METTL3 Promotes Pancreatic Tumor Progression Through Regulating the miR-196a/CPEB3 Axis

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Abstract

Background: Methyltransferase-like 3(METTL3)-mediated N6-methyladenosine (m6A) modification has been reported to regulate microRNAs maturation. Here, the study was designed to investigate the regulatory effect of m6A-dependent miRNA maturation on pancreatic cancer progression which is still limited before.

Results: We found that METTL3 significantly upregulated in the pancreatic tumor tissues. Overexpression of METTL3 promoted cancer cell proliferation and migration in vitro and tumor progression in vivo. METTL3-mediated m6A modification facilitated miR-196a maturation in pancreatic cancer cells, and miR-196a increased the proliferation and migration of cancer cells in vitro. Luciferase reporter assay verified that cytoplasmic polyadenylation element binding protein 3 (CPEB3) was a direct target gene of miR-196a. In vivo studies proved that overexpression of miR-196a inhibited the anti-tumor effect of knockdown of METTL3, and overexpression of CPEB3 inhibited the miR-196a-enhanced tumor progression.

Conclusions: We identified that METTL3 was upregulated in pancreatic cancer, leading to the upregulation of miR-196a, resulting in the downregulation of CPEB3, which promoted the pancreatic tumor progression. We first demonstrated that CPEB3 was a tumor suppressor gene in pancreatic cancer, and the METTL3 regulated miR-196a/CPEB3 axis may be a therapeutic target for pancreatic cancer therapy.

Background

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant cancers in the world, with almost 95% fatality rate of the PDAC patients [1]. The clinical outcome of pancreatic cancer is poor, because of the non-specific symptom, lack of early diagnosis and effective treatment, and its aggressive tumor biology [2, 3].Although numerous research focused on the molecular pathogenesis of pancreatic cancer development and progression [4, 5], the specific mechanism of pancreatic progression remains unclear. More studies still need to be done to complement the mechanism and contribute to the new therapeutic targets to improve the survival rate of pancreatic cancer patients. N6-methyladenosine (m6A) RNA methylation is one of the most abundant RNA modifications [6, 7]. M6A modification plays critical role in tumor progression [8]. In breast cancer, reduced m6A levels may promote tumorigenesis and predict poor prognosis [9]. In bladder cancer, m6A modification of ITGA6 mRNA promoted tumor progression [10]. Methyltransferase-like 3 (METTL3) is a methyltransferase which is identified to promote m6A modification [11]. In pancreatic cancer cells, METTL3 promoted pancreatic cancer cell proliferation and invasion through upregulation of m6A levels [12].

MicroRNAs (miRNAs) are a class of 19–22 nucleotides endogenous, small non-coding RNAs which could downregulate gene expression post-transcriptionally by targeting the 3’UTR of mRNA [13]. MiRNAs were found aberrantly expressed in cancer, and participated in various cancer development and progression

[14]. Biogenesis of miRNAs including the process of primary miRNA (pri-miRNA) into the ~ 70 to ~ 120 nucleotide-long precursor RNA (pre-miRNA), and further process into the ~ 18 to ~ 23 nucleotide-long miRNA [13]. It was reported that METTL3 could enhance miRNA maturation through m6A modification, which acted as a key post-transcriptional modification that promoted the initiation of miRNA biogenesis [15]. However, the mechanism of METTL3 regulated pancreatic cancer progression through promoting miRNA maturation has not been fully illustrated. Here, we identified that METTL3 was upregulated in pancreatic cancer, leading to the upregulation of miR-196a, resulting in the downregulation of CPEB3, which promoted the pancreatic tumor progression.

Results

METTL3 is aberrantly overexpressed in pancreatic tumors.

To explore the role of METTL3 in pancreatic cancer, we first examined the expression level of METTL3 in pancreatic tumors. We detected 36 tumor tissues and normal para cancer tissues and found that METTL3 was significantly upregulated in the tumor tissues, as indicated by qRT-PCR (Fig. 1a) and western blot (Fig. 1b and c). Moreover, High expression of METTL3 was correlated with lymph node invasion, and the diameter of tumors (Table 1), indicating that METTL3 may possess a regulatory role in the progression of pancreatic tumors.

Table 1
General clinicopathological characteristics of patients.

Characteristics	METTL3 expression level		χ^2	*P*-value
	High (n = 18)	Low (n = 18)		
Age (years)			2.786	0.095
≤ 50	7	12		
> 50	11	6		
Gender			1.870	0.171
Male	13	9		
Female	5	9		
Diameter of tumor (cm)			5.461	0.019*
≤ 2	6	13		
> 2	12	5		
Pathological grading			0.468	0.494
I/II	10	12		
III/IV	8	6		
Lymphatic metastasis			8.000	0.005*
Negative	8	16		
Positive	10	2		
Distant metastasis			2.215	0.137
Negative	15	11		
Positive	3	7		
TNM stage			0.554	0.457
I/II	14	12		
III/IV	4	6		

Knockdown of METTL3 inhibits pancreatic cancer cell proliferation in vitro and in vivo.

We then investigated the role of METTL3 in pancreatic cancer progression by overexpressing or knocking down the gene expression. The efficacy of the overexpression or knockdown of METTL3 in PANC-1 and BXPC-3 cells was verified by western blot (Fig. 2a). We found that overexpression of METTL3 significantly promoted cell proliferation (Fig. 2b) and clone formation (Fig. 2c), while knockdown of

METTL3 inhibited cell proliferation (Fig. 2b) and clone formation (Fig. 2c). Pancreatic cancer cells overexpressed of METTL3 showed increasing migration ability, which was decreased by knockdown of METTL3 (Fig. 2d). Epithelial to mesenchymal transition (EMT), in which cancer cells lose epithelial characteristics and gain a mesenchymal phenotype, plays a crucial role in the process of the initiation of metastasis for tumor cells, and predicts poor prognosis of pancreatic cancer [16]. We then detected the EMT-related protein expression by using western blot. We found that overexpression of METTL3 decreased the E-Cadherin expression and increased the expression of Vimentin and Snail, indicating the increasing level of epithelial-mesenchymal transition of the cells, while knockdown of METTL3 showed the opposite effects (Fig. 2e and f). Moreover, in vivo studies showed that PANC-1 cells overexpressed with METTL3 showed more tumor progression in mice, and knockdown of METTL3 reduced the tumor size (Fig. 2g and h). All these data suggested that METTL3 promoted the pancreatic tumor progression.

METTL3 facilitates miR-196a expression in pancreatic cancer cells.

As m6A mark acts as a key post-transcriptional modification that promotes the initiation of miRNA biogenesis [15], we wondered if METTL3 regulated the pancreatic tumor cell proliferation through regulating the maturation of miRNAs. We then analyzed the aberrantly miRNAs expression in pancreatic cancer through the public data sets (GSE41372) (Fig. 3a and b). We found five most upregulated miRNAs (miR-145, miR-27a, miR-199a-5p, miR-150, and miR-196a) in the datasets, and verified the expressions of the miRNAs by using qRT-PCR. As shown in Fig. 3c, we found that miR-27a and miR-196a were significantly upregulated in tumor tissues, and miR-150 showed a slight upregulation in tumor tissues. We then performed the methylation RIP (MeRIP) assay with the anti-m6A antibody followed by qRT-PCR. We found that overexpression of METTL3 in PANC-1 cells resulted in obvious m6A enrichment of pri-miR-196a, indicating that METTL3 may promote miR-196a biogenesis through m6A modification (Fig. 3d). We then detected the pri-miR-196a, pre-miR-196a, and miR-196a expression in cells overexpressed or knocked down of METTL3. As shown in Fig. 3e, cells overexpressed with METTL3 showed decreased pri-miR-196a level, and METTL3 knockdown cells showed increased pri-miR-196a level. Opposite to the results of pri-miR-196a level, pre-miR-196a and miR-196a showed enhanced expression level in METTL3 overexpression cells and decreased level in METTL3 knockdown cells. MiR-196a showed a significant reduction in pancreatic tumor tissues (Fig. 3h). All these data suggested that METTL3 could promote the maturation of miR-196a.

MiR-196a is required for pancreatic cancer cell proliferation and migration in vitro.

After founding that METTL3 inducing miR-196a upregulation in pancreatic cancer, we then explored the effect of miR-196a on cancer cell proliferation and migration. We found that miR-196a accelerates pancreatic cancer cell proliferation (Fig. 4a) and clone formation (Fig. 4b and c), and treatment of miR-196a restricted cell proliferation (Fig. 4a) and clone formation (Fig. 4b and c). Pancreatic cancer cells overexpressed of miR-196a showed increasing migration ability, which was decreased by miR-196a inhibitor (Fig. 4d and e). miR-196a decreased the E-Cadherin expression and increased the expression of Vimentin and Snail, indicating the increasing level of epithelial-mesenchymal transition of the cells, while

downregulation of miR-196a showed the opposite effects (Fig. 4f and g). All these data indicated that miR-196a is required for pancreatic cancer cell proliferation and migration in vitro.

CPEB3 is a direct target of miR-196a in pancreatic cancer.

To find the gene expression regulated by miR-196a in pancreatic cancer, we predicted the target gene of miR-196a by using TargetScan, miRDB, and miRTarbase (Fig. 5a). Among the 46 genes of the intersections of the three databases, we predicted that cytoplasmic polyadenylation element-binding protein 3 (CPEB3), which showed anti-tumor effects in various cancers [17, 18], may be the potential targets of miR-196a (Fig. 5b). The luciferase reporter assay showed that miR-196a overexpression reduced the luciferase activity of wide-type but not mutant-type CPEB3 3'-UTR, indicating the direct binding of miR-196a to CPEB3 3'-UTR (Fig. 5c). Western blot further proved that miR-196a negatively regulated CPEB3 expression in pancreatic cancer cells (Fig. 5d). In PANC-1 cells, knockdown of METTL3 upregulated the expression of CPEB3, which was abolished by treatment with miR-196a mimics (Fig. 5e). Knockdown of METTL3 inhibited the pancreatic cell proliferation, which was abrogated by treatment with miR-196a mimics (Fig. 5f). miR-196a promoted pancreatic cancer cell proliferation, which was inhibited by overexpression of CPEB3 (Fig. 5f). Besides, CPEB3 was significantly downregulated in the pancreatic tumor tissues as indicated by qRT-PCR (Fig. 5g) and immunochemical staining (Fig. 5h).

METTL3 regulates pancreatic tumor progression through regulating miR-196a/CPEB3 axis in vivo.

We then verified the regulatory effect and mechanism of METTL3 in vivo. Mice were divided into five groups: Control group (NC), METTL3 knockdown (KD) group, METTL3 knockdown + miR-196a overexpression (OE) group, miR-196a overexpression (OE) group, miR-196a overexpression + CPEB3 overexpression (OE) group, with 10 mice in each group. The PANC-1 cells were transfected with METTL3 or CPEB3 knockdown lentivirus or not, and were then subcutaneously injected to the mice. miR-196a angomirs (Ribo bio; Guangzhou, China) were administrated to mice by tail injection to overexpress the miRNAs in vivo. As similar to the results of the in vitro study, knockdown of METTL3 inhibited the tumor growth in mice, which were abrogated by overexpression of miR-196a (Fig. 6a and b). miR-196a overexpression promoted tumor growth in vivo, which were inhibited by overexpression of CPEB3 (Fig. 6a and b). Knockdown of METTL3 led to the upregulation of E-Cadherin and downregulation of Vimentin and Snail, which could be abolished by overexpression of miR-196a (Fig. 6c and d); miR-196a overexpression resulted in the downregulation of E-Cadherin and upregulation of Vimentin and Snail, which were abrogated by overexpression of CPEB3 (Fig. 6c and d). Immunochemical staining of CPEB3 in the tumors showed that CPEB3 expression showed the opposite trend with the tumor progression (Fig. 6e). All these results indicated that METTL3 regulated the pancreatic tumor progression through regulating miR-196a, and eventually regulated the CPEB3 expression.

Discussion

Pancreatic cancer is one of the most malignant cancers, which is the fourth leading cause of cancer death [19]. Although some substantial progress has been made in the pathogenesis of pancreatic cancer,

the specific molecular mechanism remains unclear. In the present studies, we identified the METTL3 promoted maturation of miR-196a, leading to aberrant upregulation of miR-196a, which was closely related to the malignancy of pancreatic cancer.

M6A modification is one of the most abundant RNA modifications which regulates various biological process through regulating RNA splicing [20], RNA stability [21], and mRNA translation efficiency [22]. The m6A-related mRNA signature predicts the prognosis of pancreatic cancer patients [23]. METTL3 is one of the primary subunits of the methyltransferase complex which catalyzing RNA m6A modification [24]. METTL3 was reported to related to various cancer progression, such as gastric cancer [25], hepatocellular carcinoma [26], and breast cancer [27]. In pancreatic cancer cells, METTL3 promotes pancreatic cancer cell proliferation and invasion [12]. In the present studies, we detected the METTL3 expression in pancreatic tumor tissues by using western blot and qRT-PCR. We found that METTL3 was significantly upregulated in pancreatic tumors. We then investigated its effect on tumor progression in vitro and in vivo. We found that METTL3 contributed to the malignancy of pancreatic tumors, as indicated by which increased the pancreatic cancer cell proliferation, migration, and EMT in vitro, and promoted tumor growth in vivo.

METTL3-mediated m6A mark acts as a key post-transcriptional modification that promotes the initiation of miRNA biogenesis [15]. In bladder cancer, METTL3 promoted tumor proliferation of tumor cells through facilitating miR-221/222 maturation [28]. In colorectal cancer, METTL3 promoted metastasis of tumor cells through miR-1246/MAPK signaling pathway [29]. We then analyzed the aberrant expressions of miRNAs in pancreatic cancer. According to the GEO database, we chose 5 miRNAs which change most in the pancreatic tumors. qPCR results showed that miR-27a, miR-150, and miR-196a significantly upregulated in the pancreatic cancer, and pri-miR-196a m6A enrichment was significantly increased in METTL3 overexpression cells as indicated by MeRIP assay, suggesting that METTL3-mediated m6A modification may regulate miR-196a expression. We found that overexpression of METTL3 decreased the level of pri-miR-196a, and increased the level of pre-miR-196a, and miR-196a. All these data suggesting that miR-196a expression was regulated by METTL3-mediated m6A modification.

MiR-196a was reported to function as the oncogenic factors in various cancer, including colorectal, breast cancer, and head and neck cancer [30–32]. Zhang. et al reported that cigarette smoke could stimulate excessive miR-25-3p maturation to promote tumor progression in pancreatic cancer [33]. We found that miR-196a promoted the pancreatic cancer proliferation, migration, and epithelial-mesenchymal transition. We further predicted that CPEB3 may be the potential target of miR-196a, which was then verified by luciferase reporter assay and western blot. CPEB3 has been newly acknowledged as a tumor-suppressive gene in several cancers, such as colorectal cancer, ovarian cancer, glioma, and hepatocellular carcinoma [17, 18, 34, 35]. We then verified the mechanism in which METTL3 regulated miR-196a/CPEB3 to regulate the pancreatic cancer progression in vitro and in vivo. We found that knockdown of METTL3 inhibited the pancreatic cell proliferation and tumor growth, which were abrogated by treatment with miR-196a mimics. miR-196a promoted pancreatic cancer cell proliferation and tumor growth, which were inhibited

by overexpression of CPEB3. Besides, CPEB3 was significantly downregulated in the pancreatic tumor tissues as indicated by qRT-PCR and immunochemical staining.

Conclusion

In summary, we found that METTL3 was upregulated in pancreatic cancer, leading to the upregulation of miR-196a, resulting in the downregulation of CPEB3, which promoted the pancreatic tumor progression. We first demonstrated that CPEB3 was a tumor suppressor gene in pancreatic cancer, and the METTL3 regulated miR-196a/CPEB3 axis may be a therapeutic target for pancreatic cancer therapy.

Materials And Methods

Patients and tissue samples

Tumor tissues and para cancer normal tissues were collected patients with non-metastasis pancreatic ductal adenocarcinoma between February 2017–November 2018 at the the Second Affiliated Hospital of Nanjing Medical University. The study was approved by the ethics committee of the the Second Affiliated Hospital of Nanjing Medical University. All patients were informed of the content of the studies and signed the informed consent form. The tissues were snap-frozen in liquid nitrogen immediately after removed in the surgery and kept at − 80 °C.

Cell culture and treatment

The human pancreatic cancer cell lines BxPC-3 and PANC-1 were obtained from Cell Bank of Chinese Academy of Sciences.and maintained in RPMI-1640 culture medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Thermo, USA). For in vitro transfection, 50 nM of mimic control, miR-196a mimic, or inhibitor (Ribo bio, Guangzhou, China) were transfected into cells using Lipofectamine 2000 (Invitrogen, USA) following with the manufacturer's instructions. METTL3 overexpression (METTL3 OE) and knockdown (METTL3 KD) were achieved by transfection with related lentivirus (Genepharma, Shanghai, China) into the cells.

RNA sequencing analysis

Pancreatic cancer gene expression data were downloaded from the Gene Expression Omnibus (GEO) dataset GSE41372. The data analysis was performed with R software using the DEGseq package. The threshold set for significant differences was $\log2|\text{fold change}| \geq 1$ and P-value < 0.05.

qRT-PCR analysis

Total RNA from tissues or cells was extracted using the TRIzol reagent (Invitrogen, CA, USA). Extracted RNA was reversely transcribed to cDNA using a PrimeScript RT reagent kit with gDNA Eraser (Takara, Tokyo, Japan), and real-time PCR was performed using SYBR Premix Ex TaqII (Takara), with U6 and GAPDH served as an inner control. The PCR condition was as follows: pre-denaturation at 95 °C for 15 min, followed by 40 cycles of 20 s of denaturation at 95 °C, and annealing at 55 °C for 30 s and an

extension at 70 °C for 30 s. The fluorescence signals were collected at 72–95 °C after reaction for melting curve analysis. The results were analyzed using a hyperbolic curve and the relative gene expression was determined. The primers are shown in Supplementary Table 1.

MeRIP-qPCR

Total RNAs were subjected to fragment, then incubated with protein A beads previously bound anti-m6A polyclonal antibody (Synaptic Systems) or IgG (isotype control) in RIP buffer at 4 °C for 3 h. Next, samples were washed, followed by purification with Qiagen and subjected to qRT-PCR.

Cell proliferation

Cell proliferation was analyzed by Cell Count Kit-8 (CCK-8) cell viability assay and clone formation assay. CCK-8 kit (Beyotime, Shanghai, China) was used to examine cell proliferation according to the manufacturer's instructions. Briefly, 48 h after transfection with miRNA mimics or inhibitors or lentivirus, Bxpc-3 cells were seeded in 96-well plates at the density of 1×10^3/well. 10 μL of CCK-8 solution was used to incubate the cells for 4 h every 12 hours, followed by measurement of the 450-nm OD using a microplate reader. For clone formation assay, the cells were cultured in a 6 cm culture dish at a density of 5×10^3/well for 14 d. The cells were stained with 0.5% crystal violet for 15 min. 10 random fields of view were observed under a light microscope (Olympus, Tokyo, Japan), and the number of colonies was counted.

Transwell assay

The cells were plated in the upper chambers of Transwell inserts (Corning, NY, USA) containing polycarbonate 8-μm pores filters. 500 μl of medium containing 10% fetal bovine serum was placed in the lower chambers. After incubation for 48 h at 37 °C with 5% CO2, the cells in the lower chambers were fixed and counted under a microscope (Olympus, Tokyo, Japan) using five different fields.

Western blot

The tissues or cells were collected to extract proteins using the RIPA lysis buffer (Beyotime, Shanghai, China) with protease inhibitors (Roche, China). The total proteins were measured by the BCA assay kit (Thermo, USA), and then equal protein (30 μg) were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After transferred onto the polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA, USA), the membranes were blocked with 5% skim milk for 2 h at room temperature. Then the membranes were incubated with primary antibodies against E-Cadherin (1:1000, Abcam), Vitmentin (1:1000, Abcam), Snail (1:1000, Abcam) at 4℃ overnight, followed by incubation with secondary antibody (1:5000, Abcam) for 2 h. Finally, the blots were detected by using a chemiluminescent detection system (Bio-rad, USA). GADPH (1:5000, Abcam) was served as inner control.

Dual-luciferase reporter assay

The sequences of 3'UTR of CPEB3 mRNA were chemically synthesized and introduced into the luciferase reporter vector to constructed the wild type (WT) luciferase reporter plasmids, and the seed regions of

miR-196a in the 3’-UTR of CPEB3 was mutated to constructed mutant (Mut) luciferase reporter plasmids. The co-transfections with luciferase reporter plasmids and miRNA mimics or inhibitors to PANC-1 cells were performed after reaching 70% confluence by using Lipofectamine 3000 (Life Technologies, USA) according to the manufacturer’s instruction. 48 hours later, the cells were collected and dual-luciferase activity was measured using the Dual-Luciferase Reporter Assay (Promega, Shanghai, China) according to the manufacturer's instructions and normalized to Renilla signals.

Mouse-xenograft experiments

Six-week-old male BALB/c nude mice purchased from Model Animal Research Center of Nanjing University (Nanjing, China) were used to establish the transplantation model of human pancreatic cancer. The mice were divided into 5 groups. The PANC-1 cells were stably knocked down of METTL3 or not. Then the cells were collected (1×10^7) and resuspended in phosphate-buffered saline (PBS) (100–200 μL) and were then subcutaneously injected to the mice. miR-196a angomirs (Ribo bio; Guangzhou, China) were administrated to mice by tail injection to overexpress the miRNA in vivo according to the manufacturer’s instruction. The mice were examined the tumor size every 2 days. The mice were sacrificed at the 28th day after the injection and the tumor weight was measured. The tumor tissues were fixed with 10% PFA and treated with immunochemical staining.

Immunochemical staining

Slices were treated with 3% hydrogen peroxide for 10 min at room temperature. After blocking the sections by using 1% bovine serum albumin (Gibco; Thermo Fisher Scientific, Inc.) at room temperature for 4 h, the sections were incubated with primary anti-CPEB3 antibody (1:100) at 4 ℃ overnight. Then the sections were washed with PBS for three times to remove the primary antibodies, and were incubated with biotinylated secondary antibody (Bioworld, USA) for 2 h, and 3,3N-Diaminobenzidine Tertrahydrochloride buffer (Beyotime, Shanghai, China) was used to perform the color reaction. The slices were observed under the microscope (Olympus, Tokyo, Japan).

Statistical Analysis

All values are presented as the mean ± SEM of 3 independent experiments. Statistical analysis was performed by using one-way ANOVA followed by Bonferroni test for selected pairs with GraphPad Prism 8 statistical software. $p < 0.05$ was considered statistically significant.

Abbreviations

METTL3: Methyltransferase-like 3; m6A: N6-methyladenosine; CPEB3: cytoplasmic polyadenylation element binding protein 3; PDAC: Pancreatic ductal adenocarcinoma; miRNAs: microRNAs; pri-miRNA: primary miRNA; pre-miRNA: precursor RNA; MeRIP: methylation RIP; CCK-8: Cell Count Kit-8.

Declarations

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Authors' contributions

PPG and DF performed the experiments, analyzed the data, summarized the results and edited the manuscript; LH collected and analyzed the data; QW and JS edited the manuscript; LJX and GWX conceived and designed the experiments, analyzed the data and provided the supervision and comments on the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Tissue specimens were acquired after patient consent and the study was approved by the ethics committee of the the Second Affiliated Hospital of Nanjing Medical University. All experiments were performed in accordance with the ethics code for animal experimentation. The experimental procedures were approved by the ethical guidelines of Nanjing Medical University laboratory Animal Care and Use committee.

Consent for publication

This manuscript is approved by all authors for publication.

Competing interests

All the authors have declared no competing financial interests.

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Figures

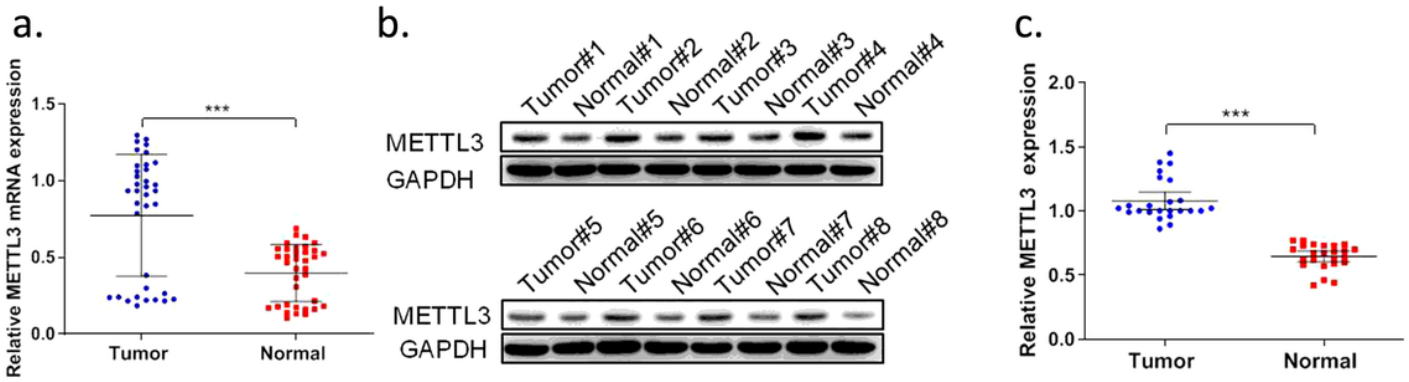
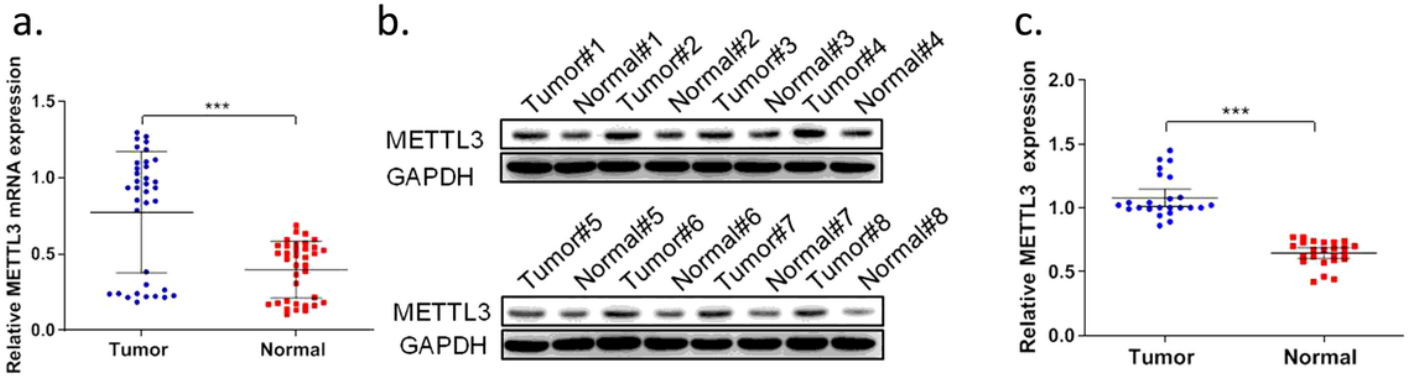


Figure 1

METTL3 is aberrantly overexpressed in pancreatic tumors. a METTL3 mRNA expression in pancreatic tumor tissues and para-tumor tissues. b METTL3 protein expression in pancreatic tumor tissues and para-tumor tissues. c Relative protein expressions were analyzed by using ImageJ. Data are presented as mean ± SEM. ###p<0.001, compared to the control group; *p<0.05, **p<0.01, ***p<0.001, compared to the borneol group. n=6 per group.



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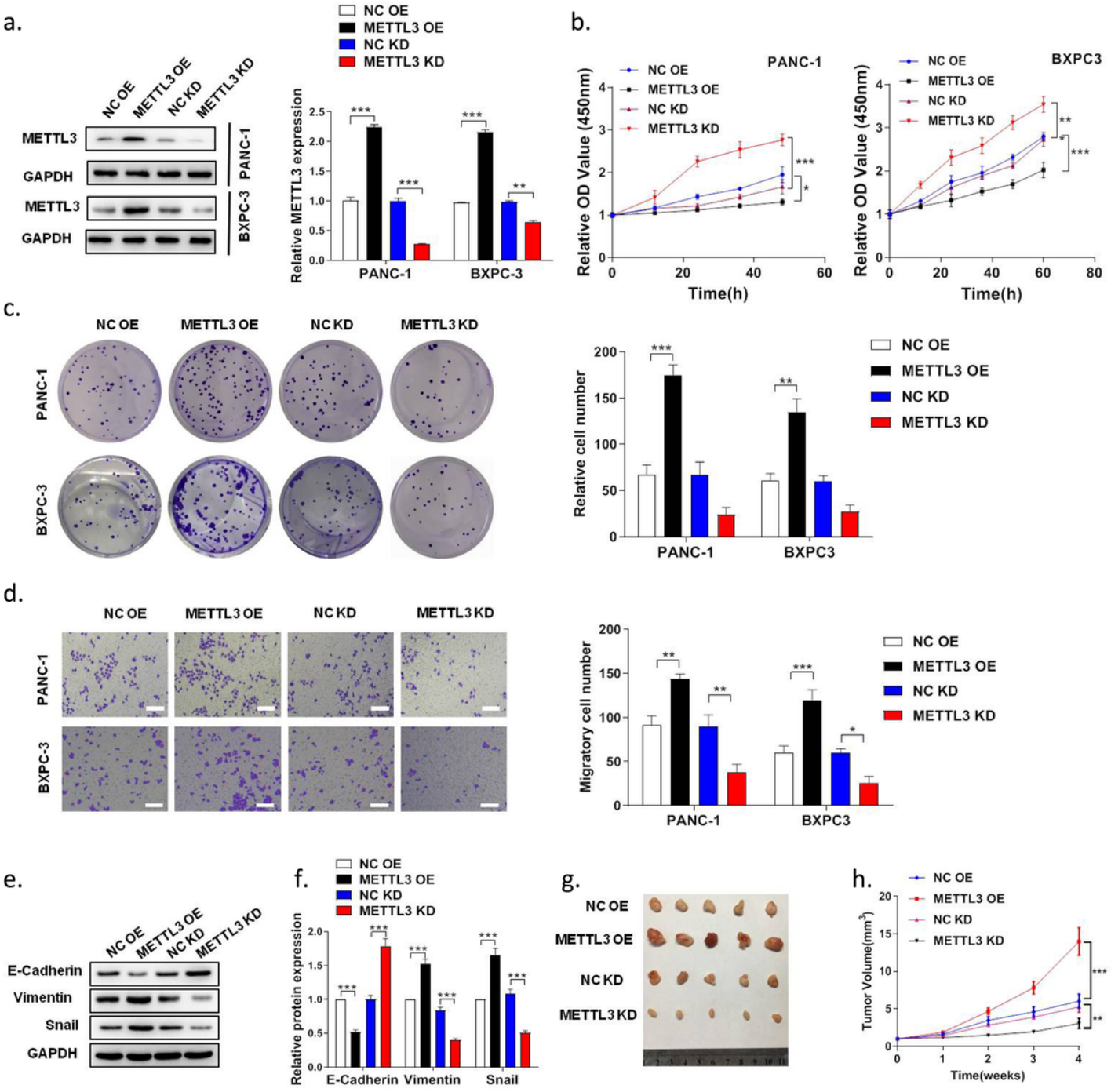

Figure 2

Knockdown of METTL3 inhibits pancreatic cancer cells proliferation in vitro and in vivo.METTL3 was overexpressed or knocked down in PANC-1 and BXPC-3 cells. a The transfection efficacy was verified by western blot. b Cell proliferation was increased by METTL3 overexpression and inhibited by METTL3

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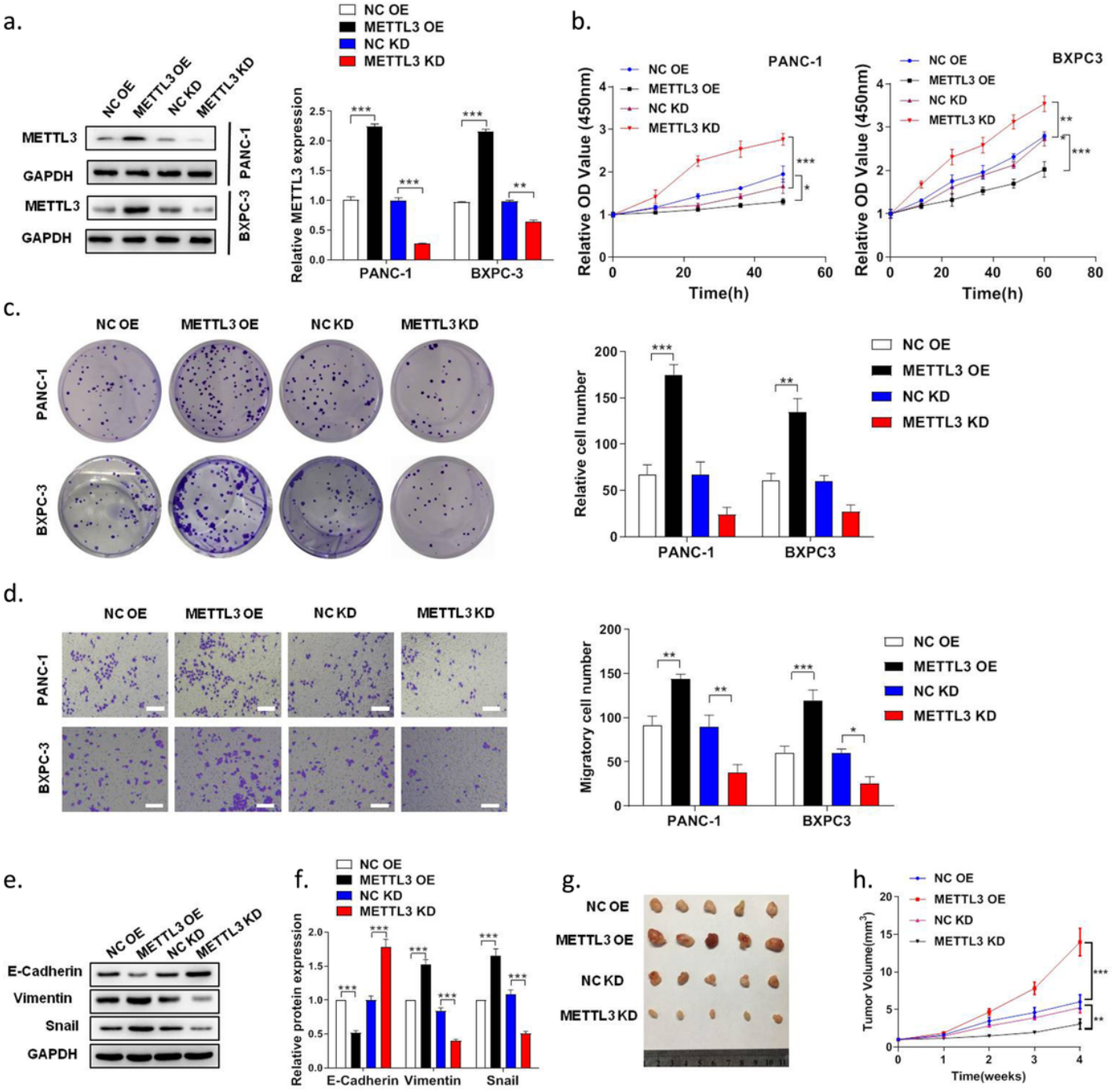

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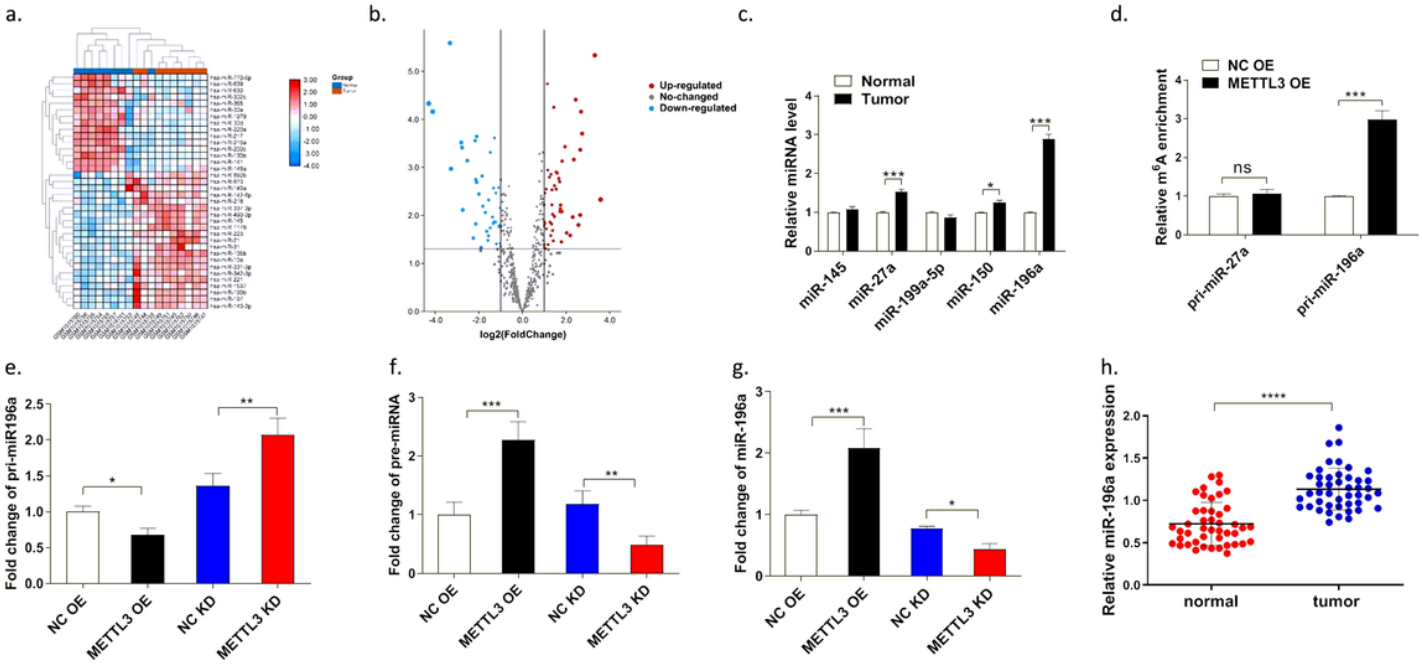
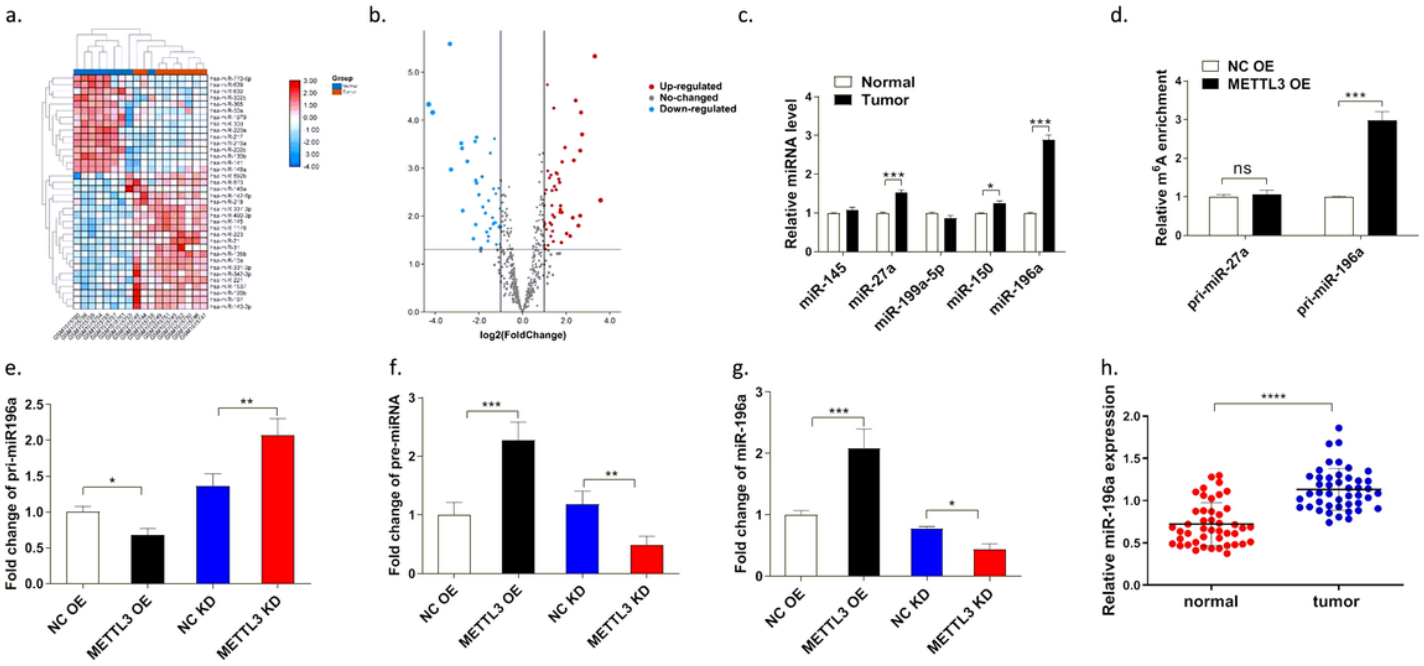


Figure 3

METTL3 facilitates miR-196a expression in pancreatic cancer cells. a Heatmap of significantly different expression of microRNAs analyzed by using GEO datasets (GSE41372). The red (higher expression) or blue (lower expression) color represents the normalized expression value of indicated microRNAs. b Volcano plots showing the expression profiles of miRNAs. c The most aberrantly expressed miRNAs in the datasets were verified by qRT-PCR. d m6A enrichment of pri-miR-27a and pri-miR-196a was detected using immunoprecipitation, followed by qRT-PCR analysis, in PANC-1 cells overexpressed with METTL3 or not. e Relative pri-miR-196a expression, f relative pre-miR-196a expression, and g relative miR-196a expression in PANC-1 cells overexpressed or knocked down with METTL3. h Relative miR-196a expression in pancreatic tumor tissues. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, compared to the relative NC group.



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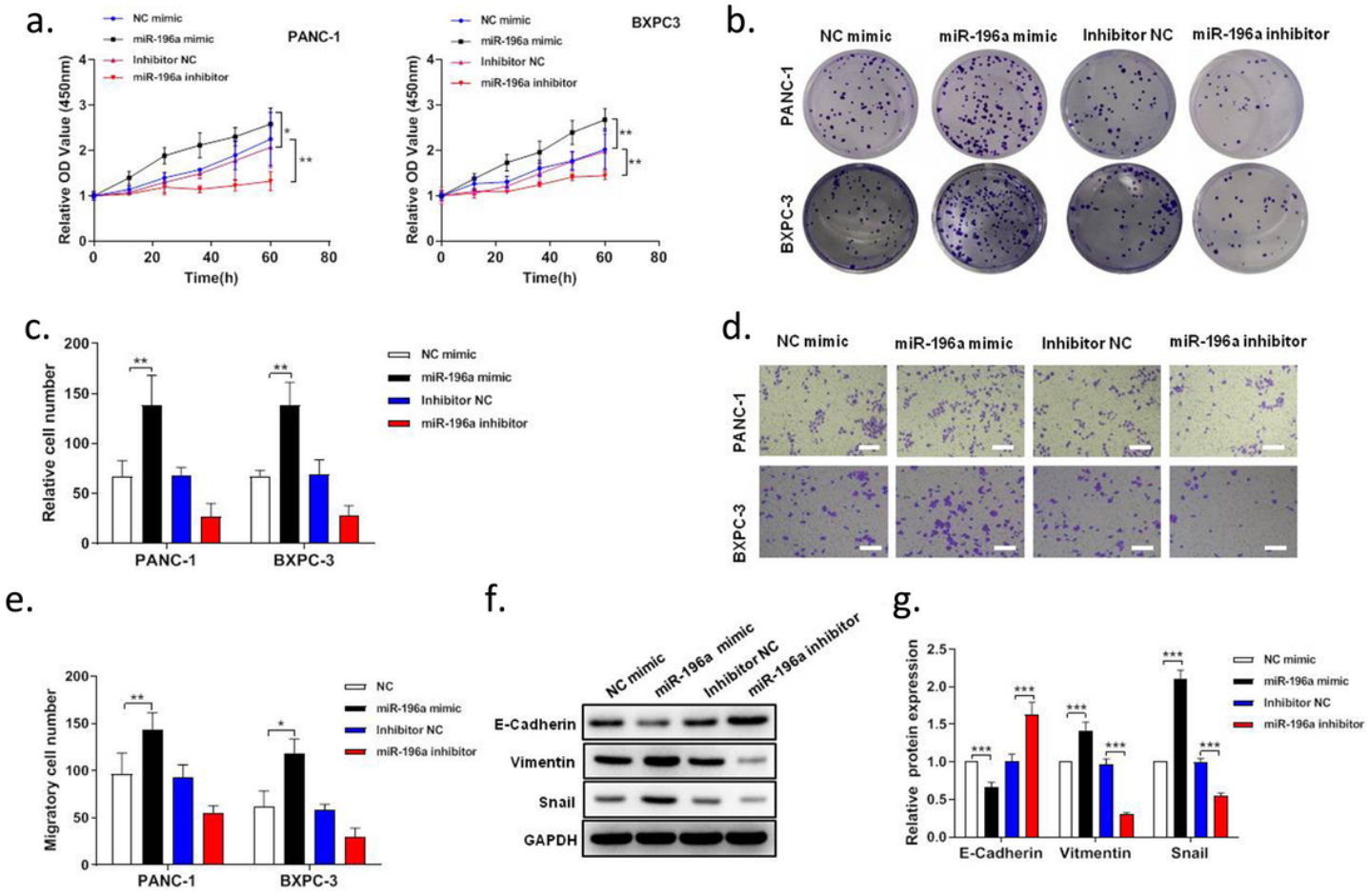
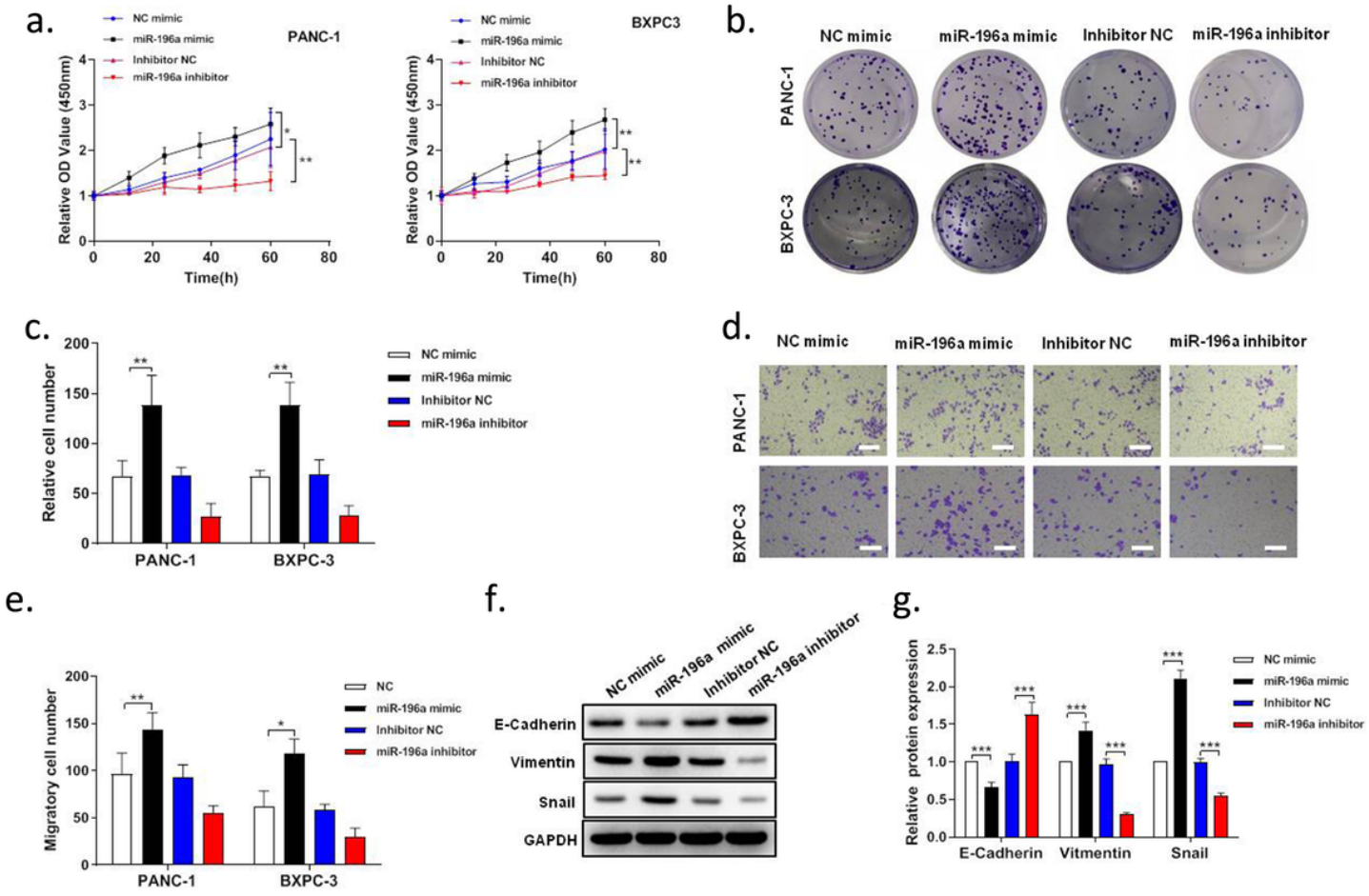


Figure 4

MiR-196a is required for pancreatic cancer cells proliferation and migration in vitro. MiR-196a was overexpressed or knocked down in PANC-1 and BXPC-3 cells. a Cell proliferation and b clone formation was increased by METTL3 overexpression and inhibited by METTL3 knockdown. c The clone number was counted. d Transwell assay showed that cell migration was increased by METTL3 overexpression and inhibited by METTL3 knockdown. e The migrated cells were counted. f, g EMT-related E-Cadherin, Vimentin, and Snail and GAPDH protein expression were detected by western blot. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, compared to the relative NC group.



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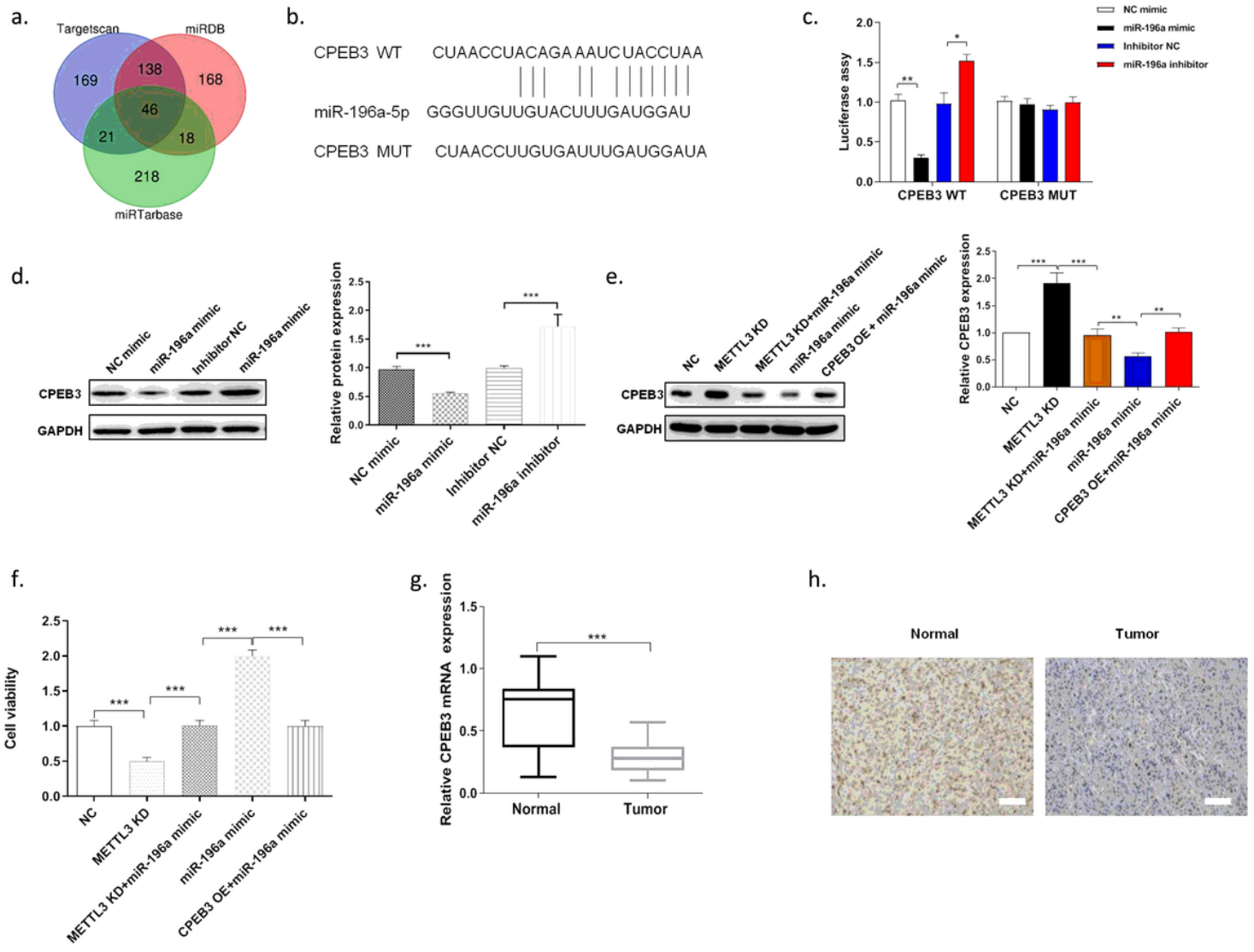
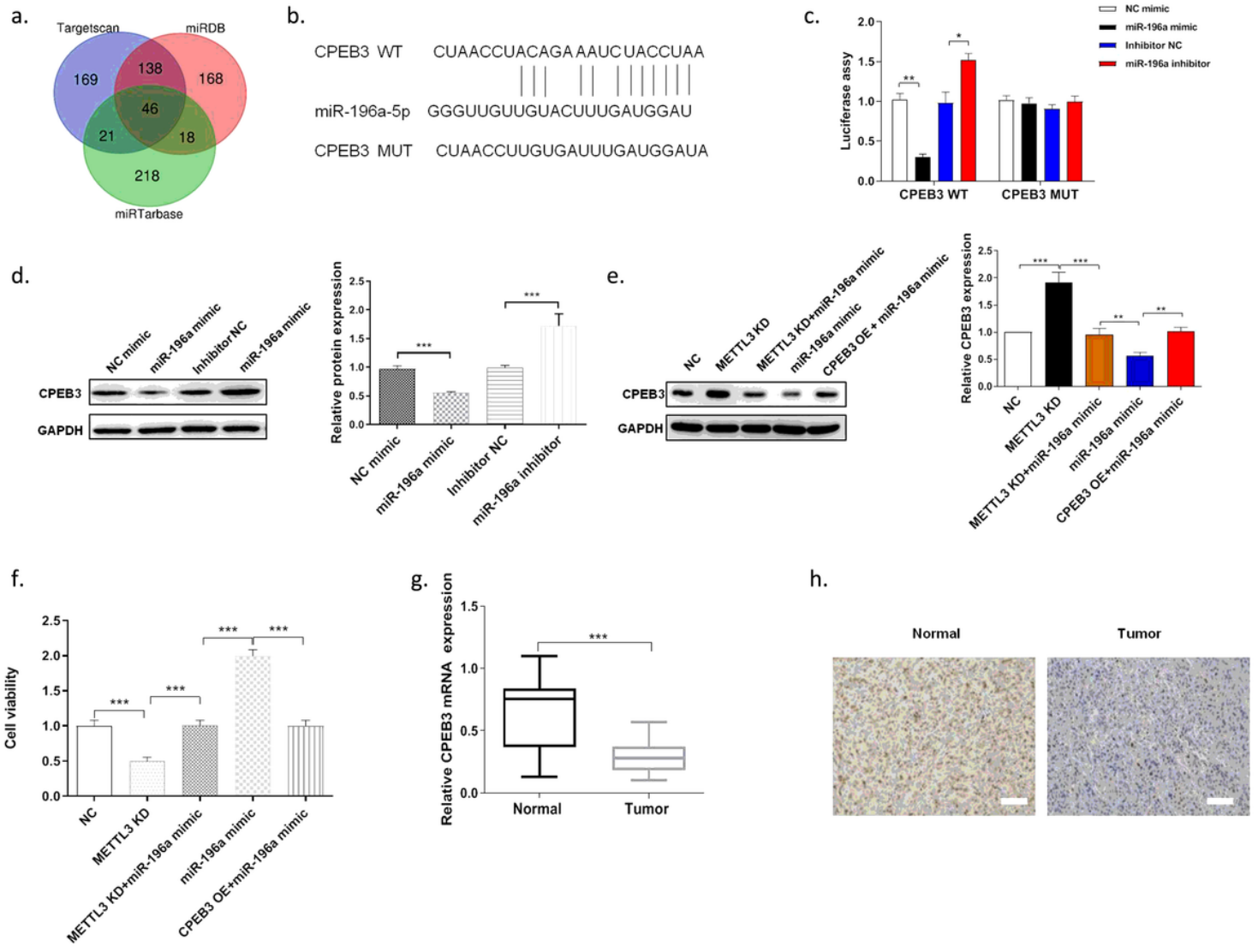


Figure 5

CPEB3 is a direct target of miR-196a in pancreatic cancer. a Venn diagram showed the intersection of the predicted target gene. b The potential binding sites of miR-196a and 3'UTR of CPEB3 mRNA. c Luciferase reporter assay was performed at 48 h with luciferase reporter plasmid containing WT or mutant form of CPEB3 3' UTR along with NC, miR-196a mimic and miR-196a inhibitor. d CPEB3 expression in the PANC-1 cells transfected with miR-196a mimic and miR-196a inhibitor for 24 h. e PANC-1 cells transfected with METTL3 knockdown lentivirus, miR-196a mimic, separately or jointly, or transfected with miR-196a mimic and CPEB3 overexpression lentivirus jointly. CPEB3 expression was detected by using western blot. f Cell viability of the cells of indicated group. g Relative CPEB3 expression in the pancreatic tumor tissues. h Immunochemical staining of CPEB3 in the pancreatic tumor tissues. Bar=20 μm. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, compared to the relative NC group.



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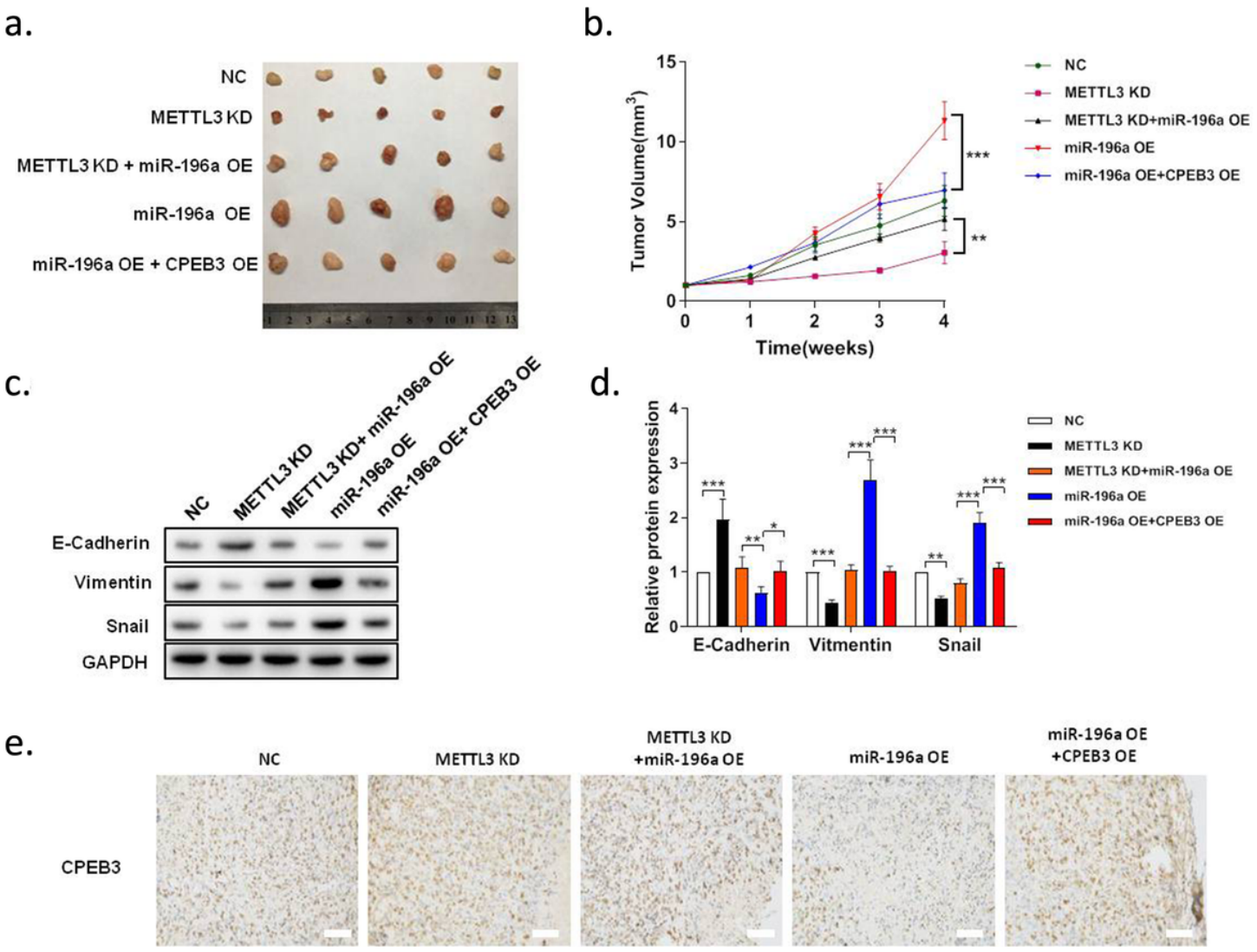
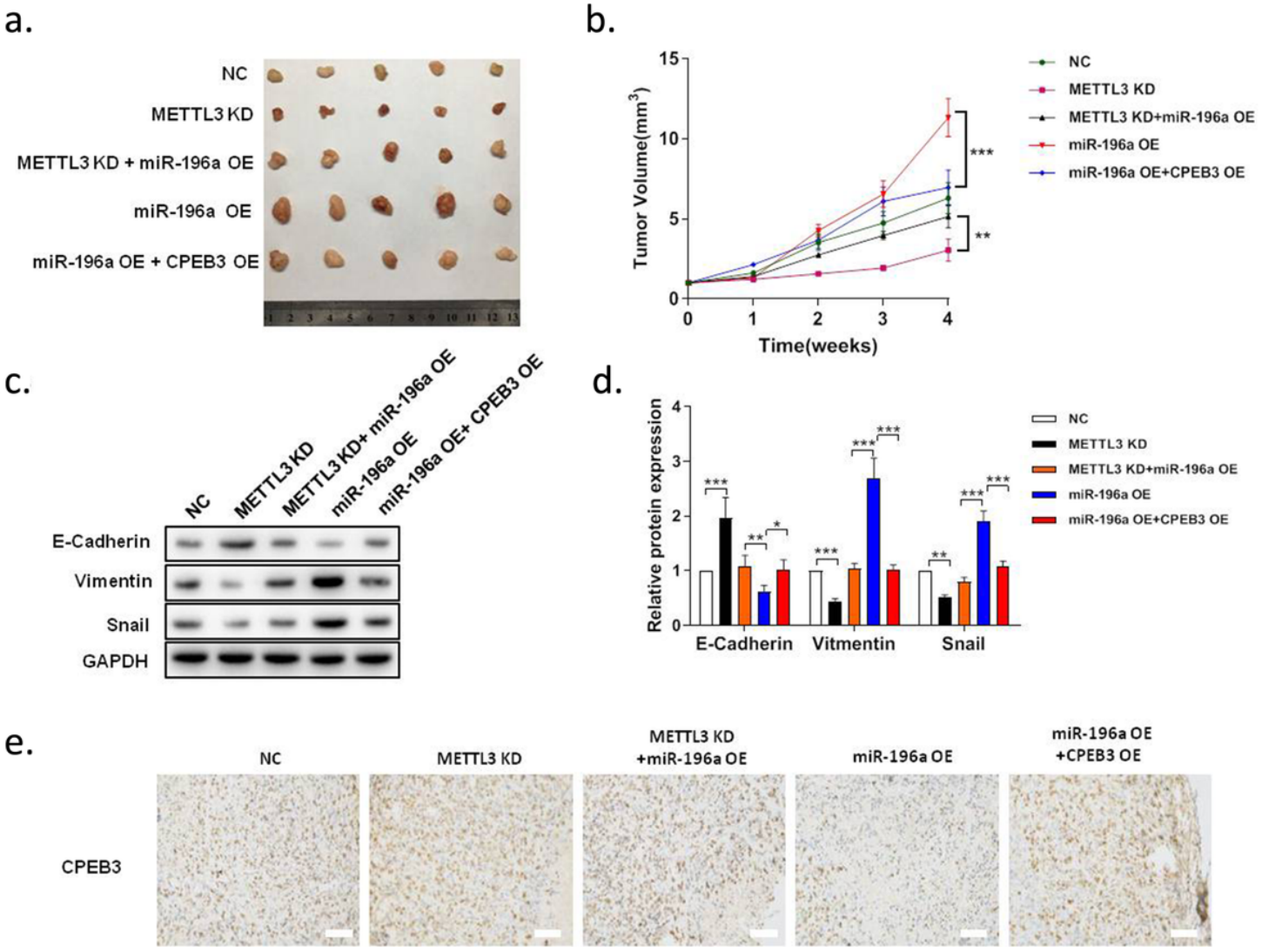


Figure 6

METTL3 regulates pancreatic tumor progression through regulating miR-196a/CPEB3 axis in vivo. The PANC-1 cells were transfected with METTL3 or CPEB3 knockdown lentivirus or not, and were then subcutaneously injected to the mice. miR-196a angomirs (80 mg/kg) were administrated to mice by tail injection to overexpress the miRNAs in vivo. a Photograph of tumor of the mice in the indicated group. b Tumor growth curve was shown. c, d EMT-related E-Cadherin, Vimentin, and Snail and GAPDH protein expression in tumor tissues were detected by western blot. e Immunochemical staining of CPEB3 in tumor tissues. Bar=20 μm. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, compared to the relative NC group.



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