Research advances of N6-methyladenosine in diagnosis and therapy of pancreatic cancer

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Abstract
Background: N6-methyladenosine (m6A) is the addition of a methyl group on the N6 position of adenosine and is the most prevalent and abundant epigenetic modification in eukaryote mRNA. m6A marks are added to mRNA by the m6A methyltransferase complex (“writers”), removed by m6A demethylases (“erasers”), and recognized by m6A-binding proteins (“readers”). Recent evidence has shown that the m6A modification plays a crucial role in the pathogenic mechanism and malignant progression of pancreatic cancer, with roles in cell survival, proliferation, migration, invasion, tumor metastasis, and drug resistance.

Methods: Literature was searched in Pubmed and Web of Science for the following keywords: "N6-methyladenosine", "pancreatic cancer", "epigenetic modification", "immunotherapy".

Results: Among classical m6A regulators, while METTL3, METTL14, WTAP, FTO, YTHDF2, IGF2BP1–3, hnRNPC, and NKAP are upregulated in pancreatic cancer, METTL16 and ALKBH5 are downregulated in pancreatic cancer. m6A modification has been investigated in pancreatic cancer therapy.

Conclusion: Dysregulated m6A and its related factors in pancreatic cancer cells and patients indicate their potential values as novel biomarkers in pancreatic cancer diagnosis and targeted therapy.

Keywords
clinical value, epigenetic modification, immunotherapy, N6-methyladenosine, pancreatic cancer

1 | INTRODUCTION

Research on RNA modification has been ongoing for more than 70 years and is currently a hot topic in scientific research. Various mRNA modifications have been identified, including N6-methyladenosine (m6A), inosine, 5-methylcytosine, pseudouridine, 5-hydroxymethylcytosine, N4-acetylcytidine, N1-methyladenosine, N6,2′-O-dimethyladenosineinosine, N7-methylguanosine, and 2′-O-methylated nucleotides. m6A is the methyl group modification on the N6 position of adenosine and is...
the most prevalent and abundant epigenetic modification in eukaryote mRNA. Approximately 0.1%–0.4% of all adenes are m6A residues in mRNA, with 3–5 m6A sites per mRNA. m6A occurs at the RRACH motif (R = A, G, or U; R = A or G; H = A, C, or U) and is enriched in the 3’ untranslated region (3’UTR) around stop codons and within long internal exons in mRNA.

m6A modifications alter the expression of specific genes, thus influencing the corresponding biological and pathological bioprocesses, including the development of embryonic stem cells, circadian rhythm control, heat shock, DNA damage response, and sex determination. Recent studies have shown that m6A modifications also play a pivotal role in cancer occurrence, progression, metastasis, and drug resistance.

Pancreatic cancer (PC) is a fatal cancer with a 5-year overall survival rate of approximately 10% in the USA. The poor survival of PC patients is from the lack of specific symptoms and early diagnosis, high aggressiveness, metastasis at an early stage, and limited availability of effective therapeutic interventions. With an incidence rising at a rate of 0.5% to 1.0% each year, PC is expected to be the second leading cause of cancer-related death by 2030 in the USA. In China, 95,000 people were diagnosed with PC and nearly 85,000 patients died of PC in 2015. The most common histological type of PC is pancreatic ductal adenocarcinoma (PDAC), which accounts for more than 90% of all confirmed PC cases. Patients with PDAC mostly present with locally advanced or metastatic disease on presentation and are not eligible for curative radical resection. From the application of FOLFIRINOX (fluorouracil, oxaliplatin, irinotecan, and leucovorin) and gemcitabine/capecitabine as adjuvant chemotherapy after resection of PDAC, the cure rate of PC has reached 10%. However, the acquired resistance to chemotherapy leads to a poor prognosis for PC patients. Emerging studies have indicated that dysregulation of the m6A machinery plays a pivotal role in the development, progression, and acquisition of chemoresistance in PC. Herein, we discuss the recent advances in m6A and PC research and the potential clinical value of m6A-related factors in diagnostic and treatment strategies for PC.

2 | REGULATORS OF M6A MODIFICATION

m6A regulators are categorized into m6A methyltransferase complex ("writers"), m6A demethylases ("erasers"), and m6A-binding proteins ("readers").

2.1 | Writers

m6A modifications are added to mRNA by the multicomponent methyltransferase complex (MTC) that consists of the core catalytic subunit methyltransferase-like 3 (METTL3) and other accessory subunits including methyltransferase-like 14 (METTL14), methyltransferase-like 16 (METTL16), Wilms’ tumor 1-associating protein (WTAP), vir-like m6A methyltransferase associated (VIRMA, also called KIAA1429), RNA binding motif 15/15B (RBM15/15B), zinc finger CCCH domain-containing protein 13 (ZC3H13), zinc finger CCHC-type containing 4 (ZCCHC4), and methyltransferase 5, N6-adenosine (METTL5). The MTC transfers the methyl group of S-adenosylmethionine (SAM) to the sixth nitrogen atom of adenosine. Using TAP-LC–MS, Knuckles and colleagues identified two stable protein complexes, which are referred to as the m6A-METTL complex (MAC) and the m6A-METTL-associated complex (MACOM).

The MAC consists of METTL3 and METTL14. METTL3, which was identified as the first SAM-binding subunit of the MTC in 1997, serves as the core catalytic component of MAC. METTL14 functions as an RNA binding scaffold to form a stable heterodimer with METTL3 and enhances the catalytic activity of METTL3. MACOM comprises WTAP, KIAA1429, RBM15/15B, ZC3H13, and HAKAI. The adaptor protein WTAP, which was identified as another critical component of MTC, interacts with both METTL3 and METTL14 to promote the localization of the heterodimer in nuclear speckles. WTAP also regulates the recruitment of selective RNA substrates and additional auxiliary proteins. Other adaptor proteins such as VIRMA, RBM15/15B, and ZC3H13 play a regulatory role in the MACOM complex. The MTC was purified from HeLa cell nuclear extracts and was separated into three components with different molecular masses of 30, 200, and 875 kDa. The MAC complex has a molecular mass of 120 kDa and is a part of the 200 kDa component. The MACOM, which belongs to the 875 kDa component, has a molecular mass of 600 kDa. Both MAC and MACOM have a smaller molecular mass than the separated enzyme component from HeLa cell nuclear extracts, which means that other unknown components of MTC need to be identified.

METTL16 is recently discovered critical subunit of the MTC. METTL16 maintains the homeostasis of intracellular SAM, the methyl group donor, and participates in the m6A modification of U6 small nuclear RNA, thus affecting the splicing of mRNA precursors by U6 snRNP. ZCCHC4 and METTL5 were found to methylate 28S rRNA and 18S rRNA, respectively.

2.2 | Erasers

The removal of m6A is catalyzed by demethylases that oxidatively reverse the m6A modifications on RNA. Fat mass and obesity-associated (FTO) and AlkB homology 5 (AlkBH5) are the only two m6A demethylases discovered so far, and both belong to the AlkB subfamily. FTO and AlkBH5 use ferrous iron as cofactor and α-ketoglutarate as co-substrate to catalyze the oxidation of the substrate.

As the first identified RNA demethylase, FTO brought research interest to m6A, as its discovery indicated that m6A modification is a reversible process similar to DNA methylation and histone modification. FTO was previously linked to human obesity and energy homeostasis. FTO is located in the nucleoplasm in a dot-like pattern and colocalizes with nuclear speckles. There are
some controversies about the real substrate of FTO. When FTO was initially identified as an m6A demethylase, Jia et al. reported that the substrate of FTO was m6A modification. However, a study by Mauer et al. in 2017 led to a different conclusion. The authors found that FTO prioritized the demethylation of N6,2'-O-dimethyladenosine rather than m6A. Later research conducted by Su et al. showed that the cellular localization of FTO determined the binding priority of m6Am and m6A: FTO tended to act on m6A in the nucleus and on m6A and m6Am in the cytoplasm. An early study found that FTO regulates adipogenesis via abrogating the m6A level of adipogenic factor RUNX1T1 mRNA and increasing the expression of the shorter isoform RUNX1T1-S. Another recent study showed that FTO interrupted YTHDF2-dependent cyclin A2 and cyclin-dependent kinase 2 decay, thereby promoting adipocyte cell cycle progression and adipogenesis. Both FTO and ALKBH5 are mainly localized in the nucleus and expressed at low levels in the cytoplasm. The mechanisms of demethylation of cytoplasmic RNA need further investigation.

2.3 | Readers

Proteins that preferentially bind to m6A-modified mRNAs and conduct downstream functions are termed m6A readers. Studies used methylated RNA as bait to obtain binding proteins and mass spectrometry to identify the binding proteins, leading to the identification of several m6A reader protein candidates in human and mouse cells.

The YTH domain–containing proteins, including YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2, are relatively well-characterized m6A readers that specifically recognize and bind m6A spots through their conserved YTH domain. The YTH domain was found in 174 different proteins in eukaryotic cells, and YTH domain–containing proteins are also abundant in plants. YTHDC1 is a nuclear m6A reader and is involved in mRNA alternative splicing, mRNA nuclear export, and X chromosome gene transcriptional silencing. YTHDF2, the first identified m6A reader protein, mediates the degradation and destabilization of target mRNAs through promoting the relocation of target transcripts from the active translation pool to decay sites and recruiting the CCR4-NOT complex. In contrast, YTHDF1 enhances the translation of methylated mRNAs through the recruitment of initiation factors (eIFs) and the facilitation of ribosome loading. YTHDF3, which shares >65% of its protein sequence with both YTHDF1 and YTHDF2, functions as a partner of YTHDF1 and YTHDF2. YTHDF3 enhances methylated mRNA translation together with YTHDF1 and strengthens mRNA decay along with YTHDF2. Similar to YTHDF3, YTHDC2 also regulates the expression and decay of target mRNAs and is involved in mammalian spermatogenesis.

Several hnRNPs are thought to be potential m6A readers, including heterogeneous nuclear ribonucleoproteins A2/B1 (HNRNPC), heterogeneous nuclear ribonucleoproteins C (HNRNPC), and heterogeneous nuclear ribonucleoproteins G (HNRNPG). Recent studies revealed that the m6A modification alters the secondary structure of target RNAs, enhancing the binding of hnRNPs and RNAs and allowing hnRNPs to specifically recognize the m6A-induced secondary structures instead of the methylated adenosine on mRNAs, which is termed as the ‘m6A switch’. As a nuclear m6A reader, HNRNPC accelerates primary microRNA processing by recruiting the microprocessor complex Drosha and DGC8 and mediates the alternative splicing of mRNAs. While HNRNPC participates in processing pre-mRNAs, HNRNPG regulates the alternative splicing of pre-mRNAs. Both HNRNPC and HNRNPG function through recognizing m6A-induced secondary structures.

As a new family of m6A readers, IGF2BPs prevent m6A-modified mRNAs from degradation by stabilizing target transcripts. Similar to the YTH domain in YTH family proteins, KH domains in IGF2BPs are required for their recognition of the m6A modification. Through studies using KH domain mutants, the essential roles of KH3-4 domains rather than KH1-2 in IGF2BP binding to m6A-modified mRNAs were confirmed. IGF2BPs maintain the stability of target mRNAs and facilitate the translation process of m6A-containing mRNAs.

ElF3 directly binds to m6A-modified sites in the 5'UTR of mRNAs and recruits the 43S ribosome complex to launch the translation process in a cap-independent pattern. FMRP was recently reported to be another m6A interactor and it regulates the stability of m6A-containing mRNAs through YTHDF2.

3 | M6A MODIFICATION AND DIAGNOSIS OF PC

Previous studies demonstrated high m6A levels in PC tissues and cell lines. However, a recent study showed lower m6A levels in PDAC samples compared with adjacent non-tumor tissues. The aberrant levels of m6A modifications result from abnormally expressed m6A-related factors, which play a vital role in PC by regulating various RNAs and downstream signal pathways (Table 1).

3.1 | Highly expressed m6A regulators in PC

3.1.1 | METTL3

As an m6A writer, METTL3 mediates the methylation process on specific sites in mRNA. METTL3 expression is enriched in PC tumor tissue and PC cell lines. Xia et al. reported a positive association of METTL3 expression with pathological stages of PC patients. Depletion of METTL3 inhibited the proliferation and invasion of PC cells by reducing m6A level. Takeo et al. knocked down METTL3...
| m6A regulators | Type      | Role           | Function in PC                                                                 | Molecular mechanism                                                                 | Ref |
|---------------|-----------|----------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----|
| Upregulated m6A regulators |           |                |                                                                                 |                                                                                      |     |
| METTL3        | Writer    | Oncogene       | Promoting proliferation and invasion                                            | -                                                                                     | 54  |
| METTL3        | Writer    | Oncogene       | Promoting chemoresistance                                                       | MAPK cascade, ubiquitin-dependent process, RNA splicing, and regulation of cellular process | 56  |
| METTL3        | Writer    | Oncogene       | Promoting proliferation, migration, and invasion                                | Processing pri-miR-25                                                                  | 57  |
| METTL3        | Writer    | Oncogene       | Promoting proliferation, migration, and invasion                                | Enhancing stability of lncRNA LIFR-A51                                               | 58  |
| METTL3        | Writer    | Oncogene       | Promoting proliferation; suppressing apoptosis induced by gemcitabine          | Regulating m6A modification of NUCB1                                                 | 55  |
| METTL14       | Writer    | Oncogene       | Promoting growth and metastasis                                                  | Increasing PERP mRNA levels                                                            | 53  |
| METTL14       | Writer    | Oncogene       | Promoting growth and metastasis                                                  | CLK1-SRSF5-METTL14<sup>stron10+</sup> axis                                           | 61  |
| METTL14       | Writer    | Oncogene       | Inhibiting apoptosis induced by cisplatin and autophagy                          | mTOR signaling-dependent pathway                                                      | 59  |
| METTL14       | Writer    | Oncogene       | Inducing resistance to gemcitabine                                               | Upregulating cytidine deaminase                                                       | 60  |
| WTAP          | Writer    | Oncogene       | Enhancing migration and invasion; inducing resistance to gemcitabine            | Fak signaling pathway                                                                  | 64  |
| WTAP          | Writer    | Oncogene       | Promoting proliferation, migration, and invasion                                | DUXAP8/miR-448/WTAP/Fak signaling                                                    | 65  |
| WTAP          | Writer    | Oncogene       | Promoting proliferation and invasiveness                                         | WTAPP1/WTAP/Wnt signaling                                                             | 66  |
| FTO           | Eraser    | Oncogene       | Promoting proliferation and inhibiting apoptosis                                | Enhancing stability of c-Myc mRNA                                                    | 69  |
| YTHDF2        | Reader    | Oncogene tumor suppressor | Promoting proliferation; inhibiting migration, invasion, adhesion, and epithelial–mesenchymal transition | Akt-GSK3β-cyclin D1 pathway; YAP signaling                                            | 71  |
| YTHDF2        | Reader    | Oncogene       | Promoting proliferation and migration                                            | Enhancing stability of PIK3CB mRNA                                                  | 72  |
| IGF2BP1       | Reader    | Oncogene       | Promoting proliferation; inhibiting apoptosis; inducing cell cycle progression   | AKT signaling pathway                                                                | 74  |
| IGF2BP1       | Reader    | Oncogene       | Promoting proliferation, migration, and invasion                                | Enhancing stability of ELF3 mRNA                                                    | 75  |
| IGF2BP1       | Reader    | Oncogene       | Promoting proliferation                                                         | HIPK2-ERK-c-myc axis                                                                | 76  |
| IGF2BP2       | Reader    | Oncogene       | Involving in apoptosis and ubiquitination                                        | PKC signaling pathway                                                                | 78  |
| IGF2BP2       | Reader    | Oncogene       | Promoting proliferation                                                         | PI3K-Akt signaling pathway                                                            | 79  |
| IGF2BP2       | Reader    | Oncogene       | Promoting proliferation and aerobic glycolysis                                  | Enhancing stability of GLUT1 mRNA                                                    | 80  |
| IGF2BP2       | Reader    | Oncogene       | Enhancing proliferation, stemness-like properties, and tumorigenesis            | Enhancing stability of lncRNA DANCR                                                  | 81  |
| IGF2BP3       | Reader    | Oncogene       | Promoting invasiveness and metastasis                                            | Facilitating local translation of ARF6 mRNA and ARHGEF4 mRNA                     | 87-88 |
Long non-coding RNAs are also downstream molecules in the oncogenic axis. Chen et al. found that METTL3 enhances the stability of LIFR-AS1 in an m6A-dependent manner; it exerts its oncogenic function via the miR-150-5p/VEGFA/Akt/mTOR signaling pathway and promotes the proliferation, migration, and invasion of PC cells.\textsuperscript{58} A recent study conducted by Hua showed the participation of METTL3 in decreasing the expression of nucleobindin 1 (NUCB1) in an m6A-dependent manner. NUCB1 was discovered to suppress growth and enhance the sensitivity of PC to gemcitabine in vitro and in vivo.\textsuperscript{55} While the exact role of METTL3 in PC has not been completely clarified, these findings indicate METTL3 as a promising biomarker in PC treatment, early diagnosis, and prognosis.

### 3.1.2 | METTL4

METTL14, another component of the MAC, plays multiple roles in PC. METTL14 is highly expressed in PC tissue and cell lines and its role in anti-cancer drugs and radiation were reversed. Microarray data and Gene Ontology functional enrichment analysis showed that MAPK cascades, the ubiquitin-dependent process, RNA splicing, and the regulation of cellular process might be potential downstream targets of METTL3.\textsuperscript{54} Furthermore, METTL3 is involved in the maturation of miRNA via m6A. Zhang et al. discovered hypomethylation of the METTL3 promoter in PDAC cell lines and control cell lines exposed to cigarette smoke condensate that resulted in the aberrant over-expression of METTL3. The dysregulated METTL3 significantly facilitates the maturation of miR-25-3p, which plays an oncogenic role in multiple cancers in an m6A-dependent manner. miR-25-3p acts as an oncogenic AKT-p70S6K signaling activator targeting PHLPP2, thus promoting the proliferation, migration, and invasion of PC cells.\textsuperscript{57} Long non-coding RNAs are also downstream molecules in the METTL3 oncogenic axis. Chen et al. found that METTL3 enhances

### TABLE 1 (Continued)

| m6A regulators | Type | Role | Function in PC | Molecular mechanism | Ref |
|----------------|------|------|----------------|---------------------|-----|
| IGF2BP3 | Reader | Oncogene | Modulating focal adhesion junction; promoting invasion | Promoting association of RISC and mRNA | 89 |
| hnRNPC | Reader | Oncogene | Promoting proliferation | Disrupting the binding site for has-miR-183-3p in the hnrNPC 3’UTR | 92 |
| hnRNPC | Reader | Oncogene | Promoting invasion and metastasis | Alternative splicing of TAF8 mRNA | 93 |
| NKAP | Reader | Oncogene | Promoting proliferation, migration, and invasion | Promoting miR-25-3p maturation | 97 |

**Downregulated m6A regulators**

| METTL16 | Writer | Tumor suppressor | Suppressing proliferation | p21 signaling pathway | 97 |
| FTO | Eraser | Tumor suppressor | Inhibiting proliferation, metastasis, and invasion | Enhancing stability of PJA2 mRNA | 70 |
| ALKBH5 | Eraser | Tumor suppressor | Inhibiting migration, invasion, and epithelial–mesenchymal transition | Increasing IncRNA KCNK15-AS1 level | 98–99 |
| ALKBH5 | Eraser | Tumor suppressor | Inhibiting proliferation, colony formation, and migration | Reducing WIF-1 m6A modification/Wnt signaling pathway | 101 |
| ALKBH5 | Eraser | Tumor suppressor | Inhibiting proliferation migration, and invasion | PER1-ATM-CHK2-P53/CDC25C signaling | 73 |
| ALKBH5 | Eraser | Tumor suppressor | Decreasing intracellular iron levels; inhibiting migration and invasion | Enhancing expression of FBXL5 and SLC25A28 | 102 |

**Abbreviations:** Akt, AKT serine/threonine kinase; ALKBH5, alk B1-hydroxylase 5; ARHGEF4, Rho guanine nucleotide exchange factor 4; ATM, ATM serine/threonine kinase; CDC25C, cell division cycle 25C; CHK2, checkpoint kinase 2; CLK1, Cdc2-like kinase 1; DANCER, differentiation and nuclear expression factor; DDXP8, double homeobox A pseudogene 8; ELF3, E74-like ETS transcription factor 3; ERK, mitogen-activated protein kinase 1; Fak, focal adhesion kinase; FBXL5, F-box and leucine-rich repeat protein 5; GLUT1, solute carrier family 2 member 1; GSK3β, glycogen synthase kinase 3 beta; HIPK2, homeodomaininteracting protein kinase 2; hnrNPC, heterogeneous nuclear ribonucleoproteins C; KCNK15-AS1, KCNK15 and WISP2 antisense RNA 1; LIFR-AS1, Leukemia inhibitory factor receptor antisense RNA 1; MAPK, p38 mitogen-activated protein kinase; METTL14exon10+, non-exon 10–skipped METTL14; miR-183, microRNA-183; miR-448, microRNA-448; mTOR, mechanistic target of rapamycin kinase; NUCB1, nucleobindin 1; p21, cyclin dependent kinase inhibitor 1A; P53, tumor protein p53; PER1, period circadian regulator 1; PERP, p53 effector related to PMP-22; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PIK3CB, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta; PJA2, praja ring finger ubiquitin ligase 2; PKC, protein kinase C; pri-miR-25, primary microRNA-25; RISC, RNA induced silencing complex; SLC25A28, solute carrier family 25 member 28; SRSF5, SR-like splicing factors 5; TAF8, TATA-box binding protein associated factor 8; WIF-1, Wnt inhibitory factor 1; WTAP, Wilms tumor 1 (WT1)-associated protein; WTAPl1, Wilms tumor 1 associated protein pseudogene 1; YAP, Yes1 associated transcriptional regulator.

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**TABLE 1**

**m6A regulators**

| m6A regulators | Type | Role | Function in PC | Molecular mechanism | Ref |
|----------------|------|------|----------------|---------------------|-----|
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| hnRNPC | Reader | Oncogene | Promoting proliferation | Disrupting the binding site for has-miR-183-3p in the hnRNPC 3’UTR | 92 |
| hnRNPC | Reader | Oncogene | Promoting invasion and metastasis | Alternative splicing of TAF8 mRNA | 93 |
| NKAP | Reader | Oncogene | Promoting proliferation, migration, and invasion | Promoting miR-25-3p maturation | 97 |

**Downregulated m6A regulators**

| METTL16 | Writer | Tumor suppressor | Suppressing proliferation | p21 signaling pathway | 97 |
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**Abbreviations:** Akt, AKT serine/threonine kinase; ALKBH5, alk B1-hydroxylase 5; ARHGEF4, Rho guanine nucleotide exchange factor 4; ATM, ATM serine/threonine kinase; CDC25C, cell division cycle 25C; CHK2, checkpoint kinase 2; CLK1, Cdc2-like kinase 1; DANCER, differentiation and nuclear expression factor; DDXP8, double homeobox A pseudogene 8; ELF3, E74-like ETS transcription factor 3; ERK, mitogen-activated protein kinase 1; Fak, focal adhesion kinase; FBXL5, F-box and leucine-rich repeat protein 5; GLUT1, solute carrier family 2 member 1; GSK3β, glycogen synthase kinase 3 beta; HIPK2, homeodomaininteracting protein kinase 2; hnrNPC, heterogeneous nuclear ribonucleoproteins C; KCNK15-AS1, KCNK15 and WISP2 antisense RNA 1; LIFR-AS1, Leukemia inhibitory factor receptor antisense RNA 1; MAPK, p38 mitogen-activated protein kinase; METTL14exon10+, non-exon 10–skipped METTL14; miR-183, microRNA-183; miR-448, microRNA-448; mTOR, mechanistic target of rapamycin kinase; NUCB1, nucleobindin 1; p21, cyclin dependent kinase inhibitor 1A; P53, tumor protein p53; PER1, period circadian regulator 1; PERP, p53 effector related to PMP-22; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PIK3CB, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta; PJA2, praja ring finger ubiquitin ligase 2; PKC, protein kinase C; pri-miR-25, primary microRNA-25; RISC, RNA induced silencing complex; SLC25A28, solute carrier family 25 member 28; SRSF5, SR-like splicing factors 5; TAF8, TATA-box binding protein associated factor 8; WIF-1, Wnt inhibitory factor 1; WTAP, Wilms tumor 1 (WT1)-associated protein; WTAPl1, Wilms tumor 1 associated protein pseudogene 1; YAP, Yes1 associated transcriptional regulator.
high expression is positively correlated with T stage and poor overall survival of patients.\textsuperscript{53,59,60} Wang and colleagues showed that the depletion or overexpression of METTL14 led to a more significant decrease or increase of m6A level, respectively, than that of METTL3 depletion or overexpression in PC cell lines. Uregulated METTL14 promotes PC growth and metastasis by targeting p53 effector related to PMP-22 (PERP) mRNA and affecting its stability in an m6A-dependent way.\textsuperscript{55} Chen et al. discovered that aberrant exon skipping of METTL14 also promotes the growth and metastasis of PC. Cdc2-like kinase 1 is aberrantly overexpressed in PC tissues and phosphorylates SRSF5 (SR-like splicing factors 5) on Ser-250. Mechanically, phosphorylation of SRSF5 on Ser-250 induced by CLK1 improves the interaction between SRSF5 and pre-mRNA of METTL14 and cyclin L2 and inhibits the skipping of exon 10 of METTL14 and promotes the skipping of exon 6.3 of cyclin L2 mRNA. The non-exon 10–skipped results in oncogenic effects of the CLK1-SRSF5 axis through m6A in PC. \textsuperscript{61} The exact mechanisms of METTL14 in PC need further exploration, and larger-scale studies with more patients are essential.

### 3.1.3 | WTAP

As a part of MACOM, WTAP plays an oncogenic role in various malignancies, including PC.\textsuperscript{62} Li and colleagues reported that both nuclear and cytoplasmic expressions of WTAP are significantly higher in PDAC tissues than in paracancerous tissues, and its high expression is related to poor overall survival and pathological characteristics.\textsuperscript{63} Further research showed that WTAP facilitates the metastasis and chemoresistance to gemcitabine in PC through stabilizing Fak mRNA and activating Fak signaling pathways. GSK2256098, a small molecular Fak inhibitor, reversed the promotion of metastasis and chemoresistance to gemcitabine induced by WTAP.\textsuperscript{64} Another study conducted by Li et al. showed the involvement of WTAP-Fak signaling in the DUXAP8-miR-448 axis. The IncRNA DUXAP8 facilitates the invasion and migration of PC cells by targeting miR-448, thus regulating WTAP-Fak signaling.\textsuperscript{65} A recent study conducted by Deng et al. indicated the critical role of WTAP in PDAC progression. WTAPP1 RNA, a transcript of the pseudogene WTAPP1, is overexpressed in cancerous tissues and positively correlated with the poor outcome of PC patients. WTAPP1 mRNA is stabilized in a METTL3-m6A-dependent manner and binds its protein-coding counterpart WTAP mRNA to recruit the eIF3 complex to enhance the translation of WTAP. Uregulated WTAP promotes the proliferation and invasiveness of PDAC cells through activating Wnt signaling.\textsuperscript{66} However, the exact function of WTAP in PC remains unclear.

### 3.1.4 | FTO

FTO, which was previously linked with human obesity and type II diabetes, was discovered to function in PC. Tang et al. found that FTO was significantly associated with a decreased or increased risk of PC in subgroups with different body mass index.\textsuperscript{67} In a case-control study conducted by Lin et al., rs9939609, the minor A allele of the FTO gene, was associated with PC risk in a Japanese population.\textsuperscript{68} Experimental studies also reported the association between FTO and PC. Tang et al. revealed that FTO was highly expressed in PC cells. Knockdown of FTO significantly reduces proliferation, increases apoptosis, and elevates m6A levels in PC cells. In addition, FTO enhances PC cell proliferation by decreasing the m6A level of c-MYC mRNA and increasing its stability.\textsuperscript{69} However, a recent study reported the opposite conclusion. Zeng et al. reported low expression of FTO both in PC cancerous tissues and cell lines, which was responsible for high m6A levels in PC. Reduced expression of FTO in PAAD was associated with poor overall survival. Functionally, FTO reduces the proliferation, metastasis, and invasion of PC cells through enhancing the stability of praja ring finger ubiquitin ligase 2 (PJA2) mRNA in an m6A-YTHDF2-dependent manner, thereby inhibiting Wnt signaling.\textsuperscript{70} More studies are needed to verify the exact role of FTO in PC.

### 3.1.5 | YTHDF2

As an m6A reader, the involvement of YTHDF2 is critical in PC. Chen et al. reported the upregulation of YTHDF2 in PC and its association with pathological stages. YTHDF2 exerts two functions in PC: promoting proliferation via the Akt/GSK3β/cyclin D1 pathway and inhibiting migration, invasion, adhesion, and epithelial–mesenchymal transition (EMT) by YAP signaling, a phenomenon which is termed migration-proliferation dichotomy.\textsuperscript{71} Tian et al. showed that a variant of PIK3CB, rs142933486-T (PIK3CB[T]), increases the mRNA and protein expression PIK3CB and decreases the m6A level of PIK3CB mRNA to enhance its stability by abolishing m6A-YTHDF2-dependent mRNA degradation. Mechanistically, overexpressed PIK3CB promotes proliferation and migration of PDAC cells.\textsuperscript{72} Guo et al. reported that YTHDF2 is involved in the repressive effect of ALKBH4 on PC growth and metastasis. By demethylating m6A on PER1 mRNA, ALKBH4 decreased PER1 mRNA degradation executed by YTHDF2.\textsuperscript{73} Another study showed that YTHDF2 mediated the degradation of NUCB1 mRNA by interacting with its m6A sites.\textsuperscript{55}

### 3.1.6 | IGF2BP protein family

In addition to YTHDF2, the IGF2BP protein family also plays a vital role in the genesis and development of PC. Wan et al. reported the overexpression of IGF2BP1 in PC tumors and showed that high IGF2BP1 expression predicts the poor prognosis of PC patients. Functionally, IGF2BP1 promotes proliferation, inhibits apoptosis, and induces cell cycle progression through the AKT signaling pathway.\textsuperscript{74} Feng et al. found that overexpressed IncRNA NEAT1 exerts oncogenic functions by recruiting IGF2BP1 and stabilizing ELF3 mRNA.\textsuperscript{75} Another study showed that the tumor suppressor LINC00261 acted
as a competing endogenous RNA of miR-222-3p and regulated the HIPK2/ERK/c-myc axis. By sequestering IGF2BP1, LINC00261 decreased the stability of c-myc mRNA and protein level.\textsuperscript{76} Lin et al. showed the potential involvement of IGF2BP2 during pancreatic islet tumor formation. Men1-loss led to decreased methylation of histone H3 at lysine 4 (H3K4me3), increased histone H3K27me3 level, and restrained IGF2BP2 expression that was reversed by ablation of RBP2 in a mouse model.\textsuperscript{77} Dahlem et al. showed that IMP2 (also known as IGF2BP2) was overexpressed in pancreatic intraepithelial neoplasia and PDAC and linked to poor prognosis. Strict correlation analysis identified 22 genes that were positively correlated with IMP2 and 9 genes that were negatively correlated with IMP2; these genes encode proteins that are involved in apoptosis, ubiquitination, and the protein kinase C (PKC) signaling pathway. Furthermore, analysis of the GDS4329 dataset revealed higher IMP2 expression in circulating tumor cells (CTCs) than in healthy pancreatic tissues and hematological cells, indicating the potential role of IMP2 in epithelial–mesenchymal transition (EMT) of PDAC.\textsuperscript{78} Another study confirmed the overexpression of IGF2BP2 in PDAC tissues and its association with clinical outcome. IGF2BP2, a target of miR-141, promotes PC growth through activating the PI3K-Akt signaling pathway both in vitro and in vivo.\textsuperscript{79} Huang et al. reported the involvement of IGF2BP2 in aerobic glycolysis in PDAC. IGF2BP2 promotes PDAC cell growth in vitro and in vivo by directly binding and stabilizing GLUT1 mRNA, which encodes a protein that functions in glucose transportation, to enhance aerobic glycolysis.\textsuperscript{80} IGF2BP2 also exerts its oncogenic function through IncRNA DANC1. By interacting with DNArc and regulating its stability in an m6A-dependent manner, IGF2BP2 enhances PC cell proliferation and stemness-like properties and promotes the tumorigenesis of PC in vivo.\textsuperscript{81} Another bioinformatics study reported the predictive potential of IGF2BP2 and its correlation with multiple biological processes. The most significant processes were related to the cell cycle, cell immortalization, and tumor immunity.\textsuperscript{82} IGF2BP3 also plays a crucial role in PC. IGF2BP3 is highly expressed in PDAC tissues but not expressed in benign pancreatic tissues.\textsuperscript{83–85} Schaeffer further confirmed that overexpressed IGF2BP3 correlates with poor survival in PDAC.\textsuperscript{86} Taniuchi and colleagues demonstrated the localization of IGF2BP3 and IGF2BP3-bound transcripts in cytoplasmic RNA granules and cell protrusions. IGF2BP3 promotes the invasiveness and metastasis of PDAC cells by locally enhancing the translation of target mRNAs in the protrusions.\textsuperscript{87} Further exploration revealed that KIF20A promoted the translocation of stress granules containing IGF2BP3 and IGF2BP3-bound transcripts to cell protrusions along microtubules. Knockdown of KIF20A decreases the protein expression from IGF2BP3-bound transcripts, including ARF6 and ARHGEF4, in cell protrusions and weakens PC cell motility and invasion ability.\textsuperscript{88} Another study conducted by Ennajdaoui et al. indicated that IGF2BP3 both promotes and represses the expression of its target transcripts, which encode proteins associated with focal adhesions, adherens junctions, actin–cytoskeleton, and cell migration, by acting as a bimodal modulator of the RISC-mRNA association in PDAC.\textsuperscript{89} Senoo et al. reported that IMP3 (also known as IGF2BP2) expression was significantly correlated with tumor differentiation in resected specimens and negatively related with PDAC patients’ prognosis. Compared with cytohistology alone, cytohistological analysis combined with IMP3 staining had substantially higher diagnostic sensitivity, specificity, and accuracy in endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) samples.\textsuperscript{85} Tadic et al. and Mikata et al. also confirmed these findings.\textsuperscript{90,91}

### 3.1.7 | hnRNPs

hnRNPs are also involved in the development of PC. Ying et al. reported the association between single-nucleotide polymorphisms of hnRNP C and the susceptibility to PDAC. The variant rs7495G allele in the 3’UTR of hnRNP C, which is common among Chinese and Japanese populations, increased the risk for PDAC by disrupting the binding site for has-miR-183-3p in the hnRNP C 3’UTR and enhancing the expression of hnRNP C. Furthermore, knockdown of hnRNP C markedly suppresses the proliferation of PDAC cells, supporting the oncogenic role of hnRNP C in PC.\textsuperscript{92} In another study, Huang et al. reported the correlation between HNRNPC expression level and clinical outcome of patients with PDAC. HNRNPC promotes PDAC cell invasion in vitro and liver metastasis in vivo through alternative splicing of TAF8 mRNA in an m6A modification-dependent manner. Mechanistically, knockdown of HNRNPC contributed to upregulation of the isoform TAF8L and downregulation of the isoform TAF8S; overexpression of TAF8L attenuated the function of HNRNPC in enhancing the invasiveness of PDAC.\textsuperscript{93} In addition to HNRNPC, other members of the hnRNPC family have also been associated with PC.\textsuperscript{94–96} Further research is needed to identify the involvement of m6A.

### 3.1.8 | NKAP

NF-κB associated protein (NKAP), a newly discovered m6A reader protein, is involved in the development of PDAC by binding both DGC8 and pri-miR-25 in an m6A-dependent manner. By promoting the maturation of miR-25-3p, NKAP activates the AKT-p70S6K oncogenic signaling pathway and enhances the malignant phenotype of PDAC cells.\textsuperscript{97} The number of m6A readers continues to grow. Further exploration of m6A readers in PC is needed to deepen understanding of m6A in PC.

### 3.2 | Downregulated m6A regulators in PC

Xie et al. reported that METTL16 was downregulated in PC tissues and cell lines and its downregulation positively correlated with the poor prognosis of PC patients. PC cells overexpressing METTL16 exhibited suppressed proliferation and enhanced cell cycle arrest through the p21 signaling pathway, a mechanism involving m6A.\textsuperscript{97}
In contrast to the m6 demethylase FTO, ALKBH5 exhibits a tumor suppressor role in PC. He and colleagues reported that ALKBH5 inhibited PC cell proliferation, invasion, and EMT by regulating the expression level of IncRNA KCNK15-AS1 in a mechanism that depends on m6A modification. Moreover, ALKBH5 induced demethylation of KCNK15-AS1, leading to its upregulation, which hampered cell proliferation and facilitated cell apoptosis in PC through repressing KCNK15 expression and inactivating the PTEN/AKT pathway.\(^9,9^9\) By evaluating expression data of PC patients from The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC), Cho et al. found that PC patients with high ALKBH5 expression had higher survival rates compared with PC patients with low expression, which suggested the potential of ALKBH5 as a prognostic biomarker.\(^10^\) Tang et al. reported that knockdown of ALKBH5 dramatically improved the proliferation, colony formation, and migration abilities of PDAC cells through reducing m6A levels in the 3′UTR of Wnt inhibitory factor 1 (WIF-1) mRNA, resulting in increased expression of WIF-1 and hindered Wnt signaling. Moreover, ALKBH5 was downregulated in patient-derived xenograft models treated with gemcitabine, which was rescued by overexpression of ALKBH5.\(^10^1\) Guo et al. further confirmed ALKBH5 as a tumor suppressor and showed that ALKBH5 was downregulated in PC tissues. ALKBH5 reduced PC cell proliferation, migration, and invasion in vivo and inhibited PC growth and metastasis through regulating m6A abundance, thus decreasing the degradation of PER1 mRNA in an m6A-YTHDF2-dependent pattern. \(^\)\(^9\) which is upregulated by PER1 through ATM-CHK2-PS3/CDC25C signaling, transcriptionally activates ALKBH5, which represents a feedback regulation of m6A in PC.\(^73\) A recent study revealed the relation between ALKBH5 and iron metabolism in PDAC. ALKBH5 removes m6A modifications in FBXL5 mRNA, SLC25A28 mRNA, and SLC25A37 mRNA, causing enhanced RNA stability and elevated expression of FBXL5 and SLC25A28 and alternative splicing of SLC25A37 mRNA. Overexpression of ALKBH5 induced a significant reduction of the iron-regulatory protein IRP2 and EMT modulator SNAI1 via FBXL5 mediation, which led to a restrained malignant phenotype of PDAC cells, including decreased intracellular iron levels as well as cell migratory and invasive abilities.\(^10^2\) Together these findings demonstrate the suppressive role and significant clinical relevance of ALKBH5 in PC.

### 3.3 m6A regulators as potential biomarkers in PC

The above dysregulated m6A regulators showed the correlation between their expression and PC patients’ clinical outcomes, suggesting the potential to be biomarkers for PC early diagnosis. Among them, IMP2 and IMP3 have gained more attention in PC diagnosis. PanIN is a well-known precursor of PDAC. Early-detection of PanIN can help interrupt the progression from PanIN to PDAC. The significant overexpression of IMP2 in PanIN lesions suggests IMP2 may be a biomarker for early stages of PDAC.\(^78\) EUS-FNA is a safe and effective technique for PC diagnosis. However, due to small specimens obtained by EUS–FNA, it is challenging in differentiating PC from benign pancreatic diseases. In EUS-FNA samples, IMP3 staining remarkably improves diagnostic sensitivity, specificity, and accuracy of cytohistological analysis of PDAC, facilitating the distinction between PDAC and benign pancreatic diseases.\(^91\) Moreover, rs9939609 and rs7495G are FTO polymorphism and hnRNP polymorphism, respectively. Both rs9939609 and rs7495G are linked to PC risk, manifesting their potential in PC early diagnosis.\(^68,9^2\)

Circulating tumor cells are a intensively studied and promising field of cancer biology, and detection of CTCs is a potential non-invasive diagnostic approach across multiple cancer types.\(^10^3\) A study showed that m6A levels in CTCs were considerably elevated in lung cancer patients,\(^10^4\) and IMP2 was upregulated in GDS4329 dataset,\(^78\) indicating the combination of CTCs and m6A may be a promising strategy in PC diagnosis.

### 4 M6A-TARGETED THERAPIES IN PC

Recently, m6A-based tumor targeting therapies, including FTO inhibitors and immunotherapy, has become a new research hotspot.

#### 4.1 FTO inhibitors and therapy of PC

Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid), a natural product, is the first identified FTO inhibitor. Through biochemical and biophysical methods, Chen and colleagues demonstrated that rhein inhibits FTO activity by competitively binding to the catalytic structural domain of FTO, thereby preventing recognition of m6A substrates.\(^10^5\) EGFR inhibitors, such as erlotinib and afatinib, are widely applied in the treatment of PC. However, acquired resistance to EGFR inhibitors limits their benefit to patients. Importantly, Yang et al. reported that the combination of rhein and EGFR inhibitors counteracts the resistance to EGFR inhibitors. Rhein induces apoptosis in PC cells by suppressing constitutive STAT3 tyrosine phosphorylation. The co-application of rhein enhances the anti-tumor activity of erlotinib/afatinib toward PC by decreasing phosphorylation of both STAT3 and EGFR in vitro and in vivo.\(^10^6\) Another recent study showed that rhein has a potentiating effect on oxaliplatin in PC treatment. Liu and colleagues found that rhein hampered cell growth and promoted cell apoptosis in PC by activating the PI3K/AKT pathway instead of the MAPK pathway. Furthermore, rhein synergistically enhanced the therapeutic effect of oxaliplatin against PC by promoting the accumulation of ROS and curbing AKT activation.\(^10^7\)

Meclofenamic acid (MA), a non-steroidal anti-inflammatory drug, was discovered as another highly effective inhibitor of FTO. Unlike rhein, MA selectively inhibits FTO, with no effect on ALKBH5, through competitive binding of m6A-containing RNA substrates.\(^10^8\) Čeponyte et al. reported the anti-tumor effect of MA on PC. MA induced significant inhibition of cell viability and antiproliferative
activity in 2D cell cultures with no effect on spheroid growth in PC cells. Furthermore, MA induced cell apoptosis and inhibited colony formation of PC cells.109

Through structure-based rational design, Huang et al. discovered two new small molecule FTO inhibitors, namely FB23 and FB23-2, which possessed high specificity and potency for FTO and its m6A demethylase activity. A derivative of FB23, FB23-2 showed higher anti-proliferative activity in AML cells than FB23 and significantly induced apoptosis and myeloid differentiation of AML cells in an FTO-m6A dependent manner.110 Whether FB23 and FB23-2 can target FTO and suppress the progression of PC needs further study.

Recently, Liu et al. discovered a new FTO inhibitor, Dac51. FTO dampened CD8+ T cell activation and effector states and promoted tumor cell glycolysis by increasing the expression of multiple bZIP transcription factors, such as Jun, Junb, and Cebpb, in an m6A-dependent manner. Moreover, the authors found that Dac51 directly binds to and stabilizes FTO. Tumor cells treated with Dac51 showed similar effects to FTO knockdown; glycolytic metabolism was inhibited, and T cell activation and infiltration were enhanced. Mice that received Dac51 combined with anti-PD-L1 blockade treatment displayed significantly inhibited tumor growth and prolonged overall survival, which suggested that Dac51 in combination with T cell function enhancers such as anti-PD-L1 drugs may dramatically improve the response to immunotherapy.111 Whether Dac51 exerts function in PC requires more investigation.

STM2457, a highly potent and selective inhibitor of METTL13, is a new treatment for myeloid leukemia. Yankova et al. demonstrated the significant anti-leukemic effect of STM2457 on AML cells and patient-derived xenografts in a mouse, such as impaired cell growth and clonogenic potential and prolonged mouse life span.112

The existing inhibitors of m6A-related factors are generally characterized by low activity, poor specificity, and overly complex effects on cell phenotypes, with undefined mechanisms. However, they have shown high potential for clinical application as treatment options. With the rapid development of drug synthesis and screening technology, targeted m6A modification drugs will be widely used as anti-tumor treatments.

4.2 m6A in PC immunotherapy

Accumulating evidence has suggested that m6A may be involved in the immune microenvironment and immunotherapy in PC. Tang et al. established a predictive model using the expression of three m6A-related genes (RBM15, HNRNPC, and IGF2BP2) that predicts the survival of patients with PC. Compared with normal pancreatic tissue, PAAD tissue has different expression level and mutation state of m6A-related genes and immune microenvironment. Moreover, the expressions of eight m6A-related genes were correlated with the number of infiltrated CD8+ T cells, suggesting that m6A might participate in the immune microenvironment of PC.113 Fang and colleagues developed an m6A scoring system using the expression value of selected genes to individually quantify m6A modification in PC patients. A cluster with a high m6A score was characterized by reduced overall survival and higher immunogenicity. T cell dysfunction and exclusion scores, which are used to assess the clinical effects of immune checkpoint blockade treatment, were lower in the high m6A score group than the low m6A score group, indicating the potential role of the m6A score in predicting immunotherapy response.114 Another group also constructed an m6A score system. Using principal component analysis, Sun et al. built an m6A scoring scheme to accurately predict m6A modification patterns in individual PC patients. The low m6A score group showed significantly better prognosis, a higher immunogenicity score in the anti-PD-1/CTLA-4 therapy alone or combination therapy groups, and lower PD-L1 expression than the high m6A score group.115

The results gained from databases are preliminary. Further experimental and clinical studies are required to clarify the roles of m6A modification in immunotherapy of PC.

4.3 m6A modification and chemoresistance in PC

Increasing studies have shown a strong association between m6A modification and drug resistance in PC, indicating the potential of targeting m6A in PC drug resistance. Knockdown of METTL3 reversed the resistance of PC cells to 5-FU, CDDP, GEM, and radiation treatment.56 Kong et al. reported that knockdown METTL14 significantly enhanced the apoptosis induced by cisplatin and thus increased the sensitivity of PC cells to cisplatin. Additionally, knockdown METTL14 remarkably facilitates autophagy and in an mTOR signaling-dependent pathway.59 Li et al. found that WTAP promotes metastasis and chemoresistance to gemcitabine in PC.64 Zhang et al. reported that the overexpression of METTL14, resulting from increased phosphorylation of p65, is linked to resistance in gemcitabine-resistant PC cells. Mechanistically, METTL14 induced resistance to gemcitabine by upregulating cytidine deaminase instead of deoxycytidine kinase. Reducing the expression of METTL14 in PC gemcitabine-resistant cell lines significantly increased sensitivity of PC to gemcitabine in vitro and vivo.60 Although more research is still needed to explore the role of m6A in drug resistance in PC, these studies have suggested that targeting m6A modification may provide a new strategy to improve treatment efficacy of PC.

5 CONCLUSION

Previous research has demonstrated the important role of m6A in various physiological and pathological processes, and therefore m6A has gained increasing attention. m6A-related factors are involved in the occurrence and development of various malignant tumors by regulating the splicing, nuclear export, degradation, and translation of mRNAs that encode critical molecules. More studies have been focusing on the mechanisms of m6A modification and identified an
expanding number of m6A-related factors, and these findings have advanced our understanding of m6A modification-related signaling pathways in tumor biology. m6A readers, which represent the largest group of m6A-related factors, can act in opposite ways. For example, YTHDF1 acts as a tumor suppressor in colorectal cancer by facilitating the expression of ankyrin repeat and LEM domain containing 1 (ANKLE1), which inhibits cell proliferation and maintains genetic stability in colorectal cancer. However, YTHDF1 promotes hepatocellular carcinoma carcinogenesis through the FZD5/WNT/β-catenin signaling pathway. More research is needed to clarify how m6A readers selectively recognize and bind to specific RNAs and whether m6A readers exert competitive or synergistic functions. Moreover, the roles of particular m6A-related factors in various cancers can vary. For example, ALKBH5 is a cancer promoter in glioblastoma tumorigenesis while it suppresses the development of PC.

With the advancement of m6A editing tools, programmable m6A modifications have been used to explore the functional roles of site-specific m6A. m6A editing systems, such as dCas9, dCas13, or dCasRx conjugated with either m6A writers or erasers, enable researchers to edit m6A modifications at targeted sites, which is key to understanding the regional effects of m6A modification. Research on site-specific m6A modifications will be a new research hotspot.

Although many studies have confirmed the close association between m6A and the development of PC, our understanding of the role of m6A in PC is still far from adequate. Further research needs to focus on identifying strategies for early diagnosis of PC through m6A modification and the clinical application of inhibitors of m6A-related factors.

ACKNOWLEDGEMENTS
This study was supported by National Natural Science Foundation of China (82102482, 82072371), Leading Talent Project of Shanghai Huangpu District (2020-1-28), Shanghai Health and Medical Development Foundation under Grant (201972), and Shanghai Science and Technology Committee under Grant (21ZR1478200).

CONFLICT OF INTEREST
The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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How to cite this article: Chen S, Ren H, Zhang X, et al. Research advances of N6-methyladenosine in diagnosis and therapy of pancreatic cancer. *J Clin Lab Anal*. 2022;36:e24611. doi: 10.1002/jcla.24611