

Role of N⁶-methyladenosine in the pathogenesis, diagnosis and treatment of pancreatic cancer (Review)

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Abstract. Pancreatic cancer (PC) ranks as the seventh leading cause of cancer-associated mortality, and is predicted to become the third leading cause of cancer-associated mortality by the year 2025. Although advanced modalities of diagnosis and treatment have been continuously emerging, the mortality rate (466,003) approximated to that of the morbidity rate (495,773) in 2020. N⁶-methyladenosine (m⁶A) has been shown to be methylated on the sixth N atom of adenine in RNA, which occurs co-transcriptionally and serves to regulate gene expression post-transcriptionally. The discovery of m⁶A has heralded a new era in the scientific investigation of PC. In the present review article, the classical conception of m⁶A and emerging hypotheses regarding its role are summarized, and the function of m⁶A in carcinogenesis and progression of PC is then discussed, followed by the potential roles of m⁶A in the diagnosis of PC and in therapeutic applications. However, this new era is only at the initial stages, and the extent to which m⁶A influences PC is still poorly understood. In view of this, the present review article also summarizes the developments at the frontier of the interaction between m⁶A and PC, and discusses strategies through which m⁶A may provide a promising avenue for anticancer therapy.

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1. Introduction

Pancreatic cancer (PC) is the most common type of malignancy affecting the digestive system, which has the characteristics of 'three low and three high'; that is, a low early diagnosis rate, a low resection rate, a low 5-year survival rate, and a high incidence, a high metastasis rate and a high mortality rate. Owing to the deficiency of specific symptoms during the early stages of the malignancy, the early diagnosis rate of PC is <5%. At the first visit, only 15-20% of patients can undergo radical resection, 30-40% of patients present with locally advanced cancer, whereas 40-50% of patients have distant metastasis. The 5-year survival rate has been found to be 7.2-9.0% (1,2). The early detection, early diagnosis and early treatment of PC has always been a pivotal and difficult challenge for PC. Hence, there is an urgent need to elucidate the underlying mechanisms of its pathogenesis, and to develop novel techniques with a greater diagnostic efficacy.

A multitude of previously published articles in the literature have demonstrated that N⁶-methyladenosine (m⁶A) not only participates in physiological processes, including neurogenesis, hematopoiesis, spermatogenesis, sex determination, response to DNA damage or heat shock, circadian rhythm, and the innate and adaptive immune response (3-7), but that it is also tightly associated with the initiation, progression (8,9), diagnosis and treatment of neoplasms (10). Accordingly, the initial part of the present review article will introduce the biological functions of m⁶A; subsequently, the present review will interpret the attributes of m⁶A in the carcinogenesis and development of PC, and finally, the prospective clinical applications of m⁶A in PC will be discussed.

2. Biology of m⁶A

Thus far, diverse chemical modifications have been detected, and m⁶A remains the most abundant post-transcriptional modification to be found in messenger RNAs (mRNAs) (3). The modification of m⁶A comprises methylation at the sixth N atom of adenine in RNA, and approximately one third of mammalian mRNAs, with an average of 3-5 m⁶A sites per mRNA, have been shown to feature this modification (11,12). m⁶A was first discovered in 1974 (13-15). The concept of reversible m⁶A modification was proposed in a stepwise manner owing to the discovery of the first methyltransferase, namely methyltransferase-like protein 3 (METTL3) in 1997 (16), and

the first demethylase, fat mass and obesity-associated protein (FTO), in 2011 (17).

In order to more clearly elucidate the functions of m⁶A, scientists have been seeking improvements in the available m⁶A-mapping methods. Meyer *et al.* (18) combined m⁶A-specific methylated RNA immunoprecipitation with next-generation sequencing (MeRIP-Seq) to depict the landscape of m⁶A at the whole transcriptome level in 2012, which heralded the creation of the ‘epitranscriptome’ (18). Since then, m⁶A has attracted extensive attention in the research community. MeRIP-Seq is able to discern the distribution of m⁶A at the single transcript level, and identify which groups of transcripts are modified by m⁶A. However, it has not been successful at counting the precise stoichiometry of m⁶A sites; neither has it been able to distinguish m⁶A from 6,2,-O-dimethyladenosine (m⁶Am). The molecular structure of m⁶Am is similar to that of m⁶A: Compared with m⁶A, the 2'-hydroxy group of adenylyl is methylated to produce this doubly methylated nucleotide.

M⁶A is conserved in eukaryotes (19-23), and m⁶A sites are preferentially enriched near stop codons, at 3'-untranslated regions (3'-UTRs) or in long exons, and they are prone to recognize a typical consensus sequence, RRACH (where ‘R’ is A/G, and ‘H’ is A/C/U) (4,16,18,21,24). Notably, m⁶A is specifically located in genes associated with development or cell fate; seldom are m⁶A sites to be found in housekeeping genes (18,24-26). Although the majority of research efforts thus far have focused on mRNAs, researchers have also identified m⁶A sites on ribosomal RNAs (rRNAs) (27,28) and transfer RNAs (tRNAs) (29). In addition, m⁶A sites have been identified in non-coding RNAs (ncRNAs), which cannot be translated into proteins, but have essential functions in gene expression and regulation, as well as long ncRNAs (lncRNAs) (30,31), microRNAs (miRNAs/miRs) (32,33), circular RNAs (circRNAs) (34), small nuclear RNAs (snRNAs) (35,36) and small nucleolar RNAs (snoRNAs) (37).

M⁶A is added by methyltransferases (termed ‘writers’), removed by demethylases (termed ‘erasers’) and identified by m⁶A-RNA-binding proteins (termed ‘readers’), which are able to affect the fate of m⁶A-RNAs, and then mediate post-transcriptional gene expression (Fig. 1 and Table I).

Writers. S-adenosyl-L-methionine (SAM) has emerged as the predominant methyl donor *in vivo*. Writers are considered to participate in a methyltransferase complex (MTC), which transfers methyl groups of SAM to target RNA in a highly specific manner. The MTC comprises METTL3, methyltransferase-like protein 14 (METTL14), an adaptor [Wilms' tumor 1-associating protein (WTAP)], a WTAP interactor [Vir-like m⁶A methyltransferase-associated (VIRMA; also known as KIAA1429)], RNA-binding motif protein (RBM)15/15B, zinc finger CCCH-type containing 13 (ZC3H13) and Casitas B lineage lymphoma transforming sequence like protein 1 [CBLL1/E3 ubiquitin-protein ligase (HAKAI)] (38). The members of this multi-subunit complex will be introduced in turn.

METTL3 and METTL14. METTL3 is the catalytic subunit of the MTC that has a SAM-binding motif and transfers methyl groups to RNA (16,39,40). METTL14 is an allosteric activator that has an interrupted SAM-binding motif, demonstrating that METTL14 is catalytically inactive (41,42). Together,

METTL14 and METTL3 form a heterodimer complex (43), which participates in recognizing substrate RNA and enhancing methyltransferase activity (44). In the absence of METTL3 or METTL14, mouse embryonic stem cells have been shown to exhibit a decrease of 99% in their m⁶A content (26). In addition to the METTL3-METTL14 heterodimer complex, there are several other critical methylation enzymes. For example, ZCCHC4 adds a methyl group on to adenylyl located in the 28S rRNA subunit (27), and METTL5-TRMT112 heterodimer complex modifies the 18S rRNA subunit (28). METTL16 independently catalyzes the formation of m⁶A in the pre-mRNAs, U6 snRNA and lncRNA.

WTAP. WTAP is the key METTL3 adaptor (43,45), which anchors METTL3-METTL14 heterodimer complex to nuclear speckles (46). Upon deletion of WTAP, the affinity of METTL3 to bind RNA is clearly decreased, indicating that WTAP recruits the METTL3-METTL14 heterodimer complex to target mRNAs (45).

VIRMA/KIAA1429. VIRMA/KIAA1429 contributes towards the modification preference of m⁶A for 3'-UTRs and in the vicinity of the stop codon. A model has been proposed wherein VIRMA may function as a scaffold to hold WTAP/HAKAI/ZC3H13 together and creates a suitable pocket mainly through WTAP to accommodate METTL3/METTL14 in order to guide m⁶A modification in the 3'UTR and around the stop codon. The 3'UTR has been shown to be shortened by VIRMA through its interactions with the polyadenylation cleavage factors, CPSF5 and CPSF6 (47).

RBM15/15B. lncRNA X-inactive specific transcript (XIST) regulates epigenetic silencing on the X chromosome. RBM15 and its paralogue, RBM15B, bind to and recruit the m⁶A methylation complex to specific sites in lncRNAs, thereby mediating the formation of m⁶A in XIST (48).

ZC3H13. ZC3H13 is considered to bridge RBM15/RBM15B to WTAP (49). The ZC3H13-WTAP-VIRMA-HAKAI complex has a profound effect on the regulation of m⁶A in RNAs. ZC3H13 plays the role of anchoring this regulatory complex in the nucleus to facilitate m⁶A modification (50).

CBLL1/HAKAI. In concert with E-cadherin, CBLL1 (also known as HAKAI) modulates cell adhesion and epithelial-mesenchymal transition (EMT) (51). The knockdown of HAKAI has been shown to reduce m⁶A levels (52). EMT provides a critical foundation for tumor metastasis, suggesting that m⁶A may be closely associated with tumor progression.

Erasers. Erasers are demethylases that catalyze the removal of the methyl group, thereby converting m⁶A into adenosine (A). At present, however, the roles of erasers under physiological conditions appear to be limited. Erasers including FTO and alkB homolog 5 (ALKBH5). ALKBH5 have been identified in a limited number of tissues such as testicles. They are also active under disease- and stress-associated conditions.

FTO. Consistently with ALKBs demethylating DNA, FTO demethylates m⁶A in mRNA in a 2-oxoglutarate (2OG)-Fe²⁺ oxygenase-dependent manner (17). Nevertheless, compared with ferrous iron, the interaction with m⁶A is stronger, and therefore, the demethylation effect of FTO in m⁶Am is more efficient, which indicates that lower substrate concentrations are required and the reaction times are shorter. Furthermore, FTO exhibits differential substrate preferences: It can demethylate m⁶Am at

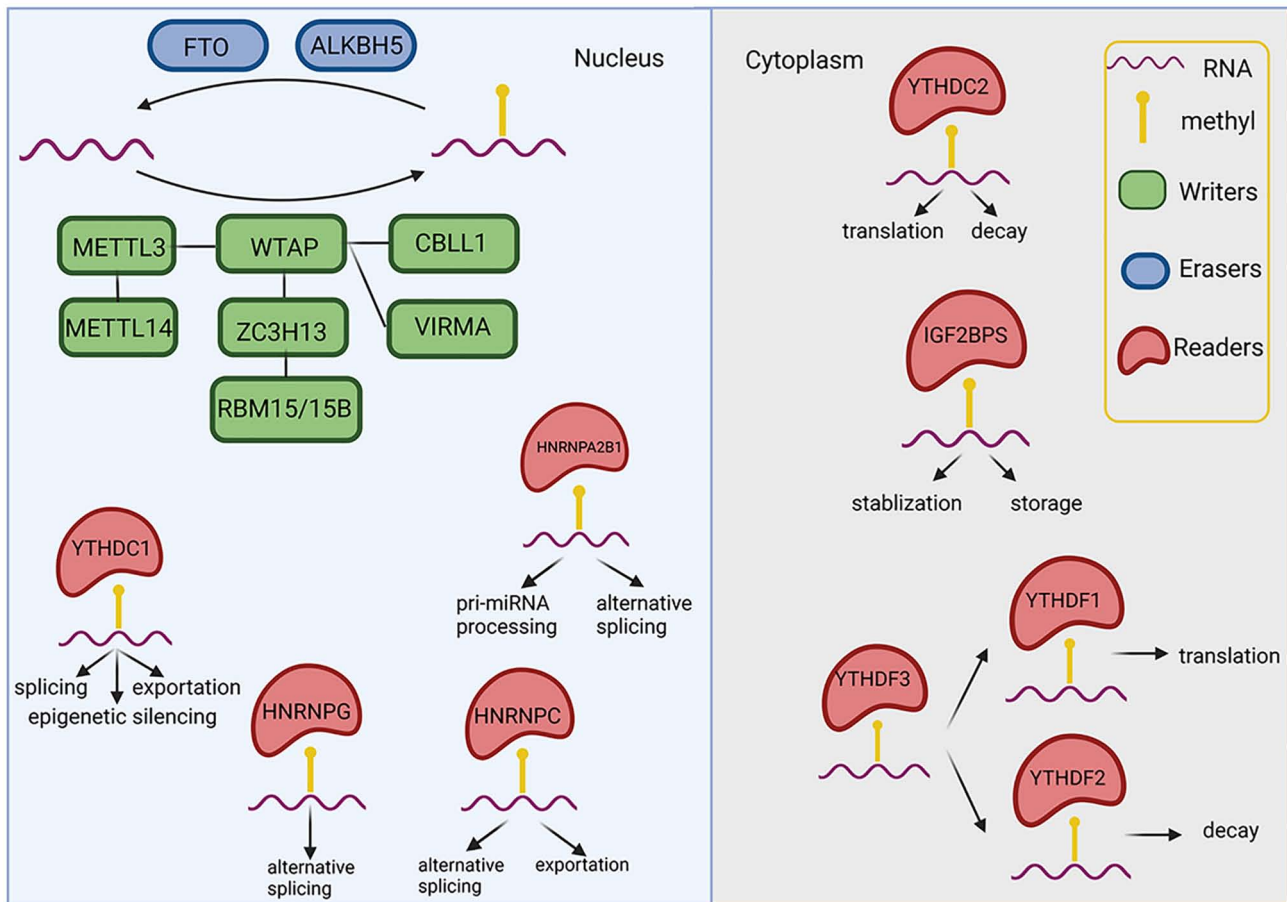


Figure 1. The biological roles of N⁶-methyladenosine. FTO, fat mass and obesity-associated protein; ALKBH5, alkB homolog 5, RNA demethylase; METTL, methyltransferase-like protein; WTAP, Wilms' tumor 1-associating protein; CBLL1, Casitas B lineage lymphoma transforming sequence like protein 1; ZC3H13, zinc finger CCCH-type containing 13; VIRMA, Vir-like m⁶A methyltransferase-associated; RBM15, RNA-binding motif protein 15; YTHDC, YT521-B homology domain-containing protein; HNRN, heterogeneous nuclear ribonucleoprotein; IGF2BPS, insulin-like growth factor-2 mRNA-binding proteins. The figure was created using <https://biorender.com>.

the 5'-cap or m⁶As in the inner region of, mRNAs; m⁶Am at the 5'-cap or in the inner regions of snRNAs; m⁶A in the inner regions of U6 RNA; and N¹-methyladenosine in tRNAs (53). It will be noteworthy to analyze this divergence in greater depth, and to identify which substrate(s) FTO truly catalyzes.

ALKBH5. ALKBH5 is also a 2OG-Fe²⁺-dependent oxidative demethyltransferase that demethylates m⁶A in mRNAs (54). Its expression is particularly enriched in the testes, where it fulfills a role in spermatogenesis (55). Moreover, the m⁶A level in the mRNA of male mice wherein ALKBH5 has been knocked down has been shown to be increased (54). These mice have been shown to be infertile, which is considered to have resulted from the apoptosis of meiotic metaphase-stage spermatocytes (54). FTO and ALKBH5 share the same sequential oxidative demethylation reaction process, which is considered to be as follows: m⁶A to N⁶-hydroxymethyladenosine to N⁶-formyladenosine to A (56).

Readers. A myriad of writers and erasers can reversibly add or remove methyl groups in target mRNA, which exerts a major influence in the fate of target mRNAs. When m⁶A-mRNA binds readers in the nucleus, this may have an impact on mRNA splicing. Once exported to the cytoplasm, m⁶A-mRNA may be recognized by various cytosolic readers, thereby influencing

the stability, translation and localization of mRNA (3). Readers comprise YTH N⁶ methyladenosine RNA binding proteins (YTHDF1/2/3), YT521-B homology domain-containing proteins (YTHDC1/2), heterogeneous nuclear ribonucleoproteins (HNRNPC/G/A2B1) and the insulin-like growth factor-2 mRNA-binding proteins (IGF2BP)-1, -2 and -3.

YTHDF1. YTHDF1 has been reported to participate in expediting the processes of translation initiation and DNA damage repair. YTHDF1 promotes the loading of ribosomes to bound mRNAs to enable the interactions with initiation factors (such as eIF3) to occur, thereby promoting m⁶A-mRNA translation initiation (57,58). Double-strand breaks (DSBs) are responsible for the most severe type of DNA damage; the METTL3-m⁶A-YTHDC1 axis has been shown to enhance the deposition of DNA-RNA hybrids at DSB sites, which enables the recruitment of breast cancer type 1 susceptibility protein and DNA repair protein RAD51 homolog 1 (RAD51) for the homologous recombination (HR)-mediated repair of DSBs (59).

YTHDF2 and YTHDF3. YTHDF2 is the most abundant protein of domain family (DF) paralogues purified from cells (60), and is crucial for the degradation of mRNA. At present, there are two prevailing hypotheses concerning the underlying mechanism. A large number of studies have confirmed that m⁶A erects a scaffold that juxtaposes DF1/2/3, enhancing the

Table I. Overview of m⁶A writers, frasers andreaders.

| Type of m ⁶ A and protein name | | | Location | Function | (Refs.) |
|---|--------------------|-------------|--------------------------|---|---------------------|
| Writers | METTL3 | | Nuclear speckle | Catalytic subunit, transfer methyl group from SAM to target RNA | (16,26,39,40,43,44) |
| | METTL14 | | Nuclear speckle | Allosteric activator, catalytically inactive recognize substrate RNA and enhance METTL3 activity | (26,41-44) |
| | WTAP | | Nucleus | METTL3 adaptor anchor METTL3-METTL14 heterodimer complex to nuclear speckles and target mRNA | (43,47,48) |
| | VIRMA/ KIAA1429 | | Nucleus | WATP interactor, modification preference of m ⁶ A in 3,UTR and near stop codon shorten 3,UTR | (49) |
| | RBM15/15B | | Nucleus | WATP interactor, regulate genetic transcription silencing | (50) |
| | ZC3H13 | | Nucleus | WATP interactor, bridge RBM15/15B toWTAPanchor ZC3H13-WTAP-VIRMA-HAKAI complex in the nucleus to facilitate m ⁶ A modification | (51,52) |
| Erasers | CBLL1/ HAKAI | | Nucleus | WATP interactor, modulate cell adhesion and EMT in cancer | (53,54) |
| | FTO | | Nucleus | Demethylate m ⁶ A/m ⁶ Am | (17,55) |
| | ALKBH5 | | Nucleus | Demethylate m ⁶ Ain the testes and play a role in spermatogenesis | (56-58) |
| Readers | YTHs | YTHDF1 | Cytoplasm | Expedite translation initiation | (59-61) |
| | | | | DNA damage repair LLPS | |
| | | YTHDF2 | Cytoplasm | Degrade mRNA, LLPS | (62-67) |
| | | YTHDF3 | Cytoplasm | Assist YTHDF1 and YTHDF2 LLPS | (68) |
| | | YTHDC1 | Nucleus | mRNA splicing nuclear exportation | (50,69-74) |
| | | YTHDC2 | Nucleus/ cytoplasm | epigenetic silencing autophagy Spermatogenesis elevate the translation efficiency decrease mRNA abundance | (75) |
| | HNRNPs | HNRNPC | Nucleus | Regulate alternative splicing mRNA,U snRNA exportation, m ⁶ A-switch | (3,35,77-80) |
| | | HNRNPG | Nucleus | Regulate alternative splicing m ⁶ A-switch | (81,82) |
| | | HNRNPA2B1 | Nucleus | Pri-miRNA processing regulate alternative splicing vesicular trafficking | (83,84) |
| | IGF2BPs | IGF2BP1/2/3 | Nucleus and cytoplasm | Stabilization and storage of mRNA in physiological condition and stress response human senescence | (80,85) |

SAM, S-adenosyl-l-methionine; 3'-UTRs, 3'-untranslated regions; EMT, epithelial-mesenchymal transition; m⁶Am, 2-O-dimethyladenosine; LLPS, liquid-liquid phase separation; METTL, methyltransferase like protein; WTAP, Wilms' tumor 1 associating protein; VIRMA, Vir like m⁶A methyltransferase associated; RBM15, RNA binding motif protein 15; ZC3H13, zinc finger CCCH type containing 13; CBLL1, Casitas B lineage lymphoma transforming sequence like protein 1; FTO, fat mass and obesity associated protein; ALKBH5, alkB homolog 5, RNA demethylase; YTHDC, YT521 B homology domain containing protein; HNRN, heterogeneous nuclear ribonucleoprotein; IGF2BPS, insulin like growth factor 2 mRNA binding proteins; YTHDF, YTH N6 methyladenosine RNA binding protein.

liquid-liquid phase separation (60-63) of RNA-protein droplets, which are subsequently partitioned into membrane-less compartments, such as processing bodies (P-bodies: mRNA decay sites) (64), or into stress granules during stress. In this

manner, mRNAs may be either degraded or stored (60-63). Furthermore, the N-terminal region of YTHDF2 recruits and interacts with the superfamily homology (SH) domain of the CCR4-NOT deadenylase complex to initiate the deadenylation

and degradation of target mRNAs (65). YTHDF3 functions in synergy with YTHDF1 to facilitate translation, thereby assisting YTHDF2 in the degradation of m⁶A-RNA (66).

YTHDC1. YTHDC1 fulfills a variety of roles in mRNA splicing, nuclear exportation, genetic transcription silencing regulated by the lncRNA XIST and autophagy. The nuclear export adaptor proteins, SRSF3 and SRSF10, identified as pre-mRNA splicing factors, competitively bind to YTHDC1. SRSF3 facilitates exon inclusion, whereas SRSF10 promotes exon skipping. YTHDC1 promotes SRSF3, whereas SRSF10 is antagonized by YTHDC1 with respect to their localization, RNA-binding capabilities and associated splicing events (67,68).

In terms of the more detailed mechanism, YTHDC1 interacts with SRSF3, thereby stimulating m⁶A-mRNA binding to SRSF3 and the mRNA export receptor, nuclear RNA export factor 1 (NXF1), which directs mRNAs into the nuclear export pathway (69). SRSF3 stands at the point of intersection between splicing and nuclear exportation, indicating that there may be some connection between them. Another study revealed that the m⁶A methylation complex stimulates the recruitment of transcription export (TREX) to target mRNAs, and TREX then additionally recruits YTHDC1 and NXF1 to trigger the effective exportation of mRNA (70). As aforementioned, RBM15 and RBM15B interact with the lncRNA XIST A-repeat region, targeting the methylation complex to lncRNA XIST, and mediating the formation of m⁶A in lncRNA XIST (48). M⁶A sites that are then identified by YTHDC1, contributing towards expediting the process of X-chromosome silencing (71). Additionally, YTHDC1 modulates autophagy by modulating the stability of nuclear mRNA of sequestosome-1, resulting in the postponement of wound healing (72).

YTHDC2. The function of YTHDC2 is currently poorly understood, although it may be instrumental to the process of spermatogenesis, elevating translation efficiency and decreasing mRNA abundance. YTHDC2 is enriched in the testes, and *Ythdc2*^{-/-} mice have been shown to be infertile (73). Since YTHDC2 has been detected in the 40-80S ribosomal fraction, it can be hypothesized that YTHDC2 may interact with translation initiation elements to accelerate translation efficiency. YTHDC2 may recruit nonsense-mediated degradation machineries (including the helicases UPF1 and MOV10, and the exoribonuclease XRN1) to promote the degradation of certain mRNAs co- or post-translation (73). Further, in-depth studies are required, however, to reveal the function of YTHDC2 in m⁶A.

HNRNPC. HNRNPC has been implicated in alternative splicing, mRNA and uridine-rich snRNA (U snRNA) exportation, and the so-called 'm⁶A switch' mechanism, in which m⁶A induces RNA unfolding and increases the accessibility of HNRNPs to single-stranded RNA. HNRNPC controls pre-mRNA processing via the regulation of alternative splicing. The individual-nucleotide resolution UV-cross-linking and immunoprecipitation method has revealed that the position of HNRNPC determines its impact on the inclusion of alternative exons. Specifically, when HNRNPC binds to exons, alternative exons are silenced; however, when HNRNPC binds to introns, the inclusion of exons is enhanced (74). The exportation of mRNAs and U snRNAs has also been shown to be associated with HNRNPC. HNRNPC functions as a 'molecular ruler'

to measure the length of the RNA polymerase II transcripts, and is involved in the sorting of transcripts into mRNA or U snRNA, depending on whether or not they are longer than the threshold length (200-300 nucleotides), leading to the export of these two different RNA species from the nucleus (75). Researchers have discovered that m⁶A modifies partial structures in mRNAs or lncRNAs, which exposes buried RBMs and increases the accessibility of HNRNPC to m⁶A-RNA, the process that is termed as the 'm⁶A-switch' (76). This structural alteration has been shown to contribute to the metastasis of PC cells, adding a new dimension to the manner in which m⁶A regulates gene expression.

HNRNPG. HNRNPG also regulates alternative splicing and the 'structural switch'. HNRNPG depends upon the Arg-Gly-Gly repeat sequence within its low-complexity region to interact with nascent m⁶A-mediated pre-mRNA and the phosphorylated C-terminal domain of RNA polymerase II, which ultimately regulates alternative splicing (77). M⁶A modification promotes the binding of HNRNPG to target RNAs in virtue of the 'structural switch' (78).

HNRNPA2B1. HNRNPA2B1 stimulates primary miRNA (or pri-miRNA) processing, participates in alternative splicing and has an essential function in vesicular trafficking. DiGeorge syndrome critical region 8 (DGCR8) serves as the miRNA microprocessor complex protein, and HNRNPA2B1 interacts with DGCR8 in order to facilitate pri-miRNA processing. In addition, HNRNPA2B1 is involved in alternative splicing (79). HNRNPA2B1 sorts specific miRNAs and lncRNAs into exosomes or microvesicles, thereby fulfilling a pivotal role in the selection of cancer-associated miRNAs and lncRNAs, emphasizing the function of HNRNPA2B1 in cancer-associated vesicular trafficking (80).

IGF2BPs. IGF2BPs participate in the stabilization and storage of mRNAs under various physiological conditions and during the stress response. The interaction between the KH3-4 di-domain of IGF2BPs and the 'GGAC' motif in m⁶A promotes the stability and storage of certain mRNAs during normal or stress conditions (81). In addition, IGF2BP2 (also known as IMP2) plays a crucial role in human senescence by recognizing and stabilizing MIS12 mRNA (82).

New readers. Recently, Wu *et al* (82) reported that proline-rich coiled-coil 2A was discovered as a new m⁶A reader that regulates myelination and oligodendroglial specification. Furthermore, Fragile X messenger ribonucleoprotein 1 (FMR1) may function as a sequence-context-dependent reader, negatively regulating mRNA translation by stalling the process of ribosome translocation. YTHDF1 and FMR1 competitively bind to m⁶A sites on mRNA. The stress granule protein G3BP is an anti-m⁶A reader that is repelled by m⁶A, enhancing the stabilization of m⁶A-modified mRNA (83).

3. Roles of m⁶A in the carcinogenesis and development of PC

As aforementioned, m⁶A is understood to play a pivotal role in post-transcriptional modification, modulating protein expression and ultimately affecting the structure and function of modified proteins in key signaling pathways. Numerous studies have confirmed that m⁶A is implicated in the proliferation, apoptosis, invasion, metastasis, EMT, metabolism and

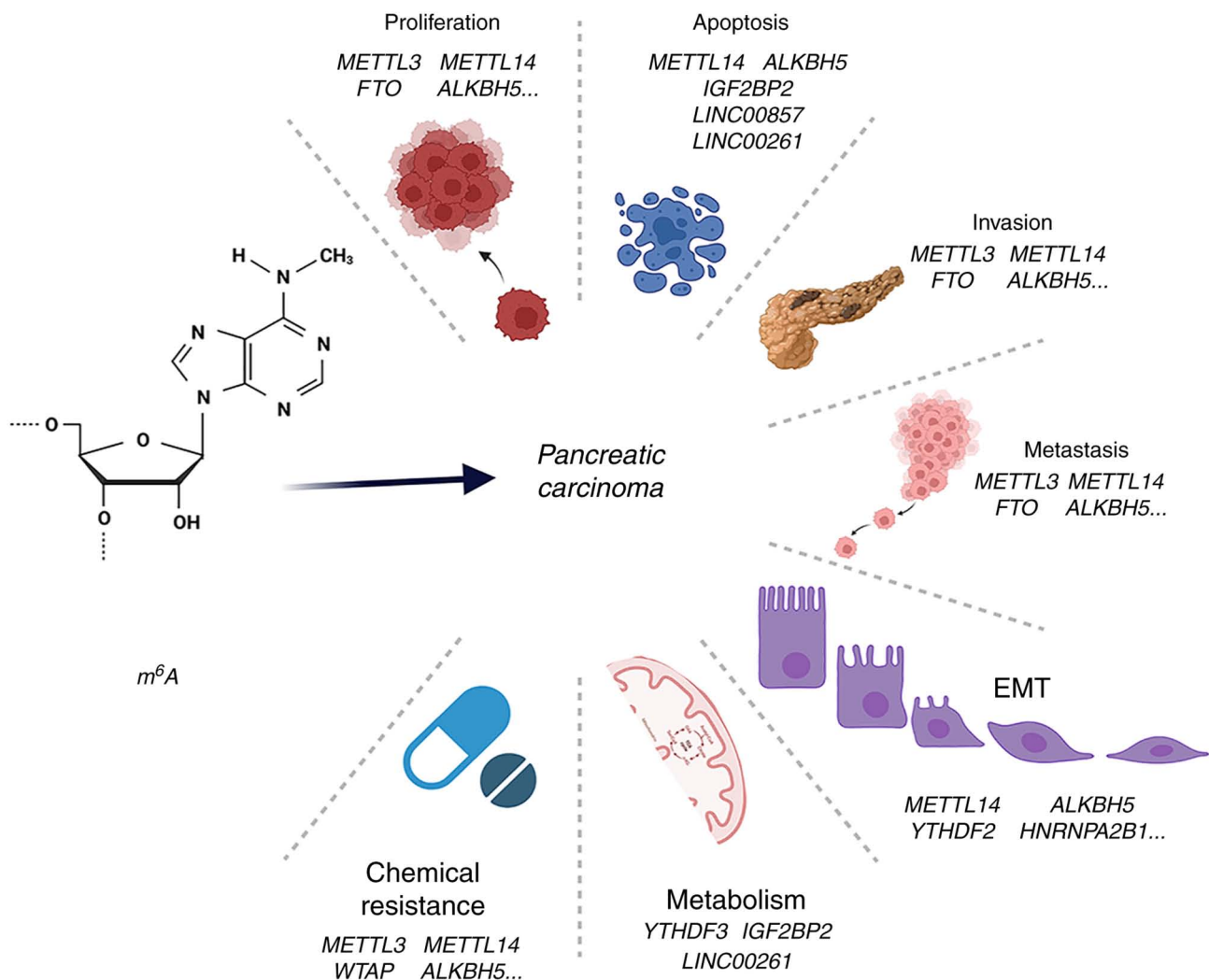


Figure 2. Role of m^6A in carcinogenesis and the development of pancreatic cancer. m^6A , N⁶-methyladenosine; METTL, methyltransferase-like protein; ALKBH5, alkB homolog 5, RNA demethylase; FTO, fat mass and obesity-associated protein; YTHDF, YTH N6 methyladenosine RNA binding protein; WTAP, Wilms' tumor 1-associating protein; IGF2BPS, insulin-like growth factor-2 mRNA-binding proteins. The figure was created using <https://biorender.com>.

chemoresistance of PC. m^6A participates in the initialization and progression of PC through altering the levels of m^6A regulators and genetic variants, and also serves an important role in aberrant alternative splicing. Consequently, in the subsequent section of the review, these studies are evaluated to disclose the function of m^6A in tumor onset and development (Fig. 2 and Table II).

Proliferation and apoptosis. By maintaining proliferation and evading subsequent apoptotic processes, m^6A effectively stimulates the progression of PC. Recently, there has been a growing understanding that m^6A modulates the proliferation and apoptosis of PC cells via alterations in the levels of both m^6A regulators and lncRNAs, and genetic variants.

A large number of studies have focused on the upregulation and oncogenic functions of METTL3 in PC. For example, cigarette smoke condensate (CSC) has been shown to induce the hypomethylation of the METTL3 promoter, which contributes to the overexpression of METTL3. Enhanced m^6A modifications caused by elevated levels of METTL3 lead to excessive pri-miR-25 maturation. PH domain leucine-rich repeat protein

phosphatase 2 (PHLPP2) is suppressed by miR-25-3p, which stimulates AKT-p70S6K signaling and ultimately promotes the proliferation of PC cells (84). In addition, the overexpression of nucleobindin 1 (NUCB1; a calcium-binding protein) has been shown to suppress cell proliferation, whereas METTL3 down-regulates the expression of NUCB1 in a YTHDF2-dependent manner (85). Taken together, these studies reveal that METTL3 is implicated in promoting oncogenic pri-miR-25 maturation and suppressing expression of the tumor suppressor NUCB1 to exert its oncogenic effects.

Reverse transcription-PCR and western blot analyses have been used to reveal that, compared with adjacent normal tissue samples, METTL14 expression is upregulated in PC samples. The protein p53 effector related to PMP-22 (PERP) functions as a p53 effector, which regulates apoptosis induced by DNA damage. The upregulation of METTL14 leads to an elevation in the m^6A level, which causes a decrease in the expression of PERP, promoting cell growth and inhibiting apoptosis (86). Hence, METTL14 has been regarded as an oncogenic regulator in PC. In addition to METTL3-METTL14, other methylation enzymes are also involved in the modulation

Table II. Role of m⁶A in the initiation and progression of pancreatic cancer.

| Initiation factor | Oncogenicity/ suppressor | Signaling or target | Effect | (Refs.) |
|-------------------|-----------------------------|--|--|---------|
| METTL3 | Oncogenicity↑ | | Proliferation↑ Metastasis↑ Invasion↑ | (103) |
| | | CSC-METTL3↑-miR-25-3p↑- PHLPP2↓-AKT-p70S6K↑ | Proliferation↑ Invasion↑ Metastasis↑ | (84) |
| | | METTL3↑-NUCB1↓ | Proliferation↑ Chemical Resistance↑ | (85) |
| | | | Chemical Resistance↑ | (112) |
| METTL14 | Oncogenicity↑ | METTL14↑-PERP↑ | Proliferation↑ Apoptosis↓ Invasion↑ Metastasis↑ EMT↑ | (86) |
| | | p65-METTL14↑-CDA↑ | Chemical Resistance↑ | (113) |
| | | METTL14↑- apoptosis; METTL14↑-mTOR- autophagy↓ | Apoptosis↓ Chemical Resistance↓ | (114) |
| METTL6 | Suppressor↓ | METTL6↓-P21 pathway↓ | Proliferation↓ | (87) |
| METTL5 | Oncogenicity↑ | METTL5↑-c-myc translation↑ | Proliferation↑ Invasion↑ Metastasis↑ | (88) |
| WTAP | Oncogenicity↑ | WTAP↑-mRNA↑ | Metastasis↑ Chemical resistance↑ | (104) |
| FTO | Suppressor↓ | FTO↓-YTHDF2-PJA2↓-Wnt signaling↑ | Proliferation↓ Invasion↓ Metastasis↓ | (89) |
| FTO | Oncogenicity↑ | | Proliferation↑ | (90) |
| ALKBH5 | Suppressor↓ | ALKBH5↓-KCNK15-AS1↓ | Apoptosis↑ Invasion↓ Metastasis↓ | (92) |
| | | ALKBH5↓-lncRNA KCNK15-AS1↓-MDM2- REST ubiquitination↓- PTEN↓-AKT pathway↑ ALKBH5↓-PER1↓-ATM- CHK2-P53/CDC25C↓ ALKBH5↓-WIF-1↓-Wnt signaling↑ | Apoptosis↑ invasion↓ metastasis↓ EMT↓ Proliferation↓ Invasion↓ Metastasis↓ Proliferation↓ Invasion↓ Metastasis↓ Chemical resistance↓ | (93) |
| | | ALKBH5↓-FBXL5↓- degradation↓ | Apoptosis↑ | (95) |
| YTHDF2 | Oncogenicity↑ | YTHDF2↑-YAP↓ | Proliferation↑ Invasion↓ Metastasis↓ EMT↓ | (105) |
| YTHDF3 | Oncogenicity↑ | YTHDF3↑- lncRNA DICER1- AS1↓-DICER1↓- maturation of miR-5586-5p↓ | Proliferation↑ Metastasis↑ Metabolism | (110) |
| HNRNPA2B1 | Oncogenicity↑ | HNRNPA2B1-ERK/snail-E- cadherin↓N-cadherin and vimentin↑ | Invasion↑ EMT↑ | (106) |
| IGF2BP2 | Oncogenicity↑ | IGF2BP2↑-DANCR↑ | Proliferation↑ | (96) |
| | | IGF2BP2↑-mRNA GLUT1↑ | Proliferation↑ Metabolism | (111) |
| | | IGF2BP2↑- protein IMP2↑ | Apoptosis↑ Metastasis↑ EMT↑ | (107) |

Table II. Continued.

| Initiation factor | Oncogenicity/ suppressor | Signaling or target | Effect | (Refs.) |
|-----------------------------|-----------------------------|---|---|---------|
| LINC00857 | Oncogenicity↑ | LINC00857↑-miR-150-5p↓-E2F3↑ | Proliferation↑ Apoptosis↓ | (97) |
| LINC00261 | Suppressor↓ | LINC00261↓-IGF2BP1↑-c-Myc↑ | Proliferation↑ Apoptosis↓ Metabolism | (98) |
| | | LINC00261↓-miR-222-3p/HIPK2/ERK↑-c-myc↑ | Proliferation↑ Apoptosis↓ Metabolism | (98) |
| rs7495 genetic variant | | hnRNPC↑ | Proliferation↑ | (100) |
| | | rs7495G allele-has-miR-183-3p-hnRNPC↑ | Proliferation↑ | (99) |
| rs142933486 genetic variant | | PIK3CB↑ | Proliferation↑ Metastasis↑ | (101) |
| CLK1↑ | | METTL14 ^{exon 10} skipping↓ | Metastasis↑ | (108) |
| | | Cyclin L2 ^{exon 6,3} skipping↑ | Proliferation↑ | (108) |
| HNRNPC | Structural switch | TAF8L↓ | Metastasis↑ | (109) |
| | | TAF8S↑ | Metastasis↑ | (109) |

‘↑’ represents upregulation and ‘↓’ represents downregulation. EMT, epithelial-mesenchymal transition; CSC, cigarette smoke condensate; NUCB1, nucleobindin 1; PERP, p53 effector related to PMP-22; PJA2, praja ring finger ubiquitin ligase 2; METTL, methyltransferase-like protein; WTAP, Wilms' tumor 1-associating protein; VIRMA, Vir-like m⁶A methyltransferase-associated; RBM15, RNA-binding motif protein 15; ZC3H13, zinc finger CCCH-type containing 13; CBL1, Casitas B lineage lymphoma transforming sequence like protein 1; FTO, fat mass and obesity-associated protein; ALKBH5, alkB homolog 5, RNA demethylase; YTHDC, YT521-B homology domain-containing protein; HNRN, heterogeneous nuclear ribonucleoprotein; IGF2BPS, insulin-like growth factor-2 mRNA-binding proteins; YTHDF, YTH N6 methyladenosine RNA binding protein.

of PC proliferation. METTL16 has been shown to suppress the proliferation of PC cells through the P21 pathway (87). METTL5 specifically catalyzes the methylation of A1832 of 18S rRNA, which is a crucial point in the decoding mechanism, indicating its importance as a regulatory protein in translation. METTL5 has been demonstrated to function as an oncogene that promotes the proliferation of PC cells by increasing c-Myc translation (88).

As regards the functions of demethylation enzymes, the impact of FTO on the proliferation of PC cells remains controversial. Zeng *et al* (89) identified that FTO was a tumor suppressor protein. Reduced FTO expression led to the upregulation of the m⁶A level of praja ringfinger ubiquitin ligase 2 (PJA2), and an enhanced rate of PJA2 degradation was shown to be dependent on YTHDF2, which promoted Wnt signaling, ultimately facilitating the proliferation of PC cells. By contrast, Tang *et al* (90) demonstrated that, when overexpressed, FTO functions as an oncogenic protein. The knockdown of FTO resulted in compromised levels of cell proliferation. The effects that are actually elicited by FTO in PC remain an interesting topic that warrants further investigation. Differently from FTO, another demethylation enzyme, ALKBH5, has over time been shown to function as a tumor suppressor (91-95), often involved in inhibiting the proliferation of PC. ALKBH5 demethylates period circadian regulator 1 (PER1), and it functions in concert with YTHDF2

to activate PER1. The overexpression of PER1 activates the ATM-CHK2-P53/CDC25C signaling pathway, which leads to the inhibition of tumor proliferation. p53 activates the transcription of ALKBH5, which subsequently functions in a feedback loop to regulate the m⁶A level (91). Furthermore, ALKBH5 has been shown to exert an anticancer effect against PC by targeting regulators of ferroptosis. Mechanistically, the ubiquitin ligase FBXL5 mediates the degradation of PC cells via ferroptosis. A previous study demonstrated that not only was ALKBH5 responsible for enhancing the stability of FBXL5, but it also led to upregulation of the expression of FBXL5 and the mitochondrial iron importer SLC25A28, and alternative splicing of SLC25A37 was also increased (95).

Although numerous studies have focused on alterations in the level of m⁶A regulators with respect to their role as initiation factors in the proliferation and apoptosis of PC, to date, relatively few studies have been performed to assess the mechanisms through which m⁶A functions in lncRNAs. Nevertheless, there is sufficient evidence to suggest that lncRNAs also function as initiation factors in the proliferation and apoptosis of PC. First, the lncRNA DANCR was shown to promote the tumorigenesis and proliferation of PC; IGF2BP2 recognizes m⁶A modified at A664 of DANCR, which plays the role of stabilizing DANCR (96). Secondly, LINC00857 has been shown to be overexpressed, thereby promoting the tumorigenesis of PC in an m⁶A-mediated manner (97). The

deletion of LINC00857 clearly hinders cell proliferation and facilitates cell apoptosis. M⁶A is pre-eminently enriched within LINC00857, which enhances its RNA stability, thereby suggesting a role for LINC00857 in modulating the miR-150-5p/E2F3 signaling axis. As a competitive endogenous RNA, LINC00857 exerts its effects through sponging miR-150-5p and upregulating the expression of E2F3 (97). Finally, LINC00261 has been reported to function as a tumor suppressor that inhibits proliferation, and accelerates the rate of apoptosis of PC tissues and cell lines. The EZH2-mediated histone H3K27 trimethylation and DNA hypermethylation both serve to downregulate LINC00261. Mechanistically, on the one hand, the downregulation of LINC00261 leads to the upregulation of the expression of c-Myc by binding to miR-222-3p, thereby activating the HIPK2/ERK/c-Myc signaling pathway. On the other hand, LINC00261 negatively regulates the expression of IGF2BP1, where the overexpression of IGF2BP1 has been shown to enhance c-Myc stability (98). From this example, it may be noted that there are links among m⁶A, DNA modification and histone modification.

Genetic variants, as another type of initiation factor, may provide novel insight into the association between m⁶A regulators and PC. For example, the genetic variant rs7495 in the 3'-UTR region of hnRNPC has been shown to disrupt a presumptive binding site for has-miR-183-3p that promotes the expression of hnRNPC, and which contributes to the susceptibility of PC (99). In another study, cell viability assay indicated that the depletion of hnRNPC hindered the proliferation of PC (100). Similarly, the rs142933486 variant in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (PIK3CB) has been observed to have decreased m⁶A levels, and its expression is increased in a YTHDF2-dependent manner. In addition, the variant of PIK3Cb[T] has been shown to activate AKT signaling, accelerating the proliferation of phosphatase and tensin homolog (PTEN)-deficient PC cells (101).

Generally, it can be seen that alterations in the levels of m⁶A regulators and lncRNAs, together with genetic variants, are intimately involved in the proliferation and apoptosis of PC.

Invasion, metastasis and EMT. As is currently understood, PC is characterized by a high metastatic rate: 40-50% of patients present with distant metastasis in their first hospital visit. Metastasis is a complex process that comprises EMT, the migration of cancerous cells, their infiltration into adjacent tissues, vascular transportation and settlement in distant organs. EMT is defined as the transformation from polar epithelial cells into interstitial cells under specific physiological and pathological conditions. The process is mainly characterized by the loss of epithelial phenotypic molecules (such as E-cadherin) and the acquisition of interstitial cell characteristics (such as vimentin), which enables tumor cells to lose their intercellular adhesive properties and become more loose, thereby gaining the abilities of invasion and metastasis (102). M⁶A regulates the invasion, metastasis and EMT of PC through alterations in the levels of m⁶A regulators, genetic variants and aberrant alternative splicing.

In addition to sustaining proliferation, METTL3 promotes the invasion and migration of PC cells (103). The CSC/METTL3/miR-25-3p/PHLPP2/AKT/p70S6K signaling

axis has been shown to accelerate invasion and metastasis (84). Furthermore, PERP serves as a tetraspan plasma membrane protein, having multifaceted roles in EMT and cell-cell adhesion. Of note, the tumor suppressor function of PERP may involve inhibition of the invasion, metastasis and EMT of PC cells. METTL4 downregulates the expression of PERP through the METTL4/YTHDF2/PERP pathway (86). Furthermore, METTL5 promotes the invasion and migration of PC cells. METTL5 functions as an oncogenic regulator by increasing the translation of c-Myc, whereas the knockdown of c-Myc has been shown to abolish the oncogenic effects induced by the upregulation of METTL5 (88). In addition, both nuclear and cytoplasmic WTAP have been shown to be upregulated, promoting metastasis by modulating the stability of downstream mRNA (104).

Studies on the function of FTO in PC metastasis are relatively limited, although one study documented that FTO facilitates the invasion and metastasis of PC cells via the FTO/YTHDF2/PJA2/Wnt signaling axis (89). Moreover, lncRNA KCNK15-AS1 inhibits the invasion and migration of PC. ALKBH5 is responsible for the demethylation and upregulation of KCNK15-AS1. In PC, ALKBH5 is downregulated, and the expression of KCNK15-AS1, lying downstream in the pathway, is subsequently also downregulated (92). He *et al* (92) also discovered that the overexpression of KCNK15-AS1 suppressed EMT and cell migration in PC. Mechanistically, KCNK15-AS1 was shown to recruit the MDM2 proto-oncogene to promote the ubiquitination of RE1 silencing transcription factor (REST), thereby inactivating the AKT pathway via the transcriptional upregulation of PTEN (93). Furthermore, the upregulation of ALKBH5 has been shown to inhibit tumoral invasive and migrative activities *in vitro*, whereas the depletion of ALKBH5 promotes tumor progression. ALKBH5 demethylates and regulates PER1 in an m⁶A-dependent manner. PER1 itself has the role of reactivating the ATM/CHK2/P53/CDC25C signaling axis, which inhibits the processes of cell invasion and migration (91).

Additionally, the upregulation of YTHDF2 in PC tissues facilitates cell proliferation, and suppresses invasion and migration, a phenomenon that has been defined as the 'migration-proliferation dichotomy'. Moreover, deficiencies in YTHDF2 have been shown to promote EMT, probably via the upregulation of Yes-associated protein 1, and not via TGF- β /Smad signaling in PC cells (105). Furthermore, HNRNPA2B1 has been shown to influence EMT progression in PC via the ERK/Snail signaling pathway. Both *in vitro* and *in vivo*, HNRNPA2B1 downregulates E-cadherin, and upregulates N-cadherin and vimentin to enhance EMT, thereby promoting the invasion of PC cell lines (106). In addition, the IGF2BP2 binding protein, IMP2, has been shown to be upregulated in PC, particularly upon the treatment of Panc-1 PC cells with TGF- β , where the induction of EMT indicates its oncogenic effectiveness (107).

Apart from alterations in m⁶A regulators, the PIK3Cb[T] genetic variant has been shown to promote metastasis of PTEN-deficient PC cells (101). Alternative splicing has also been identified as a novel means through which m⁶A may modulate gene expression. For example, Cdc2-like kinase 1 (CLK1) is an alternative splicing-associated gene that is significantly upregulated in PC tissues. An increased expression

level of CLK1 has been shown to enhance phosphorylation on SR-like splicing factor 5250-Ser (SRSF5250-Ser), which subsequently suppresses the exon skipping of METTL14^{exon10}, but facilitates the exon skipping of cyclin L2^{exoN6.3}. The alternative skipping of METTL14^{exon10} increases the abundance of m⁶A and promotes metastasis. Alternative cyclin L2^{exoN6.3} skipping promotes proliferation (108). Moreover, HNRNPC regulates alternative splicing via the 'm⁶A switch' to accelerate the invasion and liver metastasis of PC. HNRNPC inhibits the anti-metastatic isoform of TAF8 (TAF8L), but promotes the pro-metastatic isoform of TAF8 (TAF8S). Mechanistically, m⁶A increases the likelihood of pre-mRNA of TAF8 to form a linear structure, which increases the accessibility of HNRNPC to TAF8, and upregulates the expression of the pro-metastatic isoform, TAF8S (109).

Above all, various studies, as aforementioned, have described the crucial function of m⁶A in the invasion, metastasis and EMT of PC, and these findings may provide novel prospects for m⁶A therapy in the future.

Metabolism. The initiation and progression of carcinoma requires the reprogramming of metabolism. Tumor cells automatically transform their flux via diverse metabolic pathways to accommodate an elevated bioenergetic and biosynthetic requirement. The influence of m⁶A in altering metabolism has proven to be a rich avenue for scientific exploration. Scientists have presented several mechanisms to explain the mechanisms through which m⁶A may promote metabolism transformation and tumor progression.

For example, bioinformatics analysis has revealed that DICER1 antisense RNA 1 (DICER1-AS1) is overexpressed in PC. DICER1-AS1 recruits the transcription factor YY1 to the promoter of DICER1 in order to expedite the transcription of DICER1. The maturation of miR-5586-5p is promoted by DICER1 to inhibit the expression of glycolytic genes, including LDHA, HK2, PGK1 and SLC2A1. YTHDF3 forms a negative feedback loop with DICER1-AS1 to facilitate glycolysis. Moreover, the upregulation of DICER1-AS1 has been shown to inhibit the proliferation and metastasis of PC (110).

In addition, in order to meet the enhanced metabolic requirements, tumor cells are often observed to upregulate the expression of glucose transporters (GLUTs), such as GLUT1, to augment glucose uptake. IGF2BP2 overexpressed in PC has been shown to increase the rate of aerobic glycolysis and PC cell proliferation by stabilizing GLUT1 mRNA (111). Similarly, the epigenetic silencing of LINC00261 has been found to modulate the miR-222-3p/HIPK2/ERK signaling axis, and IGF2BP1 has been shown to promote c-Myc-mediated aerobic glycolysis (98).

Taken together, the studies published to date in this area have demonstrated that m⁶A plays a role of paramount importance as a regulator of metabolic alterations, and this function appears to play a crucial role in PC.

Chemoresistance. PC is characterized by a highly aggressive progression: Only 15-20% of patients with PC are identified at an early stage, whereas the majority of them (75-80%) exhibit either locally advanced or distant metastasis, and should receive chemotherapy as a first-line treatment. However, in spite of this, numerous patients fail to benefit from the all

the benefits potentially afforded by the therapy due to rapid drug resistance induced by the chemotherapeutic agents. Chemoresistance is a major impediment in terms of the efficacy of therapeutic drugs.

A previous study revealed that the overexpression of NUCB1 activated the antitumor effects of gemcitabine (GEM) by inhibiting the GEM-induced unfolded protein response (UPR) and autophagy; in PC, METTL3-YTHDF2 down-regulated NUCB1 (85). The knockdown of METTL3 has been shown to sensitize PC cells to irradiation and antitumor drugs (namely, 5-fluorouracil, GEM and cisplatin). METTL3 targets various pivotal pathways, including the ubiquitin-dependent pathway and the MAPK signaling cascades. Alterations in these pathways trigger aberrant biological behaviors, which may lead to chemotherapeutic and radiation resistance (112).

Moreover, overexpression of METTL14 is associated with resistance to GEM in PC. Cytidine deaminase (CDA) functions as an enzyme which induces resistance to GEM by inactivating GEM. METTL14 was overexpressed in PC via its promoter region binding to the transcriptional factor p65, thereby increasing the expression of CDA (113). By contrast, the upregulation of METTL14 enhances apoptosis to sensitize PC cells to cisplatin, and also promotes autophagy via an mTOR-dependent signaling pathway (114). WTAP has been identified as an oncogenic regulator, which modulate the stability of downstream mRNA, thereby promoting chemoresistance to GEM (104).

Furthermore, ALKBH5 has been shown to be down-regulated in a patient-derived xenograft model treated with GEM. ALKBH5 upregulates the expression of Wnt inhibitory factor-1, which subsequently suppresses the Wnt pathway. Wnt signaling boosts the resistance of PC cells to GEM. The increased expression of ALKBH5 has also been shown to sensitize PC cells to chemotherapy (94). Taken together, the upregulation of METTL3, METTL14 and WTAP, and the downregulation of ALKBH5 have been shown to lead to resistance to GEM. On the other hand, another study indicated that increased levels of METTL14 sensitized PC cells to cisplatin, and this apparent contradiction is worthy of further exploration in future studies.

Although the various capabilities and properties of m⁶A have been discussed separately in this review, it should be noted that the functional roles of m⁶A in terms of understanding the occurrence and progression of PC are all intrinsically associated, and the process cannot be comprehended by considering the individual capabilities of m⁶A entirely in isolation.

4. Possible clinical applications of m⁶A in PC

m⁶A is closely associated with every stage in the development of PC, and is therefore predicted to be of utmost importance in planning future diagnostic and therapeutic directions for PC. However, to date, a large number of studies have concentrated on the fundamental biological and physiological aspects of m⁶A at the laboratory level, and few studies have focused on translating these findings into clinical practice. Thus, the final section of the present review summarizes the possible clinical applications of m⁶A in PC, in an attempt to broaden the current understanding of this post-transcriptional modification (Table III).

Table III. Role of m⁶A in the pathological stage and poor prognosis of pancreatic cancer.

| Initiation factor | Oncogenicity/ suppressor | Signaling or target | Effect | (Refs.) |
|----------------------|------------------------------------|--|--------------------|---------|
| METTL3 | Oncogenicity↑ | | Pathological stage | (103) |
| | | | Poor prognosis↑ | |
| | | CSC-METTL3↑-miR-25-3p↑- PHLPP2↓-AKT-p70S6K↑ | Poor prognosis↑ | (84) |
| WTAP | Oncogenicity↑ | WTAP↑- mRNA↑ | Poor prognosis↑ | (104) |
| FTO | Suppressor↓ | FTO↓-YTHDF2-PJA2↓- Wnt signaling↑ | Pathological stage | (89) |
| | | | Poor prognosis↓ | |
| ALKBH5 | Suppressor↓ | ALKBH5↓-PER1↓-ATM-CHEK2- P53/CDC25C↓ | Poor prognosis↓ | (91) |
| | | ALKBH5↓-WIF-1↓-Wnt signaling↑ | Poor prognosis↓ | (94) |
| | | ALKBH5↓-FBXL5↓-degradation↓ | Poor prognosis↓ | (95) |
| YTHDF2 | Oncogenicity↑ | YTHDF2↑-YAP↓ | Pathological stage | (105) |
| | | | Poor prognosis↑ | |
| IGF2BP2 | Oncogenicity↑ | IGF2BP2↑-DANCR↑ | Poor prognosis↑ | (96) |
| | | IGF2BP2↑-mRNA GLUT1↑ | Poor prognosis↑ | (111) |
| | | IGF2BP2↑-protein IMP2↑ | Poor prognosis↑ | (107) |
| LINC00857 | Oncogenicity↑ | LINC00857↑-miR-150-5p↓-E2F3↑ | Poor prognosis↑ | (97) |
| CLK1□ | Phosphorylationon SRSF5250-Ser↑ | METTL14 ^{exon 10} skipping↓ | Pathological stage | (108) |
| | | | Poor prognosis↑ | |
| | | Cyclin L2 ^{exon6.3} skipping↑ | | (108) |
| Structural switch | HNRNPC | TAF8L↓ | Pathological stage | (109) |
| | | TAF8S↑ | Poor prognosis↑ | (109) |

‘↑’ represents upregulation and ‘↓’ represents downregulation. CSC, cigarette smoke condensate; PJA2, praja ring finger ubiquitin ligase 2; METTL, methyltransferase-like protein; WTAP, Wilms' tumor 1-associating protein; RBM15, RNA-binding motif protein 15; CLK1, Cdc2-like kinase 1; FTO, fat mass and obesity-associated protein; ALKBH5, alkB homolog 5, RNA demethylase; YTHDC, YT521-B homology domain-containing protein; HNRN, heterogeneous nuclear ribonucleoprotein; IGF2BPS, insulin-like growth factor-2 mRNA-binding proteins; YTHDF, YTH N6 methyladenosine RNA binding protein.

Pathological stage and the poor prognosis. Different levels of m⁶A regulators represent different tumor, node, metastasis (TNM) stages, and therefore different prognoses of PC. Higher levels of expression of the methylase METTL3 are associated with a higher pathological stage (P=0.02) and a higher N stage (P=0.02) (103). METTL3 promotes pri-miR-25 maturation. Excessive levels of miR-25-3p have been detected in PC tissues and smokers, and are associated with a worse prognosis (84). Taken together, it has been demonstrated that the survival rates are shorter in patients with a high level of METTL3 expression (84,103). Furthermore, univariate analysis has demonstrated that the overexpression of nuclear WTAP is associated with a later N stage and poor overall survival (P<0.001) (104).

The decreased expression of the demethylase FTO is indicative of advanced carcinoma stages and the nodal metastasis status. The median survival rate has been found to be significantly shorter within the low-level FTO expression group (16.90 vs. 48.67 months; P=0.0474), with a high level of LINC00857. In brief, patients with decreased levels of FTO have poor survival outcomes (89). In addition, survival analysis has revealed that the lower the level of ALKBH5 expression, the shorter the overall survival rate. Reduced ALKBH5 levels

have been shown to indicate the occurrence of PC, and poor clinicopathological manifestations (91,94,95).

Emerging evidence suggests that readers, such as YTHDF2 and IGF2BP2, are of vital importance for the prognosis of PC. The overexpression of YTHDF2 in PC tissues is associated with worse tumor stages (105). Moreover, IGF2BP2 is characterized as an unfavorable prognostic marker for PC. The overexpression of IGF2BP2 has been shown to be closely associated with a poor overall survival and disease-free survival rate (96,107,111).

As demonstrated in a previous study, the upregulation of LINC00857 activates the miR-150-5p/E2F3 signaling axis to facilitate the tumorigenesis of PC. In that previous study, the overall survival rate [hazard ratio (HR), 1.6; P=0.034] and disease-free survival rate (HR, 1.9; P=0.0046) in the high-expression LINC00857 group were found to be significantly lower (97).

The CLK1/SRSF5 signaling pathway promotes the abnormal exon skipping of METTL14 and cyclin L2 to facilitate the growth and metastasis of PC. In a previous study, univariate analysis suggested that the overexpression of CLK1 was representative of a severe TNM stage, lymphatic metastasis and tumor size (108). An increased CLK1 expression was also

shown to be associated with a lower overall survival rate (108). HNRNPC impedes anti-metastatic alternative splicing events in an m⁶A-dependent manner. The level of HNRNPC has been found to be markedly higher in hepatic metastatic tissues compared with non-metastatic tissues (P<0.01) (109). Kaplan-Meier curves were previously employed to demonstrated that the overall survival rate was significantly lower in the HNRNPC overexpression group (both log-rank P<0.05). As aforementioned, the hepatic metastasis rate was confirmed to be significantly higher in the HNRNPC overexpression group (log-rank P=0.008) (109).

However, although there is a significant difference in the abundance of m⁶A regulators between PC and normal tissues, m⁶A regulators has not been widely utilized as a diagnostic marker. However, it is considered that, with the anticipated continued improvement of mapping methods, m⁶A regulators will be able to precisely predict the occurrence, TNM stage and prognosis of PC in the not-too-distant future.

M⁶A inhibitors as potential therapeutics in PC. A wealth of studies has confirmed the complex functions and molecular mechanisms of m⁶A in PC. Targeting m⁶A regulators as a means of therapeutic invention therefore provides a promising prospect for the treatment of cancer (Table IV).

FTO inhibitors can be divided into selective or non-selective types. Rhein is a natural product that was demonstrated to be the first FTO inhibitor possessing cell activity (115). Rhein has been shown to restrict the occurrence of breast cancer cells (116). Meclofenamic acid is an example of a selective FTO inhibitor (117), which restrains the growth and self-renewal of glioblastoma stem cells (118). FB23 and its derivative (FB23-2) inhibit the proliferation, and increase the rate of apoptosis and differentiation of acute myeloid leukemia (AML) cells (119). Finally, R-2-hydroxyglutarate has been shown to exert an anti-leukemic effect by suppressing cell proliferation/viability, and enhancing the apoptosis and cell-cycle arrest of leukemia cells (120).

METTL3 is a crucial catalytic subunit, which has attracted extensive attention among all regulators. METTL3 inhibitors can be separated into nucleosides and non-nucleosides. Inhibitors in both of these categories have been shown to function as competitive substrate inhibitors of SAM. High-throughput analysis identified adenosine (1-8) as small-molecule METTL3 inhibitors which occupy the binding site of SAM. The disadvantages of these adenosine (1-8) inhibitors, however, are poor selectivity and poor cellular permeability properties. The discovery of the METTL3 non-nucleoside inhibitor UZH1a, UZH2 and UZH1a analogues, JMC-1, JMC-5, JMC-8 and JMC-10, promoted the development of METTL3 inhibitors as therapeutic targets (121). The non-nucleoside METTL3 inhibitor, STM2457, has also been shown to be effective against AML without exerting any significant effect on normal hematopoiesis (121-123).

SPI1 has been shown to inhibit METTL14 expression directly, and serves as a possible AML therapeutic target (124). Additionally, carbonic anhydrase IV has been found to suppress the tumorigenesis of colon cancer by inhibiting the WTAP-WT1-TBL1 pathway (125).

Currently, research regarding m⁶A inhibitors in PC is still at its infancy. DNA damage repair represents a prominent obstacle for evaluating the chemotherapy efficacy of PC. The knockdown

Table IV. N⁶ methyladenosine inhibitors.

| Target | Drug | Cancer type | (Refs.) |
|--------------------------|--------------------------------|------------------------|-----------|
| FTO | Rhein | Breast cancer | (115,116) |
| | MA | Glioblastoma stem | (117,118) |
| | FB23/ | Acute myeloid | (119) |
| | FB23-2 | leukemia | |
| METTL3 | R-2HG | Leukemia cell | (120) |
| | Adenosine (1-8) | | (121) |
| | UZH1a, UZH2 and UZH1a analogue | | (121) |
| | STM2457 | Acute myeloid leukemia | (121-123) |
| METTL14 | SPI1 | Acute myeloid leukemia | (124) |
| WTAP | CA4 | Colon cancer | (125) |
| DNA double-strand breaks | Fisetin | Pancreatic cancer | (126) |

MA, meclofenamic acid.

of PHF10 has been shown to result in the elevated recruitment of γ -H2AX, RAD51 and 53BP1 to DSB sites, and decreased HR repair efficiency. Fisetin induces DSBs, and suppresses HR repair of DNA through impeding the ZC3H13-mediated m⁶A regulation of PHF10. Fisetin treatment has also provided insight into novel therapeutic strategies for PC (126).

Taken together, m⁶A inhibitors present a frontier of research that may provide a novel direction in m⁶A-targeted drug therapeutics. However, numerous challenges lie ahead. The clinical application of m⁶A inhibitors in PC remains insufficient at present, and the efficacy and adverse reactions of the prospective inhibitors requires further verification.

5. Conclusions and future perspectives

In the present review, the biological functions of m⁶A were introduced, with a focus on its influence in PC. There are, however, certain divergences and discrepancies that have not been fully explored herein. Based on these unresolved discrepancies, the authors' prediction is that the future exploration of m⁶A will give attention to the following topics. First, it is possible that certain m⁶A writers, erasers and readers have not yet to be identified; therefore, reforming m⁶A-mapping methods will be necessary to promote the discovery of novel m⁶A regulators. Secondly, FTO remains controversial with respect to whether it actually targets m⁶A as a substrate, and what its precise role is in the proliferation of PC. Thirdly, the question remains as to whether erasers always function antagonistically with writers. For example, if METTL3-METTL4 serves as an oncogene, FTO may function as suppressor, although this would not be in agreement with the experimental results described above. The detailed mechanisms that account for how the actions of

different regulators are coordinated in the same tumor, and also how the same regulator may function heterogeneously in distinct type of carcinomas, need to be further elucidated. The current knowledge of m⁶A regulatory networks only represent the tip of an iceberg, and the real situation is far more complex than what has already been discovered. Fourthly, the mechanisms through which m⁶A functions in concert with DNA and histone modification, and whether there are underlying connections between them, remain unclear. Finally, the exploration of m⁶A inhibitors in PC is only at the rudimentary experimental stages at present. An extensive literature detailing their application in clinical trials does not yet exist. Efficacious and safe m⁶A inhibitors, however, do need to be developed and put forth into clinical practice.

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Authors' contributions

GZ conceived the study. TY wrote the manuscript and performed the literature search with assistance from JW, HZ and PL, and GZ supervised the whole process. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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