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Review article

Regulatory role of RNA modifications in the treatment of pancreatic ductal adenocarcinoma (PDAC)

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is an extremely life-threatening malignancy with a relatively unfavorable prognosis. The early occurrence of metastasis and local recurrence subsequent to surgery contribute to the poor survival rates of PDAC patients, thereby limiting the effectiveness of surgical intervention. Additionally, the desmoplastic and immune-suppressive tumor microenvironment of PDAC diminishes its responsiveness to conventional treatment modalities such as chemotherapy, radiotherapy, and immunotherapy. Therefore, it is imperative to identify novel therapeutic targets for PDAC treatment. Chemical modifications are prevalent in various types of RNA and exert significant influence on their structure and functions. RNA modifications, exemplified by m^6A, m^5C, m^1A, and Ψ, have been identified as general regulators of cellular functions. The abundance of specific modifications, such as m^6A, has been correlated with cell proliferation, invasion, migration, and patient prognosis in PDAC. Pre-clinical data has indicated that manipulating RNA modification regulators could enhance the efficacy of chemotherapy, radiotherapy, and immunotherapy. Therefore, targeting RNA modifications in conjunction with current adjuvant or neoadjuvant therapy holds promise. The objective of this review is to provide a comprehensive overview of RNA modifications in PDAC treatment, encompassing their behaviors, mechanisms, and potential treatment targets. Therefore, it aims to stimulate the development of novel therapeutic approaches and future clinical trials.

1. Introduction: the overview of PDAC and RNA modifications

1.1. Pancreatic ductal adenocarcinoma and its dilemma on treatments

Pancreatic ductal adenocarcinoma (PDAC) is an extremely lethal neoplasm with a 5-year survival rate of only approximately 10 % [1]. The estimated numbers of newly diagnosed PDAC case were around 49,5000 worldwide, with 59,143 in USA and 134,374 in China respectively in 2022 [2,3]. The current treatment options for PDAC are unsatisfactory, as long-term survival is only achieved in a small percentage of patients after radical surgical procedures. In addition to surgery, the standard treatment for PDAC includes both adjuvant and neoadjuvant chemotherapy and radiotherapy [4]. Although emerging therapeutic strategies such as immunotherapy and advancements in conventional treatments have gradually extended patient survival, significant breakthroughs are still urgently needed.

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A large number of studies have elucidated the reasons behind the poor therapeutic efficacy of PDAC. In essence, the intrinsic characteristics of PDAC cells and the distinctive tumor microenvironment (TME) collectively contribute to the highly malignant behaviors exhibited by PDAC [5]. During the progression of PDAC, the homeostasis of the immune system, vascularization, and stromal components underwent extensive remodeling [6]. Desmoplasia is a critical characteristic of the PDAC tumor microenvironment (TME), which is characterized by the excessive proliferation of myofibroblast-like pancreatic stellate cells (or cancer-associated fibroblasts, CAFs) and fibrosis [7]. Limited blood supply and oxygen-deficient circumstance remodels the metabolic pattern and sensitivity towards adjuvant therapy of PDAC cells. In addition, it facilitates tumor progression, compromises chemotherapeutic drug delivery and established an immunosuppressive circumstance for anti-tumor response [8,9]. The increased presence of tumor-associated macrophages, Treg cells, and myeloid-derived suppressor cells further suppresses both the function and quantity of $CD8^+$ T cells and dendritic cells.

The high incidence of metastasis and local recurrence following surgical resection significantly contributes to the poor survival outcomes in PDAC. It is suspected that micro-metastases occur ubiquitously prior to visible tumor progression [10]. Hence, relying solely on surgical intervention is insufficient to achieve long-term survival, even in resectable cases. Given the limited efficacy of current treatment regimens, it is imperative to enhance systematic treatment through diverse approaches.

1.2. Forms and functions of RNA in human

RNA is a basic form of macromolecule carrying information transcribed from DNA and were regulated from multiple aspects. Messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA) were the most abundant forms of RNA, while the biological function of other RNAs including long non-coding RNA (lncRNA), micro-RNA (miRNA), circular RNA (circRNA) and small RNA (snRNA, including small nuclear RNA and small cytoplasmic RNA) has also been gradually demonstrated in recent years. These RNAs are basic and delicate regulators of tumor behavior with much unknown. Regardless of the 3 canonical types of RNA, it is difficult to conclude the function of these RNA molecules laconically (Fig. 1).

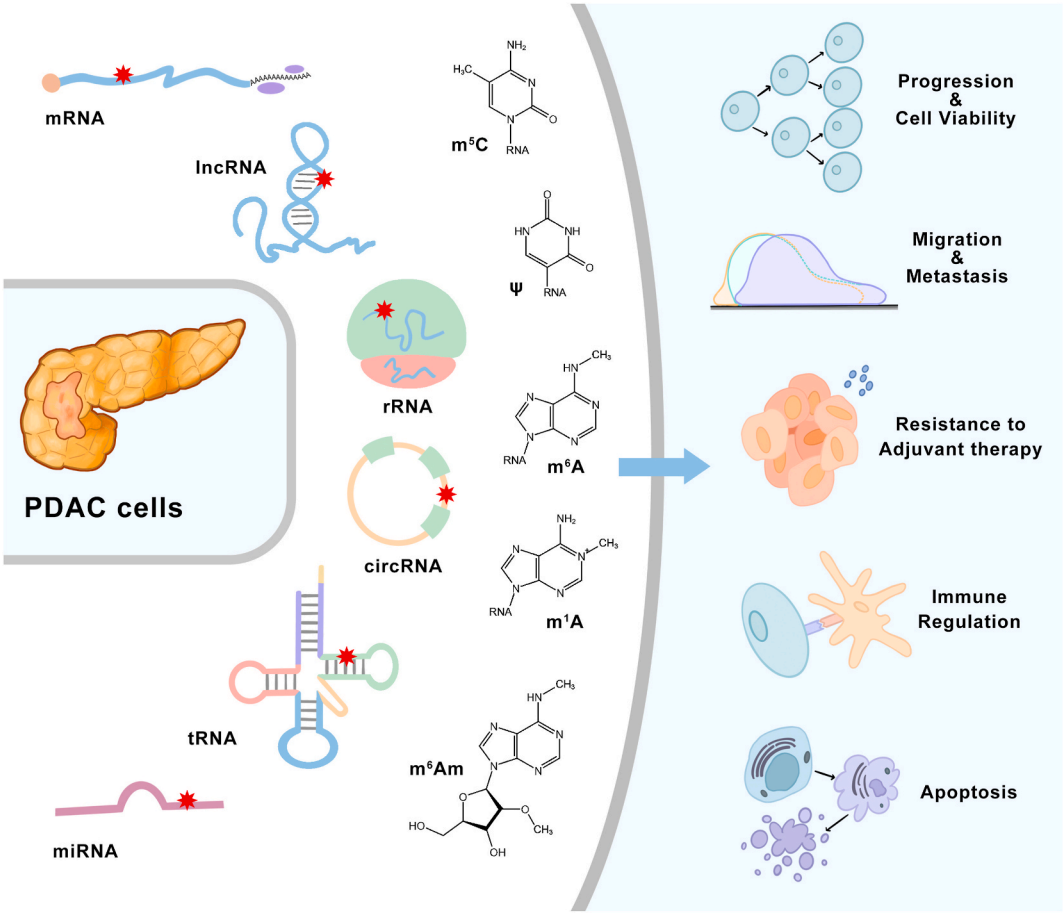

Fig. 1. Categories of functional RNA, RNA modification and their reported biological functions in malignancies. In PDAC, studies on modifications of mRNA, lncRNA, rRNA, circRNA, tRNA and miRNA have revealed their function in the regulation of tumor progression, viability, migration, metastasis, chemoresistance, immune-microenvironment regulation and apoptosis.

Messenger RNA (mRNA) has undergone extensive studies regarding its metabolism and functional processes. As the carrier of protein-coding sequences, the precursor hnRNA is synthesized in the cell nucleus. After the removal of introns, 5′ caping and 3' tailing, mature mRNA is exported to cytosol for translation. Ribosomes, composed of highly conserved rRNA and ribosomal proteins, form the primary components of the protein synthesis complex. rRNA forms intricate secondary or tertiary structures through specific sequences and modifications. During translation, tRNA functions as both a recognizer of codons on the mRNA and a carrier of amino acids. These three classic forms of RNA play crucial roles in protein synthesis, thereby significantly impacting cellular functions based on their abundance and conditions.

Long non-coding RNA is defined as RNA transcripts over 200 nucleotides without protein-coding function [11]. Similar to mRNA, lncRNA shares the same RNA polymerase II and undergoes processing through capping, tailing, and splicing [12]. The expression of lncRNA is highly dynamic and tissue-specific, suggesting its role in regulating multiple biological processes [13–15]. In cancer cells, lncRNA plays a crucial role in the regulation of tumorigenesis, development, progression, and metastasis through four pathways: acting as regulatory signals in specific pathways (signal lncRNA), inhibiting translation by competitively binding to transcription factors (decoy lncRNA), guiding regulatory protein complexes towards target genes (guide lncRNA), and interacting simultaneously with proteins and other molecules (scaffold lncRNA, especially histones and transcription factors) [16]. Through interaction with protein, DNA and other RNA molecules, lncRNA universally modulates cellular signaling pathways indirectly [17–20].

Circular RNA mainly consists of exons (sometimes with introns) alternatively spliced into single-strand circular structures. Despite sharing precursor sequences with mRNA and competing in splicing pathways [21,22], cirRNAs possess much greater stability as they are resistant to degradation by RNAse R [23,24]. Most circular RNAs are expressed in a stable and tissue-specific manner, with the regulatory function at transcription and post-transcription stages. It regulates the expression of downstream genes by either sequestering corresponding miRNA molecules (miRNA sponge) or interacting with RNA-binding proteins (RBP) [25–27]. Furthermore, by forming RNA-RNA complexes in the transcription region, circRNAs have been found to modulate alternative splicing and transcriptional efficiency [21,28]. Moreover, circRNA could be translated in certain conditions upon unconventional pathways in a modification-regulated manner [29,30]. In PDAC, over 20 microRNAs (miRNAs) have been documented to significantly contribute to the regulation of tumorigenesis, invasion, metastasis, gemcitabine resistance, and apoptosis [31]. Among these miRNAs, the majority exhibit upregulation.

Micro-RNA are small non-coding RNAs consisting of approximately 22 noncoding nucleotides [32]. Endogenous miRNAs hinder the movement of ribosomes, or triggers the cleavage or degradation of mRNA via binding to the 3′-UTR of target mRNA [33]. The highly conservative miRNAs play an important role in the regulation of oncogenesis, tumor behaviors and response towards treatments primarily through suppressing downstream gene expression [34]. Another common form of miRNA is circulating miRNA, which can be released upon cell necrosis or secreted in exosomes as intercellular signals. In PDAC, exosomal miRNAs in plasma could serve as diagnostic biomarkers or tumor-suppressive treatment [35]. For instance, miR-210 is both a hypoxia marker and a regulator of tumor microenvironment in PDAC, thereby providing a therapeutic target against chemoresistance [36–39].

1.3. RNA modification: forms, function and regulation

RNAs regulate downstream processes not only by their coding sequences or secondary structure, but also by its modifications. More than 150 types of chemical alternations have been documented on RNA residues [40]. However, only a few RNA modifications have been well characterized due to their high abundancy or stability. Among these, the most biologically significant and extensively studied modifications are 6-methyladenosine (m^6A), 5-methylcytosine (m^5C), and pseudouridine (Ψ) [41–44]. Several other representative but less extensively studied modifications include N6, 2′-*O*-dimethyladenosine (m^6Am), 1-methyladenosine (m^1A), and 5-hydroxymethylcytosine (hm^5C). These RNA modifications have been discovered to influence the secondary structure formation, RNA transportation/localization, molecular stability, RNA-protein interaction, double-strand stability, etc. [40,45–47] The landscape of RNA modifications was also described as epitranscriptome which functions as a rapid responder to the dynamic cellular environment [48,49]. Depending on the specific type of RNA, these modifications selectively modulate cellular pathways in distinctive ways.

Transfer RNA (tRNA) and ribosomal RNA (rRNA) exhibit the highest proportion of modifications, accounting for 17 % and 2 % respectively [50]. Additionally, these modifications are predominantly localized to specific regions with consistent structural characteristics. The modifications on tRNA play crucial roles in accurate recognition of anticodons, secondary structure formation, and stabilization of the tRNA molecule. Furthermore, they are dynamically regulated in response to various external stimuli [51]. Both hypomodification of tRNA and malfunction of tRNA methyltransferase are commonly observed in various types of malignant neoplasms, emphasizing the significance of tRNA modifications in tumorigenesis and regulation of tumor behaviors [51–53]. Similarly, rRNA modifications are enriched at peptidyltransferase and decoding sites, and mediated the molecular stabilization and formation of tertiary structure [43,54]. As tRNA and rRNA serve as essential components in protein synthesis, it is reasonable to assume that RNA modifications impact cellular functions indirectly.

As the template of cellular function executors, the whole lifespan of mRNA is profoundly affected by modifications [44]. Throughout the processes of nascent mRNA maturation, including splicing, nuclear export, and response to damage in transcription regions, various modifications such as m^5C, m^6A and Ψ have been shown to exert regulatory control [55–61]. Furthermore, during translation, these modifications play a pivotal role in modulating translation efficiency, fidelity, and stability by interacting with RNA-binding proteins (RBPs) [62–67]. Although the distribution pattern of mRNA modification is not as constant as that of tRNA, there is still evidence of site or sequence specificity, indicating their regulatory role in specific cellular events.

The regulatory functions of modifications on other non-coding RNAs have been gradually elucidated in recent years. Notably, the regulatory roles of m^6A modifications in specific long non-coding RNAs (lncRNAs) have been identified in various malignant tumors

and diseases [68]. Additionally, the presence of m^6A modifications in lncRNAs has been found to serve as a biomarker for disease diagnosis and prognosis prediction in PDAC, gastric neoplasms, and lung adenocarcinoma [69–72]. Similar regulatory roles have been observed in miRNAs and circRNAs, highlighting the potential of targeting RNA modifications for disease control [73,74].

1.4. Regulators of RNA modification

"Writers", "erasers", and "readers" are three pivotal regulatory RNA-binding proteins (RBPs) involved in the functional process of RNA modifications [44] "Writers" refer to enzymes that catalyze the transformation reaction of RNA from its original state. It is equivalent to the concept of RNA methyltransferase, as exemplified by m^6A writer METTL3. While "erasers" denote enzymes that mediate the reverse process (demethylation) which were also known as RNA de-methyltransferases. Fat mass and obesity-associated protein, FTO was the first discovered and most extensively studied mammalian m^6A eraser [75]. "Readers" represent a large number of proteins directly recognizing RNA modifications which mediated or triggers downstream process or pathways. As a valuable complement to RNA sequencing, researchers have been able to uncover the intricate landscape of RNA modification by studying these regulators.

Multiple routes have been applied in the study of RNA modification, each with its own specific focus. The first route focus on modification itself by direct sequencing [76]. Detection techniques for RNA modifications are tailored to each specific modification. For instance, the detection of m^6A typically involves immunoprecipitation on RNA fragments and subsequent mapping with high-throughput sequencing techniques [77]. Antibody-dependent techniques are commonly employed in most common RNA modifications, relying on the specificity and quality of the antibodies used.

In contrast to original RNA cytosine, m^5C and a few other modifications on m^5C would not be converted into uridine through bisulfite treatment. This property greatly facilitates the location of m^5C by comparing the sequences before and after bisulfite labeling [78,79]. Another sequencing strategy utilized the crosslink reaction between Ψ and N-cyclohexyl-N′-beta-(4-methylmorpholinium) ethylcarbodiimide *p*-tosylate (CMCT). This reaction inhibits reverse transcription, thereby enabling the easy identification of corresponding sites [80]. The two individualized sequencing strategies above are typical cases of RNA sequencing, while were only practicable in certain categories of modification. Compared to DNA, the lower stability of RNAs poses greater challenges in sequencing, particularly for modifications with low abundance. Hence, the identification and further exploration of novel RNA modifications remains challenging [81].

The other approach studies RNA modification by manipulating regulatory RBPs, particularly on modification writers. In most cases, this method yields data with lower technical complexity and has been widely adopted. However, it also sacrificed the reliability of results, since a single RBP may be shared by various types of RNA or DNA. Consequently, attributing observed phenotypes to a specific category of RNA becomes challenging. Increasing number of RBPs have been discovered though, "knocking out" RBPs for a single type of RNA modification still seems to be unfeasible. It is evident that many writers are yet to be identified, as a significant quantity of modifications can still be detected even when all known writers wereknocked out. Furthermore, the quantity or functions of modifications may not be reflected by the abundance of corresponding proteins. Nevertheless, a substantial number of conclusions have been drawn based on the study of RBPs instead of modification itself, for the high difficulty of high-throughput sequencing and limitation of feasible sequencing strategies.

2. Tumor behaviors regulated by RNA modification

2.1. The role of m^6A in PDAC

Several RNA modifications have been implicated in the regulation of tumor behavior specifically in PDAC (Fig. 1). Among them, m^6A is the most abundant and well-studied one. Human sequencing data has revealed the presence of over 7000 transcripts containing m^6A modifications, with a predominant localization near stop codons and 3′ untranslated regions [82,83]. Structurally, m^6A decreases the binding affinity between A and T/U, as well as the stability of specific secondary structures. Although a brief overview of its functions has been provided earlier, this section will delve into the specific role it plays in PDAC.

Current model of m^6A methylation mainly refers to a complex (methyltransferase complex, MTC) consisting of METTL3-METTL14, Wilms' tumor 1-associating protein (WTAP), and a few other cofactors [84,85]. Other m^6A methyltransferase includes ZCCHC4 (rRNA), METTL5 (rRNA, stabilized by TRMT112), METTL16 (multiple types of RNA, including U6 snRNA), etc [83,86–88]. The m^6A writers exhibit distinct target preferences, leading to varying effects on m^6A levels in PDAC. As the opposite regulator of methylation, few erasers have been reported for m^6A. FTO (obesity-associated protein) and ALKBH5 (alkB homolog 5) showed demethylase activity with limited function and low efficiency unless in specific stress-relevant conditions [89]. It is shown that the de-methyltransferase activity of FTO is even stronger in 5' cap N6, 2-*O*-dimethyladenosine (m^6Am) than m^6A, which has been mainly detected in snRNA [90,91]. Although its regulation effect on m^6A was subtle according to the sequencing result in knock-out mice system, FTO plays an oncogenic role in certain malignancies [92,93]. As for ALKBH5, it has been reported to be enriched in tissues in reproductive system, and upregulated in multiple types of neoplasm and hypoxic environment with high m^6A demethylase activity [55,94,95]. Compared with FTO, ALKBH5 seems to be a more important epitranscriptome regulator in the carcinogenesis and adjuvant therapy of PDAC.

Theoretically, any protein with the ability of recognizing and functionally affected by RNA m^6A modification directly or indirectly could serve as an m^6A reader. The YTH domain-containing proteins, such as YTHDC1, YTHDC2, and the YTHDF family, are typical examples of direct m^6A binding proteins, characterized by their "tryptophan cage" structure [96,97]. Eukaryotic initiation factor 3 (eIF3) is also a direct RNA m^6A recognizer which initiates and promotes the translation of mRNA methylated at 5′ UTR [98]. On the

contrary, a group of RNA binding proteins were repelled once their original target adenosines were methylated. Representative proteins of these "anti-readers" include G3BP1, LIN28A and EWSR1 [89,99]. Since m^6A tend to induce single-strand, linear, unfolded structural switch, indirect readers could access their original binding sites with higher affinity. These phenomena were observed in heterogeneous nuclear ribonucleoprotein family (HNRNPG, HNRNPC and HNRNPA2B1) recognizing principally non-coding RNAs [100,101]. Notably, HNRNPA2B1 had been supposed to bind m^6A according to gel-shift assay results [102]. Therefore, it is sometimes difficult to distinguish whether a protein is a direct reader.

Similar to many other types of cancer, RNA m^6A modification participates in the regulation of tumor behavior extensively with most known "writers", "erasers" and "readers" involved (see Table 1 and Fig. 2). For PDAC, high m^6A modification level in RNA is generally an oncogenic factor suggesting malignant tumor behavior and poor prognosis. The most commonly observed methyltransferases in PDAC, including METTL3, METTL14, WTAP, and their co-factors, have been found primarily to be upregulated and play oncogenic roles [103]. For instance, METTL3 was reported to promote pancreatic cancer cell proliferation and invasion in MIA PaCa-2 and BxPC-3 cell lines [104]. During adjuvant therapy, cellular m^6A level also plays an important role in the resistance of chemotherapy and radiotherapy via METTL3 dependent manner [105]. Meanwhile, m^6A is a biomarker in PDAC patients. High METTL3 level was seen in PDAC cells compared with para-carcinoma tissue, and were associated with higher pathological stage, N stage ($P = 0.02$) and poor prognosis [104,106]. Multiple pathways including E2F5, lncRNA MALAT1, miR-25-3p and miR-380-3p have been involved to elucidate how METTL3 function in these process [73,107–109]. Although less than METTL3, other studies indicated that m^6A writers worked similarly to promote pancreatic cancer malignancy and therapy resistance either *in vivo* or *in vitro*. METTL16 is an exception for its low expression in PDAC tissue and positive correlation with antitumor immunity and good prognosis [110].

On the other hand, RNA m^6A demethylases play a complex role in PDAC. At the cellular level, these erasers contribute to the regulation of cell progression, migration, and resistance to adjuvant therapy. ALKBH5, in particular, has been implicated to compromise the tumorigenesis via multiple molecular pathways. Low expression of ALKBH5 is significantly associated with poor clinicopathological features and prognosis, as evidenced by data from the International Cancer Genome Consortium (ICGC), the Cancer Genome Atlas (TCGA) database, and other cohort studies [111–113]. The targets of ALKBH5 involves mRNA and lncRNA including but not limited to PER1-ATM-CHK2-P53/CDC25C and mTOR pathways [112,114]. Other targets like FBXL5, SLC25A28, SLC25A37, KCNK15-AS1, KCNK15 and PTEN/AKT signaling were also screened by high-throughput experiments [113,115]. The tumor-suppressive role of ALKBH5 is further supported by its low expression in gemcitabine (GEM)-resistant cells in patient-derived xenograft (PDX) models [116]. FTO, another known m^6A eraser, exhibits an overall oncogenic effect by promoting cell growth, migration, and invasion [117,118]. Recent studies also primarily revealed the function of FTO in PDAC chemoresistance by influencing the mRNA stability of NEDD4 [119]. Other FTO-modulated pathways involved in PDAC drug-resistance includes DNA damage repair,

Table 1
Current reported RNA m^6A writers and erasers were listed, with their corresponding target gene, downstream pathway, and biological functions in PDAC.

RBPs	RNA involved	Regulated gene/pathway	Functions
Writer			
METTL3	Total RNA	NA	Cell proliferation, invasion, and migration [104]
METTL3	Total RNA	Mitogen-activated protein kinase cascades, ubiquitin-dependent process, RNA splicing, etc.	Resistance to chemotherapy and radiotherapy [105]
METTL3	mRNA	E2F5 stability	Cell viability, migration and invasion [107]
METTL3	lncRNA	MALAT1 - PD-L1	Cell viability and PD-L1 expression regulation [108]
METTL3	miRNA	miR-25-3p maturation	Cell development and progression [109]
METTL3	miRNA	miR-380-3p upregulation	Cancer aggressiveness [73]
METTL3	circRNA	circMYO1C and PD-L1 mRNA	Suppress tumor immune surveillance [74]
METTL14	mRNA	PERP	Cell proliferation and migration [187]
METTL14	NA	Cytidine deaminase	Gemcitabine resistance [83]
METTL14	NA	AMPKα, ERK1/2 and mTOR signaling pathways	Inhibits apoptosis induced by cisplatin and autophagy [161]
WTAP	mRNA	Stabilizing Fak pathway	Metastasis and chemo-resistance [162]
WTAP	NA	NA	T-cell-inflamed immunity [175]
METTL16	snRNA, mRNA	METTL16–RNA–MRE11	Sensitivity towards PARPi [171]
METTL16	Total RNA	Immunomodulation	Favorable outcome and antitumor immunity [110]
METTL5	rRNA	m^6A_{1832} – c-myc	Cancer progression [188]
Eraser			
ALKBH5	mRNA	PER1-ATM-CHK2-P53/CDC25C	Suppresses proliferation, migration, invasion [112]
ALKBH5	mRNA	WIF-1 transactivation & Wnt	Gemcitabine sensitivity, inhibits cell proliferation, migration, and invasion [116]
ALKBH5	mRNA	FBXL5, SLC25A28 and SLC25A37	Inhibits cell migratory and invasive abilities [113]
ALKBH5	lncRNA	DDIT4-AS1, mTOR pathway	Inhibits stemness and enhanced sensitivity to GEM [114]
ALKBH5	lncRNA	KCNK15-AS1, KCNK15 and PTEN/AKT signaling	Inhibits cancer progression and mortality [115,189]
FTO	mRNA	ADAMTS2, COL12A1, and THBS2	Migration and invasion [118]
FTO	mRNA	TFPI-2	Growth, migration and invasion [117]
FTO	mRNA	PDGFC	Progression [117]
FTO	mRNA	NEDD4	PTEN/PI3K/AKT pathway [119]

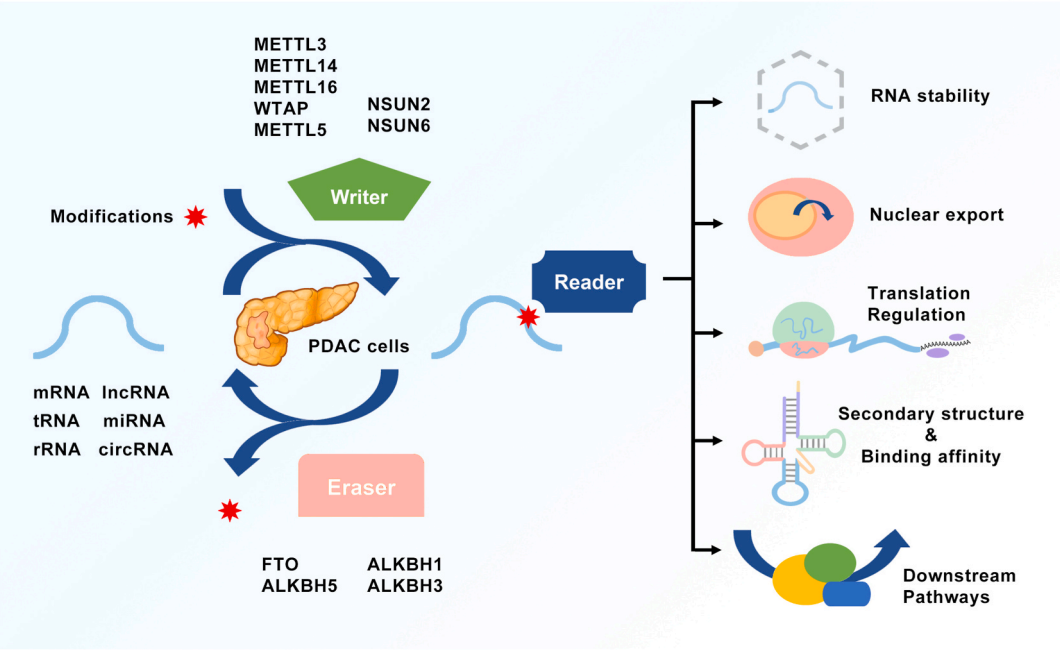

Fig. 2. Regulatory mechanisms of RNA modifications, and various pathways to generate their downstream biological effects. All identified writers and erasers functional in PDAC are listed with references in Tables 1 and 2 Writers and erasers mediated the modification and the reverse process, while readers recognized RNA modifications directly to induce downstream effects.

drug transportation, cell survival, etc [120]. Although a few targets have been identified (see Table 1), the relationship between FTO and m^6A in PDAC has not been well-established. Considering the low activity and specificity of FTO as an m^6A eraser, other functions of FTO proteins may be involved in interpreting its reverse effect in PDAC.

Given the controversial definition of m6A readers, we have chosen IGF2BP2 as an example to investigate how readers mediate the relationship between m^6A modification and downstream pathways. IGF2BP2, a protein containing the YTH domain, serves as a mediator by targeting various mRNA and lncRNA molecules, which have broad effects on cell cycle, metabolism, proliferation, apoptosis, ubiquitination, and radiotherapy resistance [103,121]. Through its ability to read and bind to modified RNA sequences, IGF2BP2 stabilizes the target genes and enhances their translation [122]. Consequently, the increased levels of downstream products from these stabilized RNAs contribute to enhanced cellular functions. Elevated levels of IGF2BP2 have been observed in both pancreatic intraepithelial neoplasia (PanIN) and pancreatic cancer tissues, indicating more rapid tumor progression and shorter overall survival [123–125]. Consistent with the phenotype of high m^6A level, the comprehensive effects of other m^6A readers (for instance, YTHDF2, IGF2BP1, IGF2BP3 and hnRNPC) were oncogenic in PDAC. Interestingly, the high expression of YTHDF2 in PDAC showed both oncogenic and tumor suppressive effects by regulating YAP and TGF-β/Smad signaling pathways [126]. Moreover, it will not be astonishing if other suppressive m^6A readers are found.

2.2. *The role of other modifications in PDAC*

This section will briefly discuss how other typical RNA modifications regulate the behavior of PDAC. Among numerous RNA modifications, three of the most extensively studied ones are m^5C, m^1A and ψ. In eukaryotic systems, the methylation process of m^5C is mainly catalyzed by the NOL1/NOP2/SUN domain (NSUN) family and TRDMT1 (also known as DNMT2) [127]. The deposition of m^5C on non-coding RNAs like tRNA and rRNA mainly stabilizes corresponding units and partakes cellular response to stress, including DNA damage, oxidative stress [128]. When it comes to mRNA, current studies have primarily revealed the function of m^5C in mRNA nuclear exportation and stabilization (Fig. 2) [57,129].

In PDAC, the copy number variations of regulatory genes of m^5C (including writers NSUN1-7, DNMT family, 5-hydroxymethylcytosine writers TET family and readers YBX1, ALYREF) were mostly correlated with their mRNA expression [130,131]. Specifically, DNMT1, DNMT3B, NSUN2, NSUN3, and YBX1 showed a negative correlation between their expression levels and patient survival, while NSUN6 and NSUN7 exhibited the opposite trend. By integrating m^5C-related differentially expressed genes, Duo et al. established a prognostic-predicting m^5C score, which is negatively correlated with overall survival probability [131]. Similarly, a high m^5C-related lncRNA signature has been shown to predict poor prognosis in PDAC patients and their response to immunotherapy [56,132].

Mechanistic investigations regarding RNPs associated with m^5C were still insufficient. Notably, the enzyme activity of several aforementioned RNPs remains unexplored and contentious, especially for DNMT1, DNMT3, most m^5C erasers and readers. By

knocking-down NSUN2 in KPC cells in mouse model, a decrease in stromal fibrosis and restoration of differentiation were observed [133]. NSUN6 exhibited inhibitory effects on PDAC by regulating cell proliferation through CDK-10 dependent pathways, as confirmed in both *in-vitro* and *in-vivo* experiments [134]. Although the involvement of TRDMT1 and other m^5C writers in various cancer types has been elucidated, their relationship with PDAC remains inadequately established (See Table 2).

Another important modification that influences PDAC is N1-methyladenosine (m^1A). Structurally, the addition of a methyl group disrupts the canonical A-T/U pair, leading to a significant change in the RNA structure [135]. Discovered as early in 1960s, it widely distributes in various types of RNA, including tRNA, rRNA, mRNA, as well as other ncRNAs [136,137]. Besides its unique structural role at certain positions, m^1A is basically a repressive factor in mRNA during transcription triggering impaired protein synthesis, cell viability, self-renewal and development process [135,137,138]. The currently identified writers include TRMT10C, TRMT61B and TRMT6/61A, while erasers only include ALKBH1 and ALKBH3 [135,139]. Readers of m^1A belong to YTH domain containing-proteins, including YTHDF1, YTHDF2, YTHDF3, and YTHDC1, with some "readers" being shared by m^6A [140]. Bioinformatic studies on these m^1A regulatory genes have indicated their value in predicting patient prognosis. Changes in copy number variation (CNV), tumor mutation burden, and mutations in the tumor suppressor gene ATM are strongly correlated with m^1A-regulating genes. Furthermore, the expression of ALKBH1 and YTHDC1 has been correlated with poor prognosis in PDAC patients [137,141]. Specifically, the function of ALKBH3 in PDAC has been studied by multiple researches: the silencing of PCA-1/ALKBH3 inhibits VEGF expression and angiogenesis, which further triggers apoptosis and cell proliferation [142]. This phenotype is consistent with studies in other malignancies [143].

Discovered in early 1950s, pseudouridine functions as a key regulator of RNA secondary structure [144]. The molecular rigidity and stability increase with the promotion of Ψ content especially in small RNA structures [145]. In addition, the conformational change induced by Ψ affects RNA-RNA interaction [146]. Therefore, it affects gene expression naturally after its formation. Lots of Ψ writers have been identified in eukaryotic system, which mainly consists of two main categories. The first type refers to RNA-dependent mechanism, which consist of four key elements: DKC1 (core protein with activity), Nhp2, Nop10 and Gar1. The other RNA-independent pathway relies on a single protein named pseudouridine synthases (PUS). At least 10 members has been identified from PUS family, which recognizes their corresponding targets [147]. The changes of gene expression and regulatory functions of DKC1 have been reported in multiple cancers, including some digestive system tumors [128]. Unfortunately, none of these proteins has been profoundly studied in PDAC.

3. Targeting RNA modifications in PDAC treatment

3.1. Mechanisms of current treatment towards PDAC

To elucidate how RNA modifications may contribute to the treatment of PDAC, it is necessary to provide a brief overview of the mechanisms underlying current therapeutic approaches. Chemotherapy is a fundamental and reliable strategy that plays a crucial role in treating PDAC at all stages. The latest guidelines recommend various chemotherapy protocols, ranging from single-drug regimens to complex combinations based on the tumor's condition and the patient's characteristics [148]. First-line chemotherapy regimens in adjuvant and neoadjuvant therapy contains gemcitabine, paclitaxel, FOLFIRINOX (combination of oxaliplatin, irinotecan, folinic acid and fluorouraci), capecitabine and S-1 [149,150]. Some of these therapeutics are analogous of deoxyribonucleotides, which hinder DNA synthesis and arrest the cell cycle at the S phase in actively proliferating cells. Gemcitabine, 5-FU (S-1), capecitabine fall into this category. Paclitaxel targets cells in mitosis, while platinum-containing drugs disrupt DNA at all stages. Furthermore, irinotecan inhibits topoisomerase I, leading to single-strand breaks and cytotoxic effects [151]. Mechanistically, these chemotherapy drugs induce DNA damage through different pathways or cause cell cycle arrest, suggesting the potential for improving current therapy by targeting DNA repair mechanisms. Novel therapeutic drugs have gradually been introduced for the treatment of locally advanced or metastatic PDAC populations. For instance, the PARP inhibitor olaparib has shown relative efficacy in patients with somatic mutations in BRCA1/2 or other deficiencies in DNA repair pathways [152]. Additionally, targeted therapy drugs such as EGFR inhibitor erlotinib have been utilized, although their effects have not been proved exact [153].

Radiotherapy is a well-established adjuvant therapy approach known for its high spatial accuracy. In the early studies of adjuvant therapy for PDAC, chemoradiotherapy was a standard protocol. PDAC has historically been considered a tumor type that is relatively insensitive to ionizing radiation and were only recommended in the therapy of advanced stage PDAC. However, advancements in radiotherapy techniques, such as stereotactic body radiotherapy (SBRT) and intensity-modulated radiation therapy (IMRT), have opened up new opportunities for adjuvant therapy, particularly in the context of neoadjuvant therapy and unresectable lesions.

Table 2
Reported regulators of other RNA modifications in PDAC, with their targets and biological functions listed.

RBPs	RNA involved	Regulated gene/pathway	Functions
Writer			
NSUN2	(m^5C) mRNA	NA	Stromal fibrosis and ductal epithelium differentiation [133]
NSUN6	(m^5C) NA	Cell cycle related genes	Suppress tumor proliferation and recurrence [134]
Eraser			
ALKBH1	(m^1A) NA	mTOR and ErbB pathway	Suppress tumor occurrence and development [137]
ALKBH3	(m^1A/m^3C) NA	VEGF	Supporting apoptotic resistance and angiogenesis [142]

Radiotherapy exerts its therapeutic effects by inducing either oxidative damage or direct breaks in macromolecules, thereby triggering apoptosis or other cytotoxic signaling pathways [154]. Given the high spatial accuracy of radiotherapy, combining it with radio-sensitizers has the potential to enhance its efficacy.

Immunotherapy has emerged as a promising approach for the treatment of various tumors, including melanoma, non-small cell lung cancer, and clear cell renal cell carcinoma [155]. However, despite the increasing number of clinical trials, immunotherapy has not yielded satisfactory results in the case of PDAC [156,157]. Several strategies have been applied in current immunotherapy: immune checkpoint inhibitors including anti-CTLA-4/anti-PD-1/anti-PD-L1 agents, tumor vaccines, adoptive cell transfer (ACT), etc. [156] The first approach aims to unleash the potential of the anti-tumor response by blocking signaling pathways that inhibit the immune system's ability to target tumors. The second method focuses on targeting specific antigens expressed by PDAC, thereby triggering a highly specific immune response. ACT involves expanding anti-tumor cells *in vitro* and subsequently infusing them back into the patient. The therapeutic effects of these approaches are influenced not only by the characteristics of the cancer cells but also by the tumor microenvironment. As previously mentioned, PDAC is characterized by a desmoplastic mesenchyme and a low level of infiltration of anti-tumor immune cells with limited activity. Therefore, RNA modifications that possess immune-promoting effects or regulate the tumor microenvironment may offer potential for improving treatment outcomes. In fact, m^6A regulators have been reported to modulate the PDAC tumor microenvironment and serve as prognostic markers for tumor progression [158]. Neoantigen-based immunotherapy holds promise for PDAC treatment, while epitranscriptome changes do not affect the formation of neoantigens directly [159].

3.2. Opportunities in PDAC treatments

Therapies targeting RNA modification in PDAC have been actively explored due to the notable functions of RNA modification in this disease. It has been observed that manipulating these RNA methylations can affect the efficacy of most adjuvant therapies [160]. Since m^6A is likely to be an oncogenic factor, suppressing its methylation has been considered a potential therapeutic target. Two main approaches have been considered in this regard. The first approach is manipulating the expression level of corresponding methylation regulators. Regardless of the pathways enrolled, the knockdown of METTL3 sensitizes PDAC cells to 5-fluorouracil, GEM, cisplatin and radiotherapy [105]. METTL14 also participates in GEM chemoresistance by regulating cytidine deaminase and inhibits cisplatin-induced apoptosis by disrupting AMPKα, ERK1/2, and mTOR signaling pathways [83,161]. Another m^6A writer, WTAP, stabilizes Fak pathway and suppresses the cell sensitivity to gemcitabine in PDAC [162]. The second approach focuses on developing specific drugs that target RNA modification-related pathways. Small molecular drugs have been designed based on the structure of the METTL3-METTL14 complex [163]. Du et al. reported an approach for screening methylation inhibitors from natural products and identified quercetin as a compound that inhibits the proliferation of MIA PaCa-2 and Huh7 cells [164]. Since METTL3 inhibitors with different chemical skeleton structures are being actively developed, clinical trials in recent future are eagerly expected [164]. Moreover, inhibitors targeting FTO and other m^6A regulators, such as rhein, MO-I-500, meclofenamic acid, fluorescein, R-2HG, and B23/FB23-2, are also available [165].

The therapeutic effects of both chemotherapy and radiotherapy were significantly influenced by cellular DNA damage response. DNA damage repair-related genes comprise 19.7 % of the most common 751 mutations in PDAC [166,167]. RNA modifications play a crucial role in the DNA damage repair process, particularly in transcriptionally active regions. Accumulation of m^6A and m^5C on mRNA were observed immediately after local DNA damage, which activate DNA polymerase κ and RAD52-RAD51 pathways respectively [61,168]. Other studies have also emphasized the significance of RNA modification-related DNA repair pathways in chemotherapy resistance. For instance, m^6A methylation on lncRNA ANRIL regulated by SRSF3 are closely related to chemotherapy resistance via homologous recombination (HR) repair [169]. The downregulation of subunit ZC3H13 in m^6A methylation compromises HR repair efficiency via PHF10 inhibition [170]. TRDMT1-mediated m^5C modification in mRNA promotes the repair process after oxidative damages by recruiting homologous-recombination related proteins [168]. Similarly, METTL16 was involved in the double-strand break repair in PDAC which sensitizes tumor towards PARPi [171]. These findings suggest that regulating RNA methylation, particularly in the germline BRCA (or other DNA-repair deficient) mutation population, will probably enhance the therapeutic benefits of chemotherapy.

RNA modifications also offer intriguing potential contributions to immunotherapy mainly by influencing the tumor microenvironment or immune cells. In PDAC, both m^6A or m^5C in lncRNA significantly modulate the tumor immune microenvironment and demonstrate prognostic value for assessing immunotherapy efficacy [56,132,141,172,173]. A lower level of m^6A is correlated with increased immune cell infiltration and a stronger response to immune checkpoint inhibitors [158]. A model based on the expression of IGF2BP2, IGF2BP3, KIAA1429, METTL3, EIF3H, and LRPPRC in PDAC demonstrates indicative value for assessing the status of the tumor microenvironment [174]. Additionally, WTAP promotes T-cell infiltration in PDAC without significantly altering the tumor microenvironment, which is contrary to the general function of m^6A [175]. Mechanistically, while METTL3 and YTHDF2 positively promote the maturation and function of NK cells, dendritic cells, and $CD8^+$ T cells, they also recruit pro-tumor macrophages and Treg cells due to cellular reprogramming and reshaping of the tumor microenvironment [165,176–178]. A m^6A-modified circRNA MYO1C also promoted the proliferation and migration of PDAC cells both *in vitro* and *in vivo*, with the ability of targeting the m^6A site of PD-L1 mRNA to increase its stability [74]. Therefore, it accelerates the immune escape. In addition to strategies that generally inhibit or promote RNA modification regulators throughout the organism, adoptive cell transfer y(ACT) allows for precise modification editing in specific cell subsets. Therefore, the efficacy of ACT or similar therapies could potentially be enhanced by regulating RNA modifications. However, experiments on PDAC immunotherapy were mostly in pre-clinical stages.

4. Discussion

RNA modification has emerged as a prominent field in the functional investigation of cancer, as evidenced by the exponential growth of related literature. In this review, we provide an overview of the various forms of RNA, the most prevalent RNA modifications, and their general roles in regulating tumor behavior. Furthermore, we review the RNA modification regulators that have been implicated in PDAC, categorized according to the specific type of modification they mediate. Although existing data has indeed established a correlation between RNA modification and tumor prognosis, only a fraction of these studies have provided reliable mechanistic insights into the underlying processes. Despite an increasing number of investigations reporting on the functional significance of RNA modification in various biological processes, it is important to note that nearly half of these studies are still focused on the protein level. By employing knockdown or knockout strategies targeting the relevant writers or erasers, similar alterations in modification levels and phenotypes have been observed. However, researchers have proposed divergent pathways to elucidate the workings of these modifications or regulators, and in some cases, conflicting findings have been reported. Consequently, the underlying mechanisms and downstream pathways remain poorly understood. Additionally, the target specificity of crucial RNA regulators, such as METTL3-METTL14, is not highly precise, leading to complex downstream effects. Consequently, simple inhibition of these proteins may elicit multifaceted outcomes. Given the formidable challenges associated with epitranscriptome studies, a multitude of mechanisms governing RNA-protein interactions may still remain undiscovered. For future clinical applications, the development of accurate manipulation methods will depend on a deeper understanding of RNA modifications.

RNA modification is a highly dynamic process that responds rapidly to changes in stimuli. Unlike mutations, RNA modifications can be easily reversed by erasers and may only be abundant under specific conditions. It highlights the importance of capturing the precise moment of observation in living cells. Even slight changes in stimulation or time can significantly alter the landscape of RNA modifications. The concept of modification readers can also be elusive. In theory, all functional proteins with binding affinity towards RNAs, regardless of their strength, can potentially be affected by modifications, as modifications alter the secondary or tertiary structure of the original nucleotides. Consequently, the compromised or enhanced interaction between RNA and protein can lead to changes in downstream protein effects. Moreover, exploring the functions of RNA-binding proteins may uncover new modification readers, which could serve as potential targets for RNA modification-based therapy.

A novel sequence-specific RNA m^6A editing system by fusing CRISPR-dead Cas9 (dCas9) and a single-chain m^6A writer/eraser was created in 2019 [179]. It allows the manipulation of RNA modifications on certain genes without interaction with original DNA sequence. *In vitro* experiments in HeLa cells validated the satisfying results both in over-modification and demethylation. With the rapid improvement of similar system in which dCas9 were replaced by dCas13 or other more advanced elements, efficient and accurate manipulation on RNA modifications is promising in the future [180,181].

The advancement of PDAC treatment relies on the progression in multiple aspects. In addition to changes in chemotherapy protocols, radiotherapy techniques, and immunotherapy, novel methods are being developed. Targeted therapy has shown benefits in patients with specific mutations in other types of malignancies, although its application in PDAC is still in its early stages. PDAC is characterized by a high mutation rate in genes such as KRAS, TP53, CDKN2A, ARID1A, KDM6A, and PREX2 [182]. A small fraction of patients also carries germline mutations in genes like BRCA1, BRCA2, p16, and PALB2, which allows for specific targeted therapies [183]. Unfortunately, patients with currently druggable mutations represent only a small minority. Similar as radiotherapy, photodynamic therapy which induces local cytotoxicity by light-activated photochemical showed potential in the desmoplastic PDAC in clinical trials, which could be further enhanced by EGFR recognition and combinational therapy with irinotecan [184,185]. This approach combines the spatial accuracy, efficient drug delivery with limited damage towards para-carcinoma tissue. As an intriguing method for cancer treatment, oncolytic viral therapy destroys cancer cells with either direct cell lysis or activated immune response [186]. In the immune-suppressive tumor microenvironment of PDAC, it may promote the enrichment of antigen-presenting cells and cytotoxic T cells, thereby facilitating immunotherapy. Given that RNA modifications are involved in the regulation of DNA damage repair, cell cycle arrest, chemotherapy resistance, and modulation of immune states, targeting these modifications holds promise for enhancing the efficacy of anti-PDAC therapy. With more breakthroughs on the basic therapeutic approaches and their promotors, the complex treatment of PDAC will further benefit patients by improving the period of survival and quality of life.

In conclusion, RNA modifications, such as m^6A and m^5C, play crucial and diverse roles in regulating tumor behaviors, therapy resistance, and disease prognosis in PDAC. Manipulating RNA modification regulators in preclinical experiments has significantly enhanced the effectiveness of PDAC treatments, including chemotherapy and immunotherapy. However, the complexity of studying the epitranscriptome has hindered our exploration of numerous RNA modifications and their specific functions. With advancements in RNA sequencing and structure prediction techniques, extensive opportunities have emerged for identifying novel targets for cancer therapy. In the foreseeable future, the investigation of RNA modifications will undoubtedly expand the therapeutic approaches accessible for cancer treatment.

Data availability statement

Data availability is not applicable to this article as no new data were created or analyzed in this study.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Hao Chen: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Wenhao Luo:** Writing – review & editing, Writing – original draft, Conceptualization. **Xiaoyue Lu:** Writing – review & editing, Writing – original draft, Visualization. **Taiping Zhang:** Writing – review & editing, Supervision.

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During the preparation of this work the authors used GPT 3.5 in order to improve the language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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