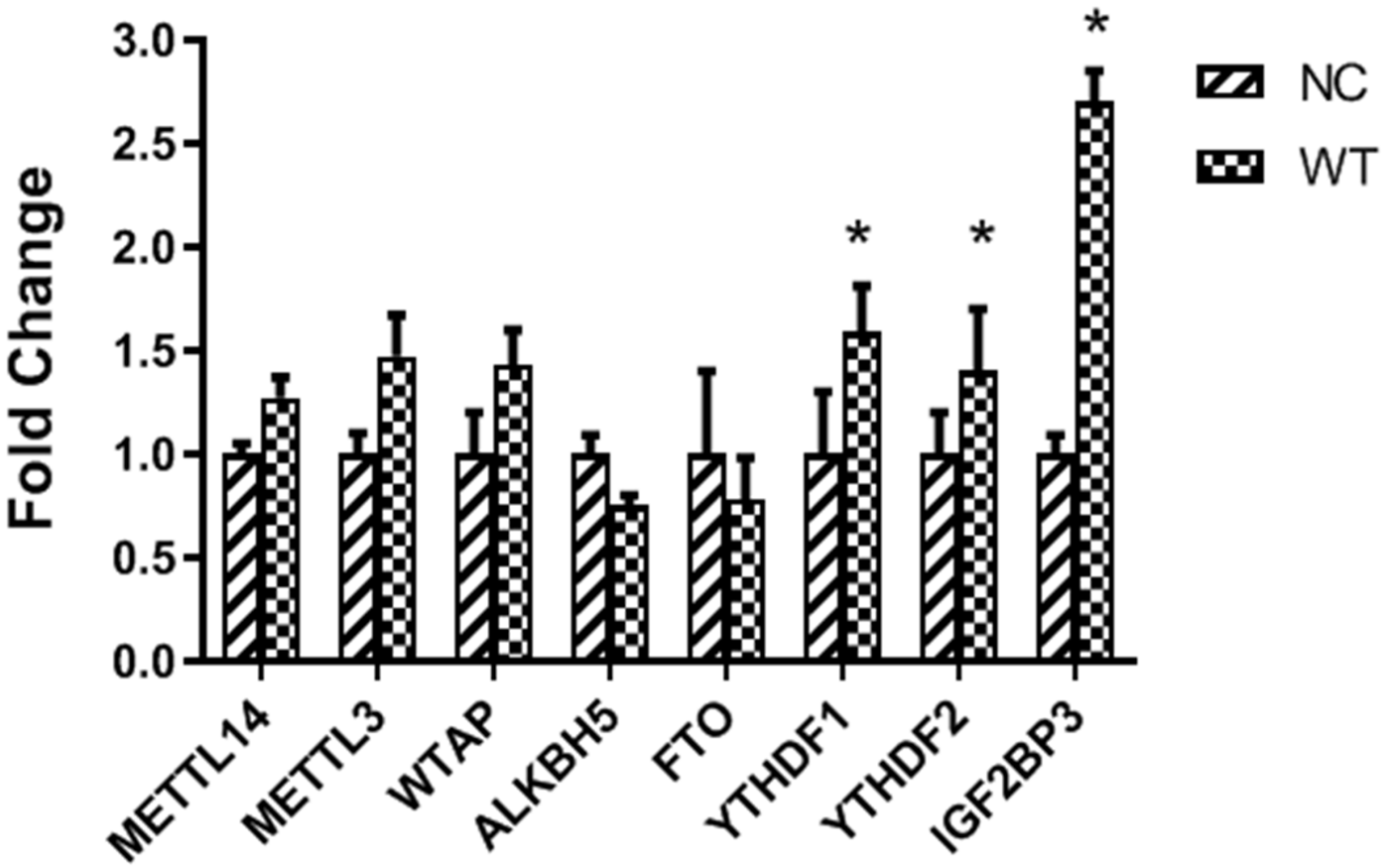


Fig.6

Figure 6

Analysis of m6A modifying enzyme levels by real time PCR in WT and NC. The expression levels of selected mRNA "writers" METTL14, METTL3, and WTAP; "erasers" ALKBH5 and FTO; and "readers" YTHDF1, YTHDF2, and IGF2BP3 was analyzed. The m6A “readers” YTHDF1, YTHDF2 and IGF2BP3 expression was statistically greater in the WT group (n=23) than in the NC group (n=23). P<0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigS1.tif
- Supplementaryinformation.docx
- TableS1.docx