

N6-methyladenosine Modifications in Pancreatic Cancer: Molecular Mechanisms and Therapeutic Implications

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Abstract. Pancreatic cancer is one of the most aggressive malignancies with a high mortality rate and poor prognosis, characterized by low early diagnosis, resection, and survival rates. Recent studies have highlighted the role of N6-methyladenosine (m6A) RNA modification in the initiation, progression, and treatment of pancreatic cancer. This review explores the molecular mechanisms of m6A modification, focusing on the regulatory roles of m6A writers (e.g., METTL3, METTL14), erasers (e.g., FTO, ALKBH5), and readers (e.g., YTHDF2, YTHDC1) in pancreatic cancer. METTL14, a core component of the m6A methyltransferase complex, is overexpressed in pancreatic cancer tissues and correlates with poor patient survival. Its depletion reduces m6A levels and inhibits cancer cell growth, identifying PERP as a key target gene regulated by METTL14. Additionally, m6A modification impacts CAR-T cell therapy and immune checkpoint inhibitors (ICIs) by influencing T cell activation, expansion, and immune evasion. Targeting m6A regulators, such as METTL3 and YTHDF2, enhances the efficacy of CAR-T cells and ICIs, offering potential therapeutic strategies. Future research should validate the clinical relevance of the m6A-METTL14-PERP axis and develop selective inhibitors to improve pancreatic cancer treatment. Understanding m6A dynamics provides new insights into the molecular mechanisms underlying pancreatic cancer and highlights the potential for m6A-based therapies to overcome current treatment challenges.

Keywords: N6-methyladenosine, pancreatic cancer, molecular mechanism, therapeutic implication.

1. Introduction

Cancer, among the world's most hazardous diseases, serves as a complex public health distress. There are many forms of cancer, and pancreatic cancer is one of the most virulent subtypes, jeopardizing the digestive tract and bearing unique clinical parameters referred to by the term 'three lows and three highs,' low early diagnosis rate, low resection rate, low 5-year survival rate, high invasiveness, high metastasis rate, and high mortality rate. It is reported by CA: A Cancer Journal for Clinicians that pancreatic cancer was the 6th leading cause of cancer death in 2022, composed of 4.8% of all cancer mortality [1]. It is predicted that it could become the 3rd leading cause of cancer death in Europe by 2025 and the 2nd leading cause in the USA by 2030. The risk factors common to all other malignant tumors, such as family history of the disease, smoking, type 2 diabetes, and obesity, are also associated with pancreatic tumors. The absence of early stage-specific symptom characteristics that contribute to the dismal rate of early diagnosis of under 5% is a critical clinical dilemma. Therefore, the 5-year survival rate remains extremely low at 7.2%-9.0% [2], owing to the fact that only about 20% of patients' diagnoses are eligible for life-extending radical resection. Clinically, it is noted that 30-40% of patients' diagnoses have been recognized as locally advanced, while a further 40-50% have distant metastasis [3].

With the situation of this death-dealing disease, urgent effective strategy must be found. In order to achieve this, recent studies show that N6-methyladenosine (m6A) modification represents a new key target for cancer therapy. m6A, which refers to the methylation of adenosine at N6 position, was discovered in the 1970s [4], since then into the most abundant internal RNA modification in eukaryotes. Functionally speaking, the m6A modification regulates diverse physiological processes, for example, neurogenesis, hematopoiesis, spermatogenesis, sex determination, and circadian rhythm regulation. More crucially, here, many studies have accumulated to point out that it correlates very closely with initiation, progression, diagnosis, and treatment of malignancies to offer the hint that m6A dynamics may be manipulated to regulate tumorigenesis and tumor progression [5]. From the

point of mechanism, m6A modification undergoes dynamic regulation through three core groups of proteins, ‘writers’ (methyltransferases, the enzymes that catalyze methylation), ‘erasers’ (demethylases, the enzymes that reverse methylation), and ‘readers’ (the proteins that recognize and interpret m6A modifications). It was shown by a recent study that m6A modification can happen aberrantly in pancreatic cancer tissues and cell lines. It was also shown that abnormal expression of m6A regulators (writers, erasers, readers) and m6A-modified genes drive the progression of pancreatic cancer. In line with this, these observations indicate the potential for m6A modification to mediate pancreatic cancer pathogenesis. Elaborating on regulatory mechanisms of m6A modification in pancreatic cancer, in a systemic manner from multiple perspectives, is the objective of this review. Correspondingly, it touches on m6A-based drugs as approaches to the world addressing this health issue.

2. m6A Modifications and METTL14 in Pancreatic Cancer

m6A marks are introduced by the methyltransferase complex harboring METTL3, METTL14, and WTAP, among others. Aberrant m6A modifications are connected with different types of cancer. To begin with them, the author observed that the m6A levels were significantly higher in five out of the seven pancreatic cancer cell lines the author studied. These m6A levels were higher compared to the instances with HPDE cells and normal pancreatic tissues. Additionally, among the correlations of m6A levels with clinicopathological parameters, m6A expression levels were shown to correlate positively with poor overall survival in patients, and significantly more lymphatic metastasis was seen in the tumors with high m6A expressions than in those with low m6A expressions. The abovementioned m6A result, as well as in pancreatic cancer cell lines and in human pancreatic cancer tissues, confirm these findings. Next, in order to unveil the mechanisms responsible for the increased m6A levels, investigators will validate the major m6A regulatory factors (METTL3, METTL14, and WTAP) in the tumor tissue samples and the normal adjacent tissues. Interestingly, real-time PCR confirmed the upregulation of WTAP, METTL3, METTL14 in pancreatic cancer significantly versus adjacent non-cancerous tissues. Likewise, Western blot analysis revealed notably increased protein levels of all three in tissues of pancreatic cancer when versus with the simple ones. However, there appears an interesting aspect among the many molecular factors: only the METTL14 level was significantly associated with the patient survival rate, worse survival with a higher METTL14 level. Thus, in conclusion, the data the author obtained supported the idea of METTL14 as the major m6A methyltransferase role occupied with the clinicopathological aspects of pancreatic cancer.

Thus, the studies were specifically targeting METTL14. Significantly reduced the amount of METTL14 in human pancreatic cancer cell lines resulted in a much lower amount of m6A. Additionally, knocking down the METTL14 levels resulted in the significant inhibition of growth and colony formation of PANC-1 and MIA PaCa-2 cells (PANC-1 and MIA PaCa-2 are two types of cells derived from pancreatic cancer). Meanwhile, METTL14 overexpression promoted the growth and colony formation of both PANC-1 and BxPC-3. The following work will be aimed at detecting the METTL14 gene through studying the expression levels of the six genes, which were found to be the most highly upregulated in the RNA-Seq and m6A-Seq studies. In these targeted molecules, it was observed that both the mRNA and protein levels of P53-associated apoptosis effectors, which are known to be involved in the expression of PMP-22 (PERP), increased in cells depleted of METTL14. The downregulation of PERP mRNA is associated with the upregulation of METTL14 mRNA in the cells. This is equivalent to saying that the METTL14 gene is a vital PERP gene that directly contributes to the progression of pancreatic cancer. And then the author will show the involvement of PERP in the function of METTL14 to promote the growth of pancreatic cancer, where silencing PERP in the METTL14-depleted pancreatic cancer cells would lead to significant increase in PANC-1 cell viability and colony formation. However, the same METTL14 knockdown also diminished the viability and colony formation of low-PANC-1 cells. In addition, PERP knockdown

reversed the effect of METTL14 depletion on the invasion of pancreatic cancer cells. In summary, the results clearly showed that PERP is an important METTL14 target gene in pancreatic cancer.

As a member of the PMP22/GAS3/EMP membrane protein family, PERP was first identified as a p53 transcriptional target that becomes induced in the apoptotic process. Subsequent investigations revealed that downregulated PERP expression in various types of cancer was associated with tumor suppressor activity endorsed by PERP. Thus, PERP plays no known role in the death of clinically aggressive uveal melanoma, but it activates feedback-amplifying processes involving p53 and p63 to reach the critical apoptosis-inducing PERP level [6].

3. m6A Writers, Erasers, and Readers in Pancreatic Cancer

m6A writers are the category of enzymes called methyltransferases, which operate using S-adenosyl methionine (SAM) as a cofactor. They are the kind of enzymes that exploit S-adenosyl methionine (SAM) as a cofactor and mediate the N6-methylation process by moving a methyl group from SAM to the adenosine substrate. In general, the writers are often multifunctional complexes formed by several critical constituents such as METTL3, METTL14, and WTAP. METTL14 is one of the names mentioned. METTL14, as it was shown to be part of the complex containing the m6A methyltransferase, also gained a lot of attention among the researchers. The presence of METTL3 and METTL14 in mammals is also highly conserved, and they show up together as well-stabilized heterodimers. Also, the METTL14 has essential functions in a number of biological events. The knockdown of METTL14 leads to the disturbance of the self-renewal process and cell differentiation of embryonic stem cells, the abnormalities in the development of embryos, and the problems in gametogenesis in some different organisms. METTL14 and METTL3 also regulate the cell cycle progression of cortical neural progenitor cells through YTHDF3-m6A-related mechanisms, contributing to neurogenesis. METTL14 performs the role of a tumor suppressor in glioblastoma; Depletion of METTL3 or METTL14 enhances the growth and self-renewal of glioblastoma stem cells and promotes tumor formation.

m6A erasers are demethylating chemicals, which are used to erase m6A building blocks and thus impact the reversing nature of m6A methylation. Notable m6A erasers are such as fat mass and obesity-associated protein (FTO) and alpha-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5). Dysfunction of these proteins is a common process in cancers, and alterations of these substances are crucial for malignancies. Primarily, FTO takes off m6A methylation from mRNA in the nucleus, and ALKBH5 acts in the cytoplasm, where it controls m6A demethylation due to the collocation with nuclear speckles. In the case of FTO, this was a demethylase acting in vitro on N3-methylthymidine in the single-stranded DNA and N3-methyluridine in the single-stranded RNA, while in vivo, FTO was found to demethylate m6A in both DNA and RNA. Knockdown of FTO leads to significantly increased m6A levels in the total polyadenylated RNA [7]. FTO promotes, in human glioblastoma stem cells, both cell development and self-renewal and is required to support full tumor progression. Loss of FTO promotes survival in glioblastoma stem cell-around humans by the way of altering crucial gene expression.

m6A readers are those RNA-binding proteins attuned to m6A modifications, which facilitate RNA-protein interactions and affect cellular pathways such as RNA splicing, export, translation, and degradation. The readers are the ones with highly specific preferences when it comes to the m6A methylation sites. YTHDC1, HNRNPA2B1, HNRNPC11, and HNRNPG are genes which have different roles in m6A recognition in the nucleus, while YTHDFs (YTHDF1, YTHDF2, and YTHDF3) and YTHDCs (YTHDC2) are cytoplasmic m6A readers. IGF2BP1, IGF2BP2, and IGF2BP3 are also mentioned as such. The recently discovered m6A readers are the KH-type splicing regulatory protein (KHSRP), which is the oncogenic factor during gastritis and pancreatic ductal adenocarcinomas (PDAC) progression. The newly identified m6A readers are FMRP (fragile X mental retardation protein), PRRC2A (proline-rich coiled-coil protein 2A), RBM33 (RNA-binding motif protein 33), and eIF3 (Eukaryotic Initiation Factor 3). Some m6A readers perform several

functions using the m6A-modified components sites, and it is evident in the example of YTHDF3 that binds to YTHDF1 to promote translation of methylated RNA, while YTHDF2 accelerates mRNA decay and also serves as a reader. YTHDF2 works differently as it stabilizes its target mRNAs in m6A-dependent fashion.

Overall, the m6A writers and erasers have the function to create the reversible methylation of m6A, while the m6A readers are involved in the fate of RNA after transcription in the post-transcriptional regulation of RNA. This interaction system provides a novel and prospective post-transcriptional mechanism for gene expression regulation. It has been noticed that the presence of m6A modification is repeatedly linked with pancreatic cancer initiation and its development. YTHDC1 is found in the breaks of the major and highly conserved YTH family gene members, which are part of the nucleus, and produces YTH bodies (compaction of RNA binding proteins) at the sites of active transcription next to processing speckles of RNA. Thus, YTHDC1 plays the role of fine-tuning splicing by causing excess of particular splicing factors, some of which are inscribed by SR proteins (SR is also known as serine-arginine repeat proteins). In addition to promoting silencing, it is also associated with the m6A present on X chromosome of XIST mediating the silencing of X chromosome of the female. Many of the properties are used to support YTHDC1 for mediation of m6A reading of XIST and interactivity with other important factors like SRSF3 and NXF1, which altogether induce m6A modifications on mRNAs, preferred for the nuclear export. This discovery indicates that YTHDC1 has other roles in mRNA metabolism other than its influence on the splicing of mRNA. YTHDC1 could actually be the tumor suppressor involved in the therapy of endometrial cancer via the interaction with different proteins of splicing [8].

4. m6A Modifications in CAR-T Cells and Immune Checkpoint Inhibitors

As a result, M6A has already become a useful treatment for cancer. In the last part, there are 2 types of treatment which are about M6A CAR-T cells with genetic engineering feature T cells that are tumor-targeting and tumor-killing specific. The m6A modification here is the key ingredient to make the CAR-T cells function more efficiently and sword-wielding for longer periods of time. Aiming at m6A regulation will not only be inconclusive with respect to the morphology of CAR-T cells, which comprise activation, expansion, and memory formation, but also these three necessary features are principles, not laws, of CAR-T cell therapies' success. Researchers have proposed that METTL3 is a deciding factor behind T cell growth in vivo, as well as for sustaining its survival. To show how METTL3 deletion in CAR-T cells affects these cells, it was found that without meaningful enhancement, they have much less persistence in tumor-bearing mice throughout their lives. This is due to the destabilization of many mRNAs during this process, such as IL-2 and IFN- γ , which are indispensable for the strong activity and proliferation of CAR-T cells [9]. YTHDF2, the factor that induces CAR-T cells' activation through the translational regulation of particular receptors for the cytokines, was found to specifically boost the stability of the respective receptors' mRNAs. This observation revealed why the signaling increased and hence led to the accumulation of the CAR-T cells, suggesting that the existence of YTHDF2 promotes the anti-cancer ability of CAR-T cells. This piece does so by shedding light on one method – a modulation of m6A pathway – used in CAR-T cell therapies, which could be an exemplary manipulation to overcome the hurdles of CAR-T cells and make the therapy a triumph by enhancing the CAR-T cells' longevity and activation. This research suggests that depletion of enzymes such as METTL3 through the use of methyltransferases inhibitors or interfering with other components of the m6A can be a very useful therapeutic tool to escalate CAR-T cell efficacy against the two major antagonistic factors: the short-lived nature of CAR-T cells and their limited persistence in the tumor microenvironment.

The concept of tumor immunotherapy is now firmly established as one of the key approaches incorporated in cancer therapy, with some positive successes to talk about. Immune checkpoints inhibitors (ICIs) represent one of the major categories of immunotherapy drugs, with their target molecules being CTLA-4, PD-1, and PD-L1. These strategies in the last decades mostly have resulted

in a small but steady rise in the prognosis of patients with the most aggressive cancers. Unfortunately, however, the same thing that potentially makes this combined therapy effective and workable is the fact that some patients do not respond to this multimodal approach or only partially, which leads to treatment resistance and recurrence problems. Recent studies have shown that tumor immune evasion is a pivotal function of m6A modifications in many aspects. The changes this entail can influence the level of target molecules for ICIs (PD-1, PD-L1, CTLA4, and other timepoints) or pathways, which are coupled with changes in the potency of the ICIs. A distinguishable influence might arise from m6A modification on the immune response or vice versa, which could have an impact on the development of immunotherapy agents, like PD-1 and PD-L1 antibodies, that are widely employed for cancer management. The research has revealed m6A marks with increased frequency to modify the levels of immune checkpoints like PD-L1, which is a classical tumor immune evasive strategy, that is frequently overexpressed in the tumor cells to inhibit the host immune response. The research team noted that YTHDF2, a protein that binds m6A and regulates PD-L1 mRNA decay, was a factor for PD-L1 mRNA stabilization in order to synthesize it in tumor cells. The researchers showed that m6A regulatory proteins, like YTHDF2, were most successful in associating with the anti-tumor immunity. This result also indicates that the m6A modification could affect T cell activation and immune checkpoint molecule presentation on tumor cells, which eventually resulted in diminishing ICI effectiveness. One of the studies therefore accentuates the possibility of blocking the m6A pathway to achieve better ICI therapy outcome. T cell responses can be maximized by modulating m6A expressions of known regulators like METTL3 or YTHDF2, among others, which also affects tumor-induced immune suppression. Over time there is the observed possibility of increasing the effectiveness of PD-1/PD-L1 biomarkers as well as other checkpoint immune therapies [10].

5. Conclusion

It is clear that N6-methyladenosine modification has been regarded as the main regulatory process from the initiation of pancreatic cancer to therapeutic development. In this context, this review summarizes the main messages regarding the way how m6A dynamics lead to the formation and development of pancreatic cancer, and the possible use of these insights to improve clinical outcome. During the molecular level, m6A modification sits under a collaborative system of writers (such as METTL3, METTL14, and WTAP methyltransferases) and erasers (like FTO and ALKBH5 demethylases) and readers (such as YTHDF2 and YTHDC1 recognition proteins). These elements are key in post-transcriptional control of RNA metabolism, from splicing to translation and degradation, and any deregulation of their function will lead to pancreatic carcinoma pathogenesis. Experimental data indicates the majority of pancreatic cancer cell lines have upregulated m6A levels compared to normal pancreatic ductal epithelial cells and healthy diseases; this is the first fact that I would like to highlight. Clinically, the m6A greater expression associates to poor overall survival and pathologic lymphatic metastasis in pancreatic cancer patients, which was directly related to their disease aggressiveness. Notable among these key modulators is METTL14, which is specific: The gene is uniformly overexpressed in pancreatic cancer tissues (both by real-time PCR and Western blot methods) and, uniquely, is associated with patient prognosis—higher tumor levels of METTL14 correlates with worse cancer survival. Furthermore, its functional studies confirm its role: Ingrowth inhibition and decreased global m6A levels occur when METTL14 is depleted (downregulation), and PANC-1 and MIA PaCa-2 cells are affected; PERP is upregulated, a tumor suppressor in this case. mRNA and protein levels of PERP that are negatively regulated by METTL14 are linked through m6A modification, and a METTL14-PERP axis is shown to be critical in promoting pancreatic cancer progression.

However, in addition to this, the m6A modification has an additional role in providing treatment approaches. The immune-therapy process is quite significant as in CAR-T cell therapy, targeting m6A regulators enhances the efficacy, and examples include METTL3 deletion that impairs CAR-T cell expansion and persistence in vivo by destabilizing mRNAs of key cytokines (IL-2 and IFN- γ); the

YTHDF2 supports CAR-T activation through stabilization of the mRNAs of ligand binding receptors, prolonging the anti-tumor activity, these things highlight what the CAR-T therapeutic option can do and solve the problem of short cell lifespan and limited tumor infiltration in pancreatic cancer. The case is no different for immune checkpoint inhibitor therapy where dependent proteins (YTHDF2, for instance) exploit immune evasion through regulating PD1 of tumor cells. Progress in reducing PD-L1 expression, generating anti-tumor immune response, and improving the efficacy of PD-1 blockade was achieved through inhibiting YTHDF2, which has a direct impact on overcoming ICI resistance, a factor that has been a major hindrance to effective pancreatic cancer treatment.

Seeing the future, the validation of the axis, m6A-METTL14-PERP, in the larger patient cohort will be a major next step in order to firmly establish its clinical relevance. Furthermore, scientists can enlighten new mechanism layers of the disorder by understanding the functions of underappreciated m6A regulators, WTAP as an example of the writer. This area is also translationally important—the design of the selective METTL14 inhibitors and YTHDF2 modulators as well as optimization of their concentration to preferentially target pancreatic cancer cells is major objectives to be achieved. The combination of m6A-driven therapies with standard treatments, such as CAR-T or ICIs, may generate the multi-mechanisms counter cancer approach that could be useful in overcoming pancreatic cancer. Finally, with the implementation of m6A modification through the diagnostics field and medicine, the most promising outcomes can be observed for the management of pancreatic cancer onset of new biomarkers for diagnosis and effective therapies, which ultimately will result in new hope in the struggle of patients with this disease against the "three lows and three highs".

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