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The RNA m6A methyltransferase METTL3 promotes pancreatic cancer cell proliferation and invasion

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ABSTRACT

Epigenetic modifications are involved in carcinogenesis and METTL3 is involved in RNA methylation. This study aimed to explore the role of the RNA m6A methyltransferase METTL3 in pancreatic cancer cells. The m6A modification was analyzed in human pancreatic cancer and paracancerous specimens, as well as in the normal HPDE6-C7 pancreatic cell line and the MIA-PaCa-2 and BxPC-3 pancreatic cancer cell lines. Immunohistochemistry (IHC), western blotting, and RT-qPCR were used to detect the expression of METTL3. Cell lines were transfected with siRNAs against METLL3. Proliferation, invasion, and migration were examined. The functions of METTL3 were predicted by bioinformatics analysis. In the 40 patients included, high METTL3 expression was associated with high pathological stage ($P = 0.02$) and high N stage ($P = 0.02$). Survival was better in patients with low METTL3 expression compared with those with high MTTL3 expression ($P < 0.01$). METTL3 and CIITA expression levels were inversely correlated ($r = -0.71$, $P < 0.01$). RNA m6A content in tumor specimens was significantly higher than that in paracancerous specimens. METTL3 protein and mRNA levels were significantly higher in tumor specimens compared with paracancerous specimens, as well as in cancerous cell lines vs. normal cells. METTL3 knockdown in MIA PaCa-2 and BxPC-3 cells decreased RNA m6A modifications. Cell proliferation, invasion, and migration were decreased by METTL3 knockdown in cancerous cell lines. A total of 673 differentially expressed genes were identified by bioinformatics: 659 were upregulated and 14 were downregulated. In conclusion, METTL3 is probably involved in pancreatic carcinogenesis. It could eventually be a prognostic marker or a treatment target.

1. Introduction

Pancreatic cancer is one of the most aggressive cancers and is associated with poor outcomes [1]. Pancreatic cancer exhibits rapid progression with late onset of symptoms, and it is often diagnosed at an advanced stage [2,3]. Only 15%–20% of patients are diagnosed with resectable pancreatic cancer, and most patients are diagnosed with unresectable locally advanced or metastatic disease, and have a poor prognosis [4]. Pancreatic cancer is highly invasive and metastatic and is highly resistant to all forms of existing therapies [5]. Pancreatic cancer is characterized by its insidious symptoms, low rate of surgical resection, high risks of local invasion, metastasis, and recurrence, and overall dismal prognosis [6]. Metastasis is an early event in the progression of pancreatic cancer and is an independent factor of poor prognosis [7]. Despite the best management (including surgery, chemotherapy, and radiation therapy), the 5-year overall survival is approximately 8% [8]. Therefore, novel therapeutic strategies to fight pancreatic cancer are urgently needed.

The N6-methyladenosine (m6A) was first reported in a study of hepatoma cells in 1974 [9], where m6A is involved in the methylation of mRNA, leading to the selective processing of specific mRNAs. Later, the understanding of RNA m6A modification was improved by the discovery of the methyltransferase complex and several m6A-binding proteins [10,11]. m6A modification plays an important role in the regulation of mRNA stability, decay, nucleus retention, and translation control [12]. The m6A modification is catalyzed by the

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methyltransferase complex, which includes WTAP, METTL3, and METTL14 [13]. The m6A methyltransferase complex catalyzes adenosine methylation at the N6 position of RNA molecules [14].

METTL3 was originally identified as a methyltransferase responsible for m6A modification [15]. METTL3 is part of the methyltransferase complex [16]. METTL3 promotes mRNA translation in human cancer cells [17]. In addition, METTL3 promotes growth, survival, and invasion of lung cancer cells [16,18]. METTL3 also promotes the translation of oncogenes such as EGFR and TAZ [17]. On the other hand, in renal cell carcinoma and glioblastoma, METTL3 inhibits tumor growth [19,20]. Therefore, METTL3 could have different roles in different cancer types. A recent study reported that METTL3 promotes chemo- and radio-resistance in pancreatic cancer cells [21]. Whether METTL3 regulates pancreatic cancer m6A modification and cell motility remains unclear.

Therefore, this study aimed to explore the role of the RNA m6A methyltransferase METTL3 in pancreatic cancer and in pancreatic cancer cell lines. The results could suggest METTL3 as a novel prognostic marker or therapeutic target for pancreatic cancer.

2. Materials and methods

2.1. Patients and specimens

Cancerous and paracancerous specimens were collected at the same time from patients with pancreatic ductal adenocarcinoma between January 2017–December 2017 at the Pancreas Center of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province, China). Patients diagnosed with distant metastasis were excluded because they are not eligible for tumor resection. The characteristics of the patients are shown in Table S3. There were five females and thirty-five males. The study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (2017-SR-171). All patients signed the informed consent form. After collection, all specimens were snap-frozen in liquid nitrogen and kept at −80 °C.

Patient follow-up was performed once a month within one year after surgery, and every 3 months thereafter. The patients were followed by outpatient visit, letters, and telephone calls. Follow-up was censored in March 2019. Overall survival time was defined as time from surgery to death or the end of follow-up.

2.2. Cell culture

The normal human pancreatic ductal cell line HPDE6-C7 was obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) and cultured as previously described [22]. Human pancreatic cancer cell lines (MIA PaCa-2 and BxPC-3) were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Those two cell lines were selected because our previous studies already showed that the levels of m6A modification were high in those cell lines [23]. BxPC-3 cells were cultured in RPMI 1640 (GIBCO, Invitrogen Inc., Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (GIBCO, Invitrogen Inc., Carlsbad, CA, USA) and 100 U/ml penicillin and 100 U/ml streptomycin (GIBCO, Invitrogen Inc., Carlsbad, CA, USA). MIAPaCa-2 cells were cultured in DMEM (GIBCO, Invitrogen Inc., Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (GIBCO, Invitrogen Inc., Carlsbad, CA, USA), 2.5% horse serum (GIBCO, Invitrogen Inc., Carlsbad, CA, USA), and 100 U/ml penicillin and 100 U/ml streptomycin (GIBCO, Invitrogen Inc., Carlsbad, CA, USA). All cells were incubated in humidified air with 5% CO_2 at 37 °C.

2.3. m6A detection

Total RNA m6A modification in specimens and cells were detected using the EpiQuik m6A RNA Methylation Quantification Kit (EpiQuik, USA), according to the manufacturer's instruction. Briefly, total RNA is bound to wells using the RNA high binding solution. m6A is detected using capture and detection antibodies. The detected signal is enhanced and then quantified by reading the 450-nm absorbance using a microplate spectrophotometer (Bio-Tek, Winooski, VT, USA). The amount of m6A is proportional to the OD intensity.

2.4. Real-time PCR (RT-qPCR)

Total RNA was extracted from tissues and cultured cells with TRIzol (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Total RNA was reverse transcribed using a reverse transcription kit (Takara, Otsu, Japan), according to the manufacturer's protocol. The SYBR Green Master Mix Kit (Takara, Otsu, Japan) was used for qRT-PCR. β-actin was used to normalize the RNA levels using the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers for RT-qPCR were: METTL3-F: 5′-CAA CAT ACC CGT ACT ACA GGA-3′; METTL3-R: 5′-TTC ATC TAC CCG TTC ATA CCC-3′; METTL14-F: 5′-TAC AGA GAA ACT GGC ATC ACT-3′; METTL14-R: 5′-CAA CCC CTC CCC AGA ACC A-3′; WTAP-F: 5′-AAG TTT ACG CCT GAT AGC CAA-3′; WTAP-R: 5′-AAG TTG TGC AAT ACG TCC CT-3′; FTO-F: 5′-AGT CAC GAA TTG CCC GAA C -3′; FTO-R: 5′-AGG CAT CGA AGC ATC ATC C-3′; ALKBH5-F: 5′-ATC TTC GAG CGC CCC ATC GT-3′; ALKBH5-R: 5′-TTC CGA CAC CCG AAT AGG CTT G-3′; β-actin-F: 5′-GTG GCC GAG GAC TTT GAT TG-3′; and β-actin-R: 5′−CCT GTA ACA ACG CAT CTC ATA TT-3′.

2.5. Cell transfection

METTL3 siRNAs were purchased from GenePharma (Shanghai, China). The siRNAs were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The expression of METTL3 was confirmed by RT-qPCR or western blot. The sequences of the siRNAs for knocking down METTL3 were: #1: 5′-GCU CAA CAU ACC CGU ACU ATT-3′; #2: 5′-GGU UGG UGU CAA AGG AAA UTT-3′; and #3: 5′−CCU GCA AGU AUG UUC ACU ATT-3′.

2.6. Western blot

Cells were lysed in RIPA lysis buffer (KeyGEN, Nanjing, China). After measuring the protein concentration using a BCA kit (Pierce antibodies, Thermo Fisher Scientific, Waltham, MA, USA), equal amounts of protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and consequently transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk in TBST buffer containing 0.1% Tween-20 and incubated with the indicated antibody at 4 °C overnight. Appropriate secondary antibodies (ab6721, Abcam, Cambridge, MA, USA) were incubated at 37 °C for 1 h, followed by detection with enhanced chemiluminescence. The primary antibodies were METTL3 (ab195352, Abcam, Cambridge, MA, USA) and β-actin (ab8227, Abcam, Cambridge, MA, USA).

2.7. Immunohistochemistry

Pancreatic ductal adenocarcinoma and paracancerous specimens were deparaffinized in xylene and rehydrated in a series of graded alcohol. Endogenous peroxidase was blocked using 3% H_2O_2. Antigen retrieval was completed after heating in citrate buffer. The sections were incubated with the primary antibody against METTL3 (dilution 1:50, ab195352, Abcam, Cambridge, MA, USA) at 4 °C overnight. Horseradish peroxidase-conjugated secondary antibody was added and incubated at room temperature for 30 min, and 3,3′-diaminobenzitine (DAB) solution was used for color reaction (Dako, Glostrup, Denmark).

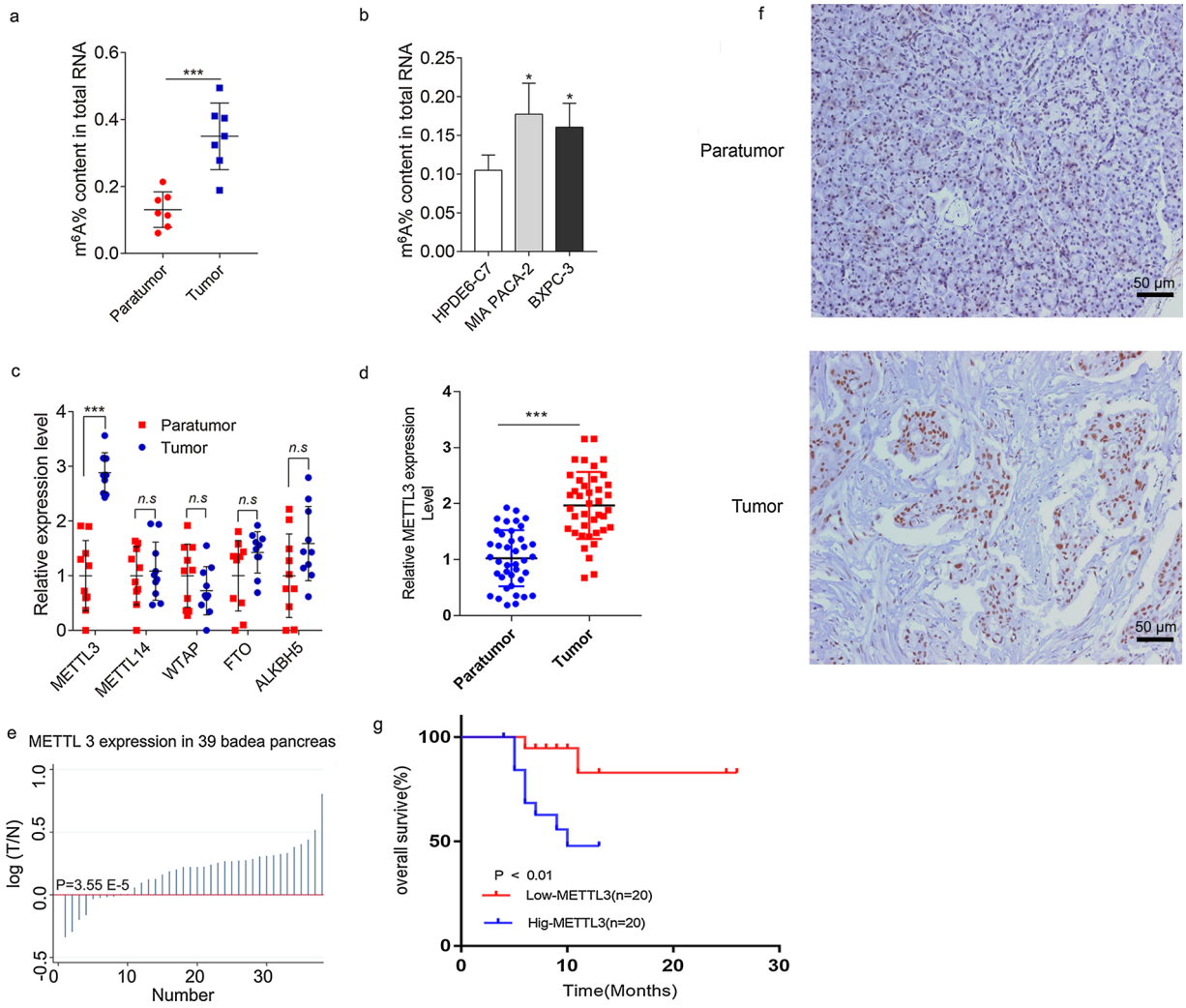

Fig. 1. RNA m6A modification and METTL3 are increased in pancreatic cancer tissues and cells. (a) Relative RNA m6A modification was higher in pancreatic cancer tissues. Seven pancreatic cancer tissue and adjacent normal tissue were subjected to RNA m6A measurement. (b) Relative RNA m6A modification was higher in pancreatic cells. Normal pancreatic cell HPDE6-C7 and cancer cells MIA PaCa-2 and BxPC-2 were subjected to RNA m6A detection. (c) m6A modifiers were examined in pancreatic cancer tissues. Ten paired pancreatic cancer tissue and adjacent normal tissue were subjected to RT-qPCR to detect the mRNA expression levels of METTL3, METTL14, WTAP, FTO, and ALKBH5. (d) METTL3 was upregulated in pancreatic cancer tissues. Forty paired pancreatic cancer tissue and adjacent normal tissue were subjected to RT-qPCR to detect the expression of METTL3. (e) Oncomine pancreatic cancer database showed that 31/39 pancreatic cancer patients exhibited increased METTL3. (f) Immunohistochemistry was used to examine the METTL3 expression in pancreatic cancer tissue (magnification 200 ×). METTL3 protein was upregulated in pancreatic cancer tissues. (g) Patient survival according to low/high METTL3 expression. For A, C and D, paired Student's *t*-test, *** $P < 0.001$; for B, unpaired Student's *t*-test,* $P < 0.05$.

2.8. Cell proliferation assay

Cell Count Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies (Tokyo, Japan) and used to examine cell proliferation, according to the manufacturer's instructions. Briefly, 48 h after transient siRNA transfection, MIA-PACA2 and Bxpc-3 cells were seeded at 1×10^3/well in 96-well plates. Each day, three wells per cell line were selected for the CCK-8 assay using 10 μL of CCK-8 solution incubated for 4 h, followed by measurement of the 450-nm OD using a microplate reader.

2.9. Migration and invasion assays

The cell invasion ability was examined using Matrigel-coated Transwell inserts (Corning Inc., Corning, NY, USA) containing polycarbonate 8-μm pores filters. Briefly, the chambers were first coated with 1:9 diluted Matrigel (Sigma, St Louis, MO, USA) for 1 h. Then, 2×10^4 MIA PaCa-2 and 3×10^4 BxPC-3 cells were seeded in the upper chambers with 200 μl of 30 μg/ml mitomycin C pure medium, while 500 μl of medium containing 10% fetal bovine serum was placed in the lower chambers as a chemo-attractant. After incubation for 48 h at 37 °C with 5% CO_2, non-invading cells were removed, and migrated or invading cells were fixed and counted under a microscope (Olympus, Tokyo, Japan) using five different fields. The invasion assay was similar to the invasion assay except that the transwell insert was not coated with Matrigel. The invasion assay used 3×10^4 MIA PaCa-2 cells and 4×10^4 BxPC-3 cells.

2.10. Bioinformatics

RNA libraries were constructed using rRNA-depleted RNAs and the TruSeq Stranded Total RNA Library Prep Kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. Libraries were controlled for quality and quantified using the BioAnalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). Libraries were reverse transcribed into single-strand DNA molecules, captured on

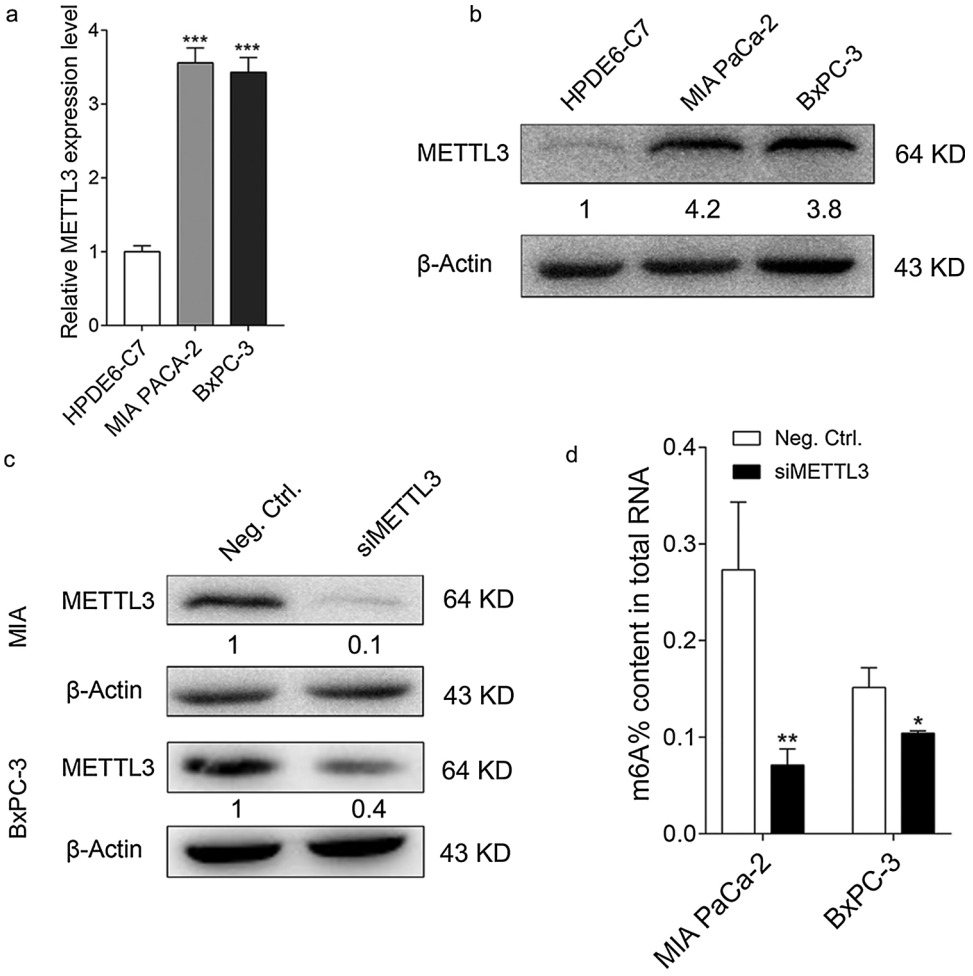

Fig. 2. METTL3 regulates RNA m6A modification in pancreatic cancer cells. (a) METTL3 RNA levels were upregulated in pancreatic cancer cells. HPDE6-C7, MIA PaCa-2, and BxPC-3 cells were subjected to RT-qPCR to detect the METTL3 RNA levels. β-Actin was used as internal control. (b) METTL3 protein levels were upregulated in pancreatic cancer cells. HPDE6-C7, MIA PaCa-2. and BxPC-3 cells were subjected to western blotting to detect the METTL3 protein levels. (c) METTL3 was interfered successfully in pancreatic cancer cells. MIA PaCa-2 and BxPC-3 cells were transfected with METTL3 siRNAs for 48 h and then western blotting was used to examine the efficiency of METTL3 knockdown. (d) m6A was decreased in METTL3 knockdown cells. Cells treated in C were subjected to m6A detection. For A and D, unpaired Student's *t*-test,* $P < 0.05$,** $P < 0.01$,*** $P < 0.001$.

Illumina flow cells, amplified in situ as clusters, and finally sequenced for 150 cycles on an Illumina HiSeq Sequencer, according to the manufacturer's instructions. Raw data were generated after sequencing, image analysis, base calling, and quality filtering on an Illumina HiSeq 4000 sequencer. Q30 was used to perform quality control. After adaptor trimming and low-quality reads removing using the cutadapt (v 1.9.2) software, high quality reads were generated. These reads were aligned to the reference genome (UCSC hg19) guided by the Ensembl GFF gene annotation file (v 70) with hisat2 software (v 2.04). The cuffdiff software (part of cufflinks, v 2.2.1) was used to get the gene level FPKM as the expression profiles of mRNA, and fold changes and P values were calculated using FPKM to identify differentially expressed mRNA. GO and KEGG Pathway enrichment analyses were performed based on the differentially expressed mRNAs.

2.11. Statistical analysis

Data are presented as mean ± SD, and were analyzed using the Student *t*-test or ANOVA with the SNK post hoc test, as appropriate. Categorical data are presented as frequencies and were analyzed using the Fisher's exact-test. Correlations were examined using the Pearson coefficient. Survival was analyzed using the Kaplan-Meier method and the log-rank test. Statistical analysis was performed using SPSS 21 for Windows (IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. m6A and METTL3 are enriched in pancreatic cancer

To assess the RNA m6A levels between pancreatic tumor and adjacent paracancerous tissues, specimens from seven patients (Table S1) were subjected to RT-qPCR analysis. The results indicated that the RNA m6A content in tumor tissues was significantly higher than in paracancerous tissues (Fig. 1A). Next, we examined the m6A content in normal pancreatic ductal epithelial cells (HPDE6-C7) and pancreatic cancer cell lines (BxPC-3 and MIAPACA2). The m6A levels in the pancreatic cancer cells were significantly higher than in the normal pancreatic ductal epithelial cells (Fig. 1B). Taken together, these results indicate that the RNA m6A modification is higher in pancreatic cancer tissues and cells than in normal tissues and cells.

To study the regulators associated with high RNA m6A modification in pancreatic cancer tissues, the transcriptional levels of some m6A modifiers were examined in the specimens 10 patients (Table S2). METTL3 levels were significantly higher in tumor specimens compared with paired paracancerous specimens (Fig. 1C). The increase in the METTL3 levels was confirmed using 40 patients (Table S3 and Fig. 1D). High METTL3 expression was associated with high pathological stage ($P = 0.016$) (Table S3) and high N stage ($P = 0.017$) (Table S3). We searched the METTL3 profile in the oncomine pancreatic cancer database (https://www.oncomine.org/) and the results revealed that 79% (31/39) of pancreatic cancer patients exhibit an increased METTL3 expression (Fig. 1E). In addition, we observed the overexpression of METTL3 in pancreatic cancer tissues by immunohistochemistry (Fig. 1F). Survival was better in patients with low METTL3 expression

compared with those with high MTTL3 expression ($P < 0.01$) (Fig. 1G); the median survival time in the high-METTL3 group was 10 months, but the median survival was not reached in the low-METTL3 group. Taken together, these results indicate that the expression of METTL3 is higher in pancreatic cancer tissues than in normal tissues.

3.2. METTL3 increases the m6A modification in pancreatic cells

Consistent with the abnormal expression in pancreatic tissues, METTL3 mRNA levels were higher in pancreatic cancer cells (MIA PaCa-2 and BxPC-3) compared with normal pancreatic cells (HPDE6-C7) by using RT-qPCR (Fig. 2A). Similarly, the upregulated METTL3 protein levels were observed in the different cell lines by western blotting (Fig. 2B). We used siRNAs to knockdown METTL3 in MIA PaCa-2 and BxPC-3 cells (Fig. 2C). Subsequently, the total RNA m6A modifications were decreased significantly in MIA PaCa-2 and BxPC-3 cells (Fig. 2D), indicating that METTL3 was responsible for RNA m6A modifications in the pancreatic cancer cells.

3.3. METTL3 positively regulates pancreatic cancer cells proliferation and motility

We observed decreased cell proliferation in METTL3 knockdown cells (Fig. 3A). METTL3-mediated cell motility was not investigated because pancreatic cancer has a high metastatic potential. We used the transwell migration assay and Matrigel invasion assay to monitor the effect of METTL3 on cell invasiveness. Cell invasiveness was significantly inhibited by METTL3 knockdown (Fig. 3B and C). These findings indicate that METTL3 is involved in the metastatic potential of pancreatic cancer.

3.4. Bioinformatics for potential targets of METTL3

Kosuke et al. [21] reported the potential targets of METTL3 by microarray analysis. To expand the METTL3 regulation networks, RNA sequencing was applied in METTL3 knockdown MIA PaCa-2 cells. A total of 673 differentially expressed genes were identified, of which 659 were upregulated and 14 were downregulated. GO and KEGG pathway analyses were applied to illuminate the functional and pathway enrichment of the differentially expressed genes. The upregulated and downregulated genes were associated with cellular response to epinephrine stimulus, neutrophil degranulation, neutrophil-mediated immunity, and potassium ion import (Fig. 4A), partially consistent with the microarray derived METTL3 GO analysis [21]. The METTL3 regulated genes were also associated with cellular component (Fig. 4B) and molecular function (Fig. 4C). In addition, the KEGG pathway analysis revealed that the upregulated and downregulated genes were associated with dysregulation in cancer (Fig. 4D).

To verify the RNA sequencing results, we selected five upregulated and five downregulated genes for RT-qPCR confirmation. As shown in Fig. 5, six of the ten genes were consistent with the RNA sequencing results, suggesting that various assays are necessary before getting an accurate conclusion.

3.5. Correlations

METTL3 expression was higher in tumor tissues than in paracancerous tissues (Fig. 6A), while CIITA expression was lower in tumor

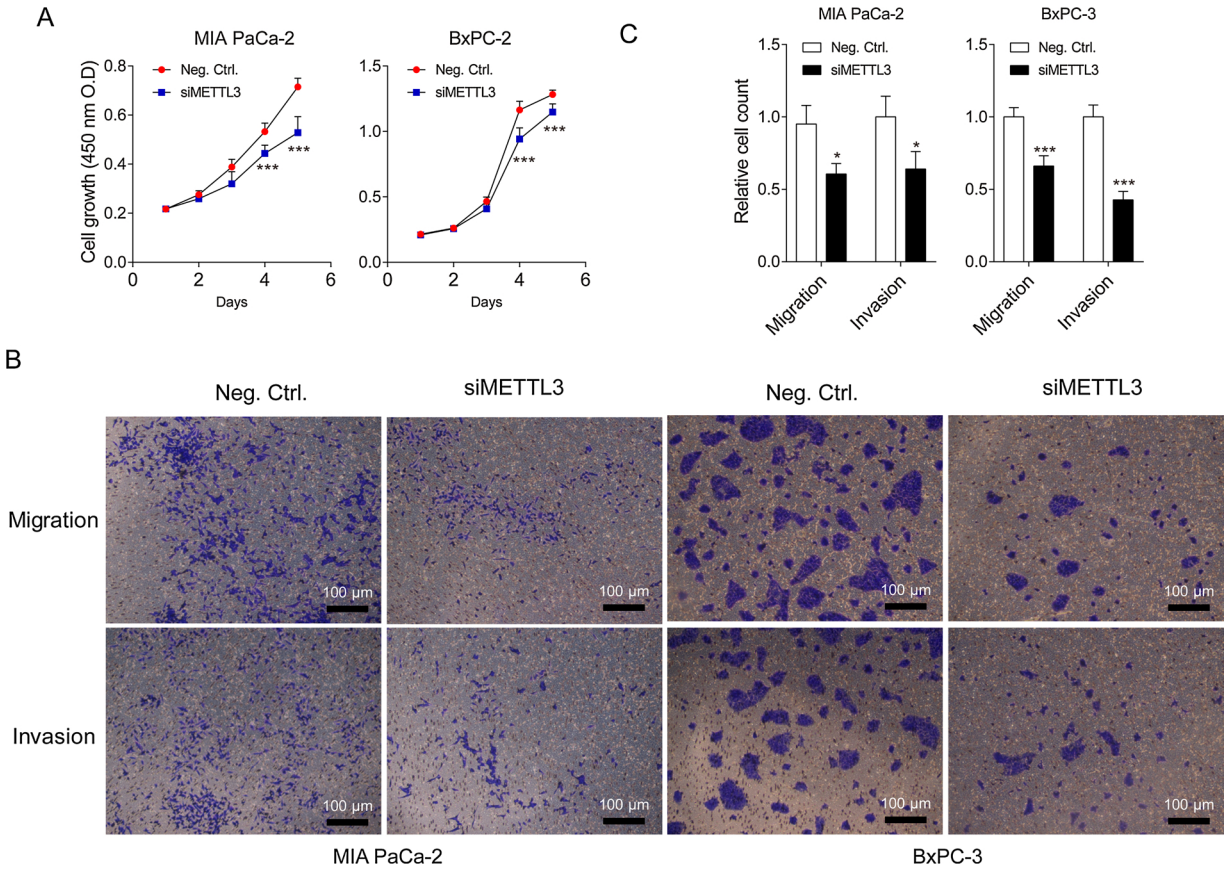

Fig. 3. Cell proliferation and migration were inhibited when METTL3 was knocked down. (a) Pancreatic cell proliferation was inhibited when METTL3 was knocked down. CCK-8 assay was used to monitor cell proliferation after METTL3 knockdown in MIA PaCa-2 (left) and BxPC-3 cells. (b) Pancreatic cell migration was inhibited when METTL3 was knocked down. Migration (upper) and invasion (lower) assays were performed to detect cell motility after METTL3 was knocked down in MIA PaCa-2 and BxPC-3 cells. (c) The statistic result for B, unpaired Student's *t*-test,* $P < 0.05$,*** $P < 0.001$.

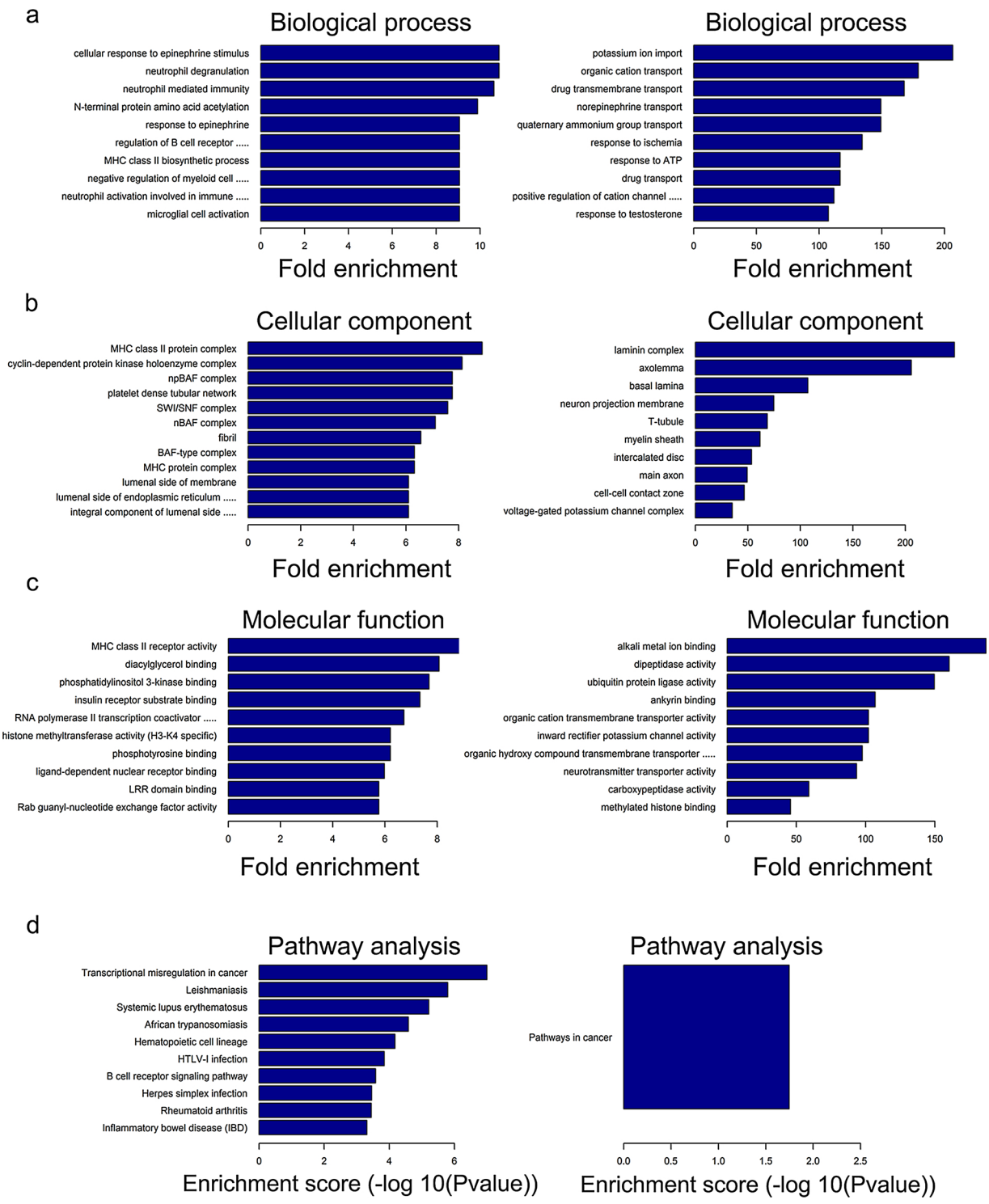

Fig. 4. The GO and KEGG pathway analyses for METTL3 regulated genes. (a) The GO biological process analysis revealed that METTL3 upregulated genes are associated with cellular response to epinephrine stimulus (left) and downregulated genes are associated with potassium ion import (right). (b) GO cellular component analysis revealed that METTL3 upregulated genes are associated with MHC II protein complex (left) and downregulated genes are associated with laminin complex (right). (c) GO molecular function analysis revealed that METTL3 upregulated genes are associated with MHC class II receptor activity (left) and downregulated genes are associated with alkali metal ion binding (right). (d) KEGG pathway analysis revealed that METTL3 upregulated genes are associated with transcriptional misregulation in cancer (left) and downregulated genes are associated with pathways in cancer (right).

tissues than in paracancerous tissues (Fig. 6B). The Pearson analysis showed that METTL3 and CIITA relative expressions were inversely correlated ($r = -0.71$, $P < 0.01$) (Fig. 6C).

4. Discussion

Epigenetic modifications such as RNA modifications are involved in carcinogenesis. Among them, m6A is an important factor involved in mRNA translation regulation [12]. This study aimed to explore the role

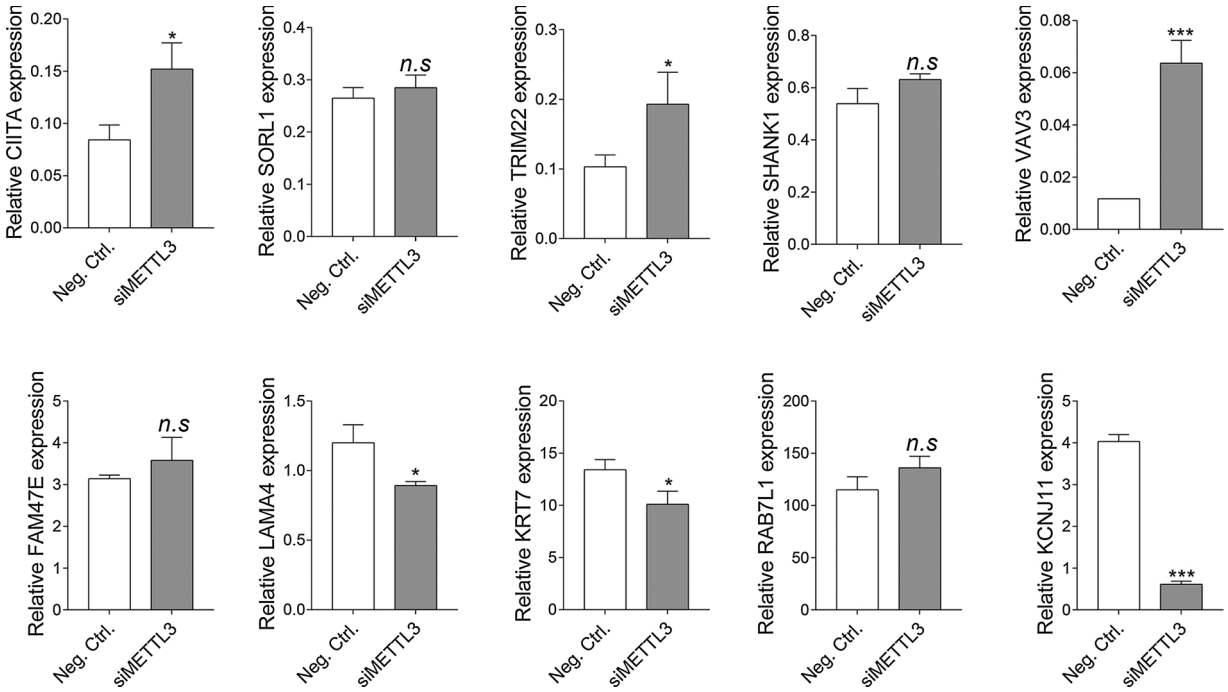

Fig. 5. Six of ten genes were consistent with the METTL3 RNA sequencing results. Ten genes were examined in METTL3 KD MIA PaCa-2 cells by RT-qPCR. β-actin was used as internal control. Only CIITA, TRIM22, VAV3, LAMA4, KRT7, and KCNJ11 exhibited consistency with the RNA sequencing results. Unpaired Student's *t*-test,* $P < 0.05$,*** $P < 0.001$.

of METTL3, an RNA m6A methyltransferase, in pancreatic cancer cells. In the present study, we found that the RNA m6A modification was higher in pancreatic cancer specimens compared with paracancerous normal specimens. These results imply that the m6A modification may participate in pancreatic oncogenesis. Subsequently, we examined the methyltransferase and demethylase in the cancerous and paracancerous tissues. Indeed, METTL3 is reported to promote translation of several oncogenes through translation in lung cancer cells, implying the importance of METTL3 in cancer development [17]. METTL3 promotes cell growth, survival, and invasion of lung and gastric cancer [16,18,24]. On the other hand, in renal cell carcinoma and glioblastoma, METTL3 inhibits tumor growth [19, 20]. Therefore, METTL3 could have different roles in different cancer types. In pancreatic cancer cells, METTL3 has been shown to promote chemo- and radio-resistance [21]. Nevertheless, whether METTL3 regulates pancreatic cancer m6A modification and cell migration remains unclear. In the present study, the results suggest that METTL3 is involved in pancreatic carcinogenesis, including proliferation and metastatic potential of pancreatic cancer cells. Therefore, MTTL3 could eventually be a prognostic marker or a treatment target. CIITA is an element for the recognition of cells by Th cells and it is usually not expressed in solid tumors as it is involved in the immune recognition of cancer cells [25]. The present study showed that MTTL3 and CIITA were inversely correlated, supporting the role of MTTL3 in pancreatic cancer.

Previous study reported that morphology and proliferation were unaffected after METTL3-knockdown in pancreatic cancer cells, but METTL3-depleted cells showed higher sensitivity to anticancer reagents such as gemcitabine, 5-fluorouracil, cisplatin, and irradiation [21]. In the present study, susceptibility to treatment was not examined, but the results showed that cell invasiveness was significantly inhibited by METTL3 knockdown, in contradiction to the previous study [21]. This discrepancy could be due to the cell lines or the proliferation assay.

Mechanistically, METTL3 has been reported to enhance the expression of HBXIP through m6A modification and promote the malignant growth of breast cancer [23]. METTL3 promotes the protein expression of a variety of genes involved in cancer cell invasiveness [17,26]. METTL3 also promotes the translation of oncogenes such as EGFR, TAZ, and WTAP [17, 27]. In ovarian cancer, METTL3 has been

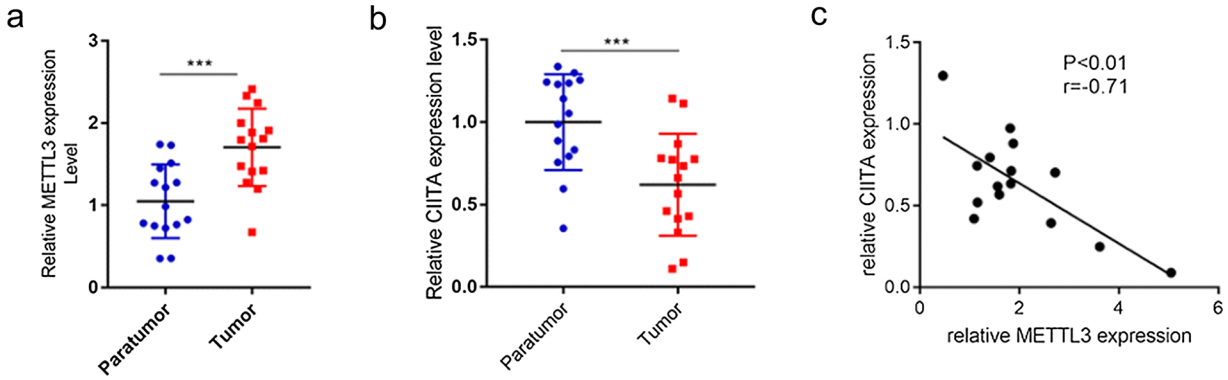

Fig. 6. METTL3 and CIITa expressions in tumor and paracancerous tissues. (a) METTL3 expression was higher in tumor tissues than in paracancerous tissues. (b) CIITA expression was lower in tumor tissues than in paracancerous tissues. (c) METTL3 and CIITA relative expressions were inversely correlated (Pearson coefficient; $r = -0.71$, $P < 0.01$).

shown to promote the epithelial-to-mesenchymal transition (EMT), and cells that underwent the EMT gain invasiveness characteristics [28]. m6A methylation has been shown to promote endometrial cancer through Akt [28]. In the present study, the targets of METTL3 were not identified, but the GO and KEGG analyses showed that METTL3 was involved in pathways such as cellular response to epinephrine stimulus, neutrophil degranulation, neutrophil mediated immunity, potassium ion import, cellular component, molecular function, and cancer dysfunction. Although METTL3 was reported to promote chemo- and radioresistance of pancreatic cancer cells as well as morphologic and proliferative changes [28], the exact mechanisms on whether METTL3 regulates metastasis remain largely elusive. Additional studies are necessary to identify the exact targets of METTL3 as well as the exact mechanisms involved in increased proliferation and migration on pancreatic cancer cells in response to high levels of METTL3.

We showed for the first time that RNA m6A modification was dysregulated in pancreatic cancer and that METTL3 is involved in pancreatic cancer pathogenesis. Our data is in agreement with other study showing that METTL3 promotes the progression of other types of cancer [26, 29]. These findings unveiled that METTL3 could be a potential novel hallmark in pancreatic cancer metastasis, which may lead to novel prognostic markers and new drugs.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152666.

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