

Role of RNA methylation in the regulation of pancreatic cancer stem cells (Review)

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Abstract. Pancreatic cancer stem cells (CSCs) play a key role in the initiation and progression of pancreatic adenocarcinoma (PDAC). CSCs are responsible for resistance to chemotherapy and radiation, and for cancer metastasis. Recent studies have indicated that RNA methylation, a type of RNA modification, predominantly occurring as m6A methylation, plays an important role in controlling the stemness of cancer cells, therapeutic resistance against chemotherapy and radiation therapy, and their overall relevance to a patient's prognosis. CSCs regulate various behaviors of cancer through cell-cell communication by secreting factors, through their receptors, and through signal transduction. Recent studies have shown that RNA methylation is involved in the biology of the heterogeneity of PDAC. The present review provides an update on the current understanding of RNA modification-based therapeutic targets against deleterious PDAC. Several key pathways and agents that can specifically target CSCs have been identified, thus providing novel insights into the early diagnosis and efficient treatment of PDAC.

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1. Introduction

Pancreatic adenocarcinoma (PDAC) is a life-threatening condition, the incidence of which has been increasing, and is now predicted to be the second leading cause of cancer-associated death in certain regions of the world (1). Given that patients have no apparent symptoms specific to PDAC and that PDAC usually shows aggressive invasion, distant metastasis, and chemotherapeutic resistance even in the initial stages of carcinogenesis (2), a lack of early diagnosis is considered a leading challenge in the management of PDAC (3). Since PDAC is commonly diagnosed in the first instance at an advanced stage, when most treatment regimens are ineffective, investigating PDAC and understanding the mechanism of how it is biologically aggressive is indispensable for the development of innovative therapeutic approaches against PDAC (4).

Previous efforts in understanding the therapeutic resistance of tumors led to the identification of cancer stem cells (CSCs), and there is overwhelming evidence that virtually all cancers are clonal and represent a single-cell progeny (5-7). A recent study demonstrated that the identification of pre-leukemic hematopoietic stem cells in acute leukemia and mapping of the evolutionary trajectory derived from the first somatic mutation to the eventual development of cancer resulted in an increased understanding of the mechanistic underpinnings of CSC lineages (8). Given that CSCs are biologically malignant in nature, exhibiting high invasive and metastatic potential, increased survival, and possessing enhanced therapeutic resistance (9), understanding the stemness mechanism may highlight novel avenues for overcoming PDAC, a life-threatening disease. Here, the intracellular and intercellular signaling pathways employed by CSCs and their surrounding cells in the tumor microenvironment of the pancreas are discussed.

2. Recent studies on pancreatic cancer stem cells

The study of gastrointestinal cancer identified the presence of CSCs in colon cancer (10) and hepatocellular cancer (11,12). The study of PDAC indicated that CSC-specific markers include prominin 1 (CD133), small cell lung carcinoma cluster 4 antigen (CD24), hyaluronate receptor (CD44), leukocyte-derived seven transmembrane domain receptor

(CXCR4), epithelial cell adhesion molecule (EpCAM), ATP-binding cassette subfamily G member 2 (ABCG2), MET proto-oncogene, receptor tyrosine kinase (c-Met), aldehyde dehydrogenase 1 family member A1 (ALDH1), and nestin (Table I) (13).

Although the role of RNA methylation in the control of CSC markers of PDAC is under investigation, previous reports demonstrated that METTL3 improves oxaliplatin resistance of CD133+ CSCs in the stomach by promoting mRNA stability of poly(ADP-ribose) polymerase 1 (PARP1) (14). Alteration of the m6A modification reportedly reduces the efficacy of drugs by regulating the expression of several drug efflux transporters, including ABCG2, and altering the m6A modification may prevent drug-mediated cell death through regulation of DNA damage repair in the tumor microenvironment (15). m6A methylation-regulated AF4/FMR2 family member 4 (AFF4) was reported to enhance the self-renewal of bladder CSCs as identified by ALDH activity (16). Therefore, m6A modifications are proposed to be intricately associated with the functions of CSCs.

Recent technological advancements have allowed the study of RNA expression profiles in each cell, highlighting the true heterogeneity of a cancer (17,18). A recent study indicated that single-cell RNA sequencing analysis of PDAC from patients and control pancreatic tissues revealed the transformation process of CSC-like ductal cells into ductal cells with invasive potential and determined CSC-related prognostic genes associated with significantly worse overall survival, suggesting an insight into the invasive trajectory for the treatment of PDAC (19). Moreover, a recent study of RNA transcription mechanisms revealed that polymerase II-associated factor 1 (PAF1), an RNA PAF, forms a complex to maintain CSC pluripotency by interacting with DEAD (Asp-Glu-Ala-Asp) box helicase 3, X-linked (DDX3), and PHD-finger protein 5A (PHF5A, a subunit of the splicing factor 3b protein complex) to regulate the expression of self-renewal markers (homeobox transcription factor NANOG, SRY-box transcription factor 9 (SOX9), and β -catenin), in therapeutic-resistant phenotypes (CD44v6 and ALDH1), and other metastasis-associated gene signatures (20). The results of a knockdown experiment of PAF1 in mice suggested that strategies targeting the PAF1-PHF5A-DDX3 complex may reduce or inhibit PDAC progression (20).

During the early elongation stage of RNA transcription, the PAF1 complex is required to determine its association with compass-orientational RNA polymerase II (21). Given that monoubiquitination of histone H2B, catalyzed by ubiquitin-conjugating enzyme E2 (Rad6)-E3 Ubiquitin-Protein Ligase BRE1, is required for histone H3 methylation on lysine residues 4 and 79, catalyzed by the Set1-containing complex, the Paf1 complex is required for Rad6-Bre1 catalytic activity (21). Dysregulation of the human RNA polymerase-II-associated factor complex was observed in the cancer cells (22). Although the significance of the PAF1 complex in CSCs remains to be completely understood, its biological function may be observed by binding with partners such as PHF5A in CSCs.

Although the significance of the relationship between pattern recognition against pathogenic organisms to cellular RNA modification by PHF5A requires further elucidation, a recent study suggested that the data in colorectal cancer

(CRC) may support this theory regarding roles of PHF5A in CSCs. A study on CRC reported that PHF5A trans-activated superoxide dismutase 2 (SOD2) by regulating lysine acetyltransferase 2A [with an alternative name of GCN5, a histone acetyltransferase (HAT) that primarily functions as a transcriptional activator] messenger RNA alternative splicing after being exposed to enterotoxigenic *Bacteroides fragilis* (ETBF) was strongly associated with the occurrence of inflammatory bowel disease (IBD), colitis-associated colorectal cancer, and CRC (23). Interestingly, PHF5A upregulation was associated with miR-149-3p downregulation, and this was dependent on N6-adenosine-methyltransferase subunit METTL14-mediated N6-methyladenosine methylation (23). Targeting the ETBF/miR-149-3p pathway presents a promising approach for treating patients with IBD and CRC with higher ETBF expression levels (23), suggesting that pattern recognition against pathogenic organisms may be associated with cellular RNA modification (24).

3. Recent studies on RNA methylation in PDAC

Studies of RNA modifications demonstrate that RNA modification is performed by writers [addition of CH₃- to RNA by the enzymatic reactions of methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit (METTL3), METTL14, and Wilms tumor 1 associated protein (WTAP)], erasers [removal of CH₃- from RNA by the enzymatic reactions of fat mass and obesity-associated protein (FTO) and α -ketoglutarate-dependent dioxygenase AlkB Homolog (ALKBH5)], and readers [recognition of methylated RNA by RNA binding proteins, such as heterogeneous nuclear ribonucleoprotein (hnRNP) and N6-methyladenosine RNA binding, YTH domain family protein (YTHDF)] (25). In cancer, cell-cell communication in the tumor microenvironment, such as that between epithelial and mesenchymal cells induces the signaling pathways that result in RNA modifications (25). In 1974, m6A was detected in poly(A) RNA fractions (26,27), and recent sequencing methods revealed that m6A levels in mRNA appeared to be dynamic, with levels varying in terms of development and response to cellular stresses (28,29). Furthermore, the enzyme FTO, which is associated with human obesity (30), could reportedly demethylate m6A. Therefore, aside from methylation, m6A can be dynamically regulated through removal (31). The biochemical process of RNA modification mediated by methylation, demethylation, and recognition of the methylation status has been studied, whereas the mechanisms of up or downregulation of gene expression of the enzymes which are involved in the RNA modification process remain to be fully understood (25). In gastrointestinal cancer, the MYC proto-oncogene and BHLH transcription factor oncogene are shown to promote the expression of RNA modification readers at m6A, which can contribute to the imbalance of the epitranscriptome system in cancer (25). Considering that methylation, demethylation, and recognition of changes are involved in the regulation of the cellular process, recent studies of PDAC have emerged in recent years and indicated that METTL3 largely plays a role in promoting PDAC, although it is also involved in the counterbalance via a complex mechanism, summarized in Table II.

Table I. Markers of cancer stem cells in pancreatic ductal adenocarcinoma.

Marker	Description	Structure	Function	(Refs.)
CD133	Prominin 1	Pentaspans transmembrane glycoprotein	Suppressing differentiation	(64)
CD24	Small cell lung carcinoma cluster 4 antigen	Anchored via a glycosyl phosphatidylinositol link to the cell surface	Preventing terminal differentiation	(65)
CD44	Hyaluronate receptor	Cell-surface glycoprotein, with many functionally distinct isoforms by complex alternative splicing	Cell-cell interactions, cell adhesion, migration, and regulation of metabolism	(65)
CXCR4	Leukocyte-derived seven transmembrane domain receptor	Seven transmembrane regions on cell surface	G protein-coupled receptor activity and ubiquitin protein ligase binding for stem cell signaling	(66)
Epcam/ESA	Epithelial cell adhesion molecule	Type I membrane protein	Providing immunological barrier, and proliferation and differentiation	(65)
ABCG2	ATP binding cassette subfamily G member 2	Superfamily of this protein transports various molecules across extra- and intra-cellular membranes	Function as a xenobiotic transporter which may play a major role in multi-drug resistance	(67,68)
C-Met	MET proto-oncogene, receptor tyrosine kinase	A prototypical receptor tyrosine kinase, whose ligand is hepatocyte growth factor	This protein plays a role in cellular survival, embryogenesis, and cellular migration and invasion, including cancer	(69,70)
ALDH1	Aldehyde Dehydrogenase 1 Family Member A1	This belongs to the ALDH family.	This encodes the next enzyme after alcohol dehydrogenase in the major pathway of alcohol metabolism.	(71)
NES	Nestin	A member of the intermediate filament protein family	This is involved in structural molecule activity and intermediate filament binding.	(72)

Importantly, quantification of the m6A RNA methylation modulator pattern has been demonstrated to allow precise evaluation of the tumor microenvironment of PDAC, including immune response, suggesting the usefulness of a potential biomarker for prognosis (32). RNA modification is associated with the progression of tumor heterogeneity (25). A study of data obtained from TCGA (185 samples) indicated that m6A regulatory genes played an important role in the prognosis, progression, and regulation of the immune microenvironment in PDAC (33). In the tumor microenvironment, glutamate from the nerve cells upregulated the expression of hexokinase 2 (HK2) through METTL3-mediated mRNA m6A modification, N-methyl-D-aspartate receptor (NMDAR2B), and downstream Ca^{2+} -dependent calcium/calmodulin-dependent protein kinase (CaM Kinase) II/mitogen-activated protein

kinase 1 (ERK)-mitogen-activated protein kinase (MAPK) pathway in PDAC (34).

Recent studies have suggested that m6A RNA alterations play essential physiological and pathological roles, particularly in the initiation and progression of various types of cancer, such as those of hematopoietic malignancies, central nervous tumors, and reproductive cancers (35). Particularly in PDAC, the formation, and evolution of the disease show both common and unique pathways when compared with other malignancies (36). Although the common pathways are responsible for regulating the balance of methylation and demethylation, the unique pathways include the mechanism through which ALKBH5 suppresses period circadian regulator 1-ataxia telangiectasia mutated-checkpoint kinase 2-tumor protein P53 (TP53)-cell division cycle 25C signaling in an m6A-YTHDF2-dependent manner, and TP53-induced

Table II. RNA methylation in PDAC.

First author, year	Gene	Function	Mechanism in related pathway	Change in expression	Oncogene or tumor suppressor	(Refs.)
Xia <i>et al.</i> , 2019	METTL3	M6a writer	METTL3 promotes PDAC proliferation and invasion	Upregulated	Oncogene	(73)
Zhang <i>et al.</i> , 2019	METTL3	M6a writer	METTL3-dependent excessive miR-25-3p maturation promotes PDAC	Upregulated	Oncogene	(74)
Tang <i>et al.</i> , 2022	METTL3	M6a writer	METTL3 promotes PDAC by regulating stability of E2F5	Upregulated	Oncogene	(75)
Guo <i>et al.</i> , 2022	METTL3	M6a writer	METTL3-IGF2BP3-axis regulates spermine synthase m6a modification	Upregulated	Oncogene	(76)
Li <i>et al.</i> , 2022	METTL3	M6a writer	Increased expression of METTL3 in PDAC associates with poor survival of the patients	Upregulated	Oncogene	(77)
Song <i>et al.</i> , 2022	METTL3	M6a writer	lncRNA MALAT1 regulates METTL3-mediated PD-L1 expression in PDAC	Upregulated	Presumably Oncogene	(78)
Taketo <i>et al.</i> , 2018	METTL3	M6a writer	METTL3 promotes chemo- and radioresistance in PDAC	Upregulated	Oncogene	(79)
Jiang <i>et al.</i> , 2022	METTL3, METTL14	M6a writer	M6a-mediated miR-380-3p maturation and upregulation promotes PDAC	Upregulated	Oncogene	(80)
Chen <i>et al.</i> , 2021	METTL3	M6a writer	M6a-mediated up-regulation of lncRNA LIFR-AS1 enhances PDAC via miR-150-5p/VEGFA/AKT signaling	Upregulated	Oncogene	(81)
He <i>et al.</i> , 2023	METTL3	M6a writer	Linc-UROD stabilized by IGF2BP3/METTL3 contributes to glycolysis and malignant phenotype of PDAC	Upregulated	Presumably Oncogene	(82)
Tatekawa <i>et al.</i> , 2022	METTL3	M6a writer	M6a regulates polo like kinase 1 cell cycle homeostasis as a target of radiotherapy in PDAC	Upregulated	Presumably Oncogene	(83)
Ye <i>et al.</i> , 2022	METTL3	M6a writer	Increased m6a modification of lncRNA DBH-AS1 suppresses PDAC via miR-3163/USP44 axis	Downregulated	Tumor suppressive	(84)
Huang <i>et al.</i> , 2022	ZC3H13	M6a writer	ZC3H13 mediates m6a modification of PHF10, which induces DNA damage repair as a barrier	Upregulated	Oncogene	(85)
Hou <i>et al.</i> , 2020	KIAA1429	M6a writer	A promising prognostic biomarker	Upregulated	Presumably Oncogene	(86)
Chijimatsu, <i>et al.</i> , 2022	FTO	M6a eraser	Targeting FTO suppresses PDAC	Upregulated	Oncogene	(50)

Table II. Continued.

First author, year	Gene	Function	Mechanism in related pathway	Change in expression	Oncogene or tumor suppressor	(Refs.)
Wang <i>et al</i> , 2022	FTO	M6a eraser	FTO promotes the progression of PDAC through reducing m6a/YTHDF1 mediated TFPI-2 mRNA stability	Upregulated	Oncogene	(87)
Garg, <i>et al</i> , 2022	ALKBH5	M6a eraser	ALKBH5 deficiency reduces the mRNA stability of key pancreatic transcription factors	Upregulated	Oncogene	(49)
Huang, <i>et al</i> , 2021	ALKBH5	M6a eraser	ALKBH5 protects against PDAC	Downregulated	Tumor suppressive	(88)
Hou <i>et al</i> , 2020	HNRNPC	M6a reader	A promising prognostic biomarker	Upregulated	Presumably Oncogene	(86)
Hou <i>et al</i> , 2020	IGF2BP2	M6a reader	A promising prognostic biomarker	Upregulated	Presumably Oncogene	(86)

PDAC, pancreatic ductal adenocarcinoma.

ALKBH5 activation acts as a feedback loop regulating m6A modification in PDAC (37). These studies suggest that RNA alterations are potential therapeutic targets and early diagnostic and prognostic cancer biomarkers (35-37). Previous studies have shown the significant role of RNA modifications in whole cancer tumor tissues, but not in the CSC fraction, in several types of cancer (35), such as PDAC (36), through RNA processing (37). However, the RNA processing-dependent mechanism in a CSC fraction in PDAC remains to be fully understood. Here, an update on the current body of knowledge regarding recent advances in studies on RNA methylation in the CSC fraction in tumor tissues of PDAC is provided.

4. Significance of RNA methylation in CSCs

Previous studies demonstrated that RNA modification plays a role in the initiation and maintenance of CSCs in several types of cancer (38), including hematopoietic malignancies (39,40) such as breast cancer (41,42) and brain tumors (43-45). m6A modification affects the properties of CSC, including tumor progression and treatment responses (38). m6A modification is involved in the function of protein-coding mRNAs and non-coding RNAs in CSCs (46). A recent study revealed that m6A methylation is associated with cancer, contributing to the self-renewal of CSC, promotion and initiation of cancer progression, and resistance to radiotherapy or chemotherapy (47).

Nevertheless, the significance and implication of RNA modifications in CSCs of PDAC remain to be fully investigated, although several reports have provided critical insights into the multifaceted roles of CSCs in PDAC (48,49). This may be associated with the insufficient power of single-cell analysis

technology for RNA modification to investigate tumor heterogeneity in PDAC. Given that mRNA profiling was successfully performed with single-cell analysis technology (50), the significance and implications of RNA modification in CSCs of PDAC may be elucidated in the near future.

5. Possible therapeutic targeting of RNA methylation of CSCs in PDAC

To understand the intractability of PDAC and create innovative therapeutic approaches to overcome this disease, the molecular and cellular biological properties of pancreatic CSCs should be elucidated. As mentioned before, CSCs have self-renewal, pluripotent, and tumorigenic properties under the control of the epigenome, and this is dependent on the metabolic characteristics of cancer (such as the presence of a hypoxic environment) in the tumor microenvironment (9,39), and this allows a cancer to exhibit resistance to anticancer drugs and radiation therapy (51).

The findings of previous studies have emphasized the importance of RNA methylation in the pathogenesis of several types of cancer, including pancreatic cancer (14,15,25). In particular, a recent study showed the role of RNA methylation in CSCs, and its importance in pancreatic CSCs is expected to be elucidated (25) (Fig. 1).

Furthermore, RNA methylation is hypothesized to play an important role in pancreatic CSCs for the following reasons: i) The epigenome reflects the extracellular environment and is destined for intracellular metabolism. RNA methylation enzymes convert the target RNA into a methylation donor using S-adenosylmethionine (SAM) produced under inflammatory stimuli (52). RNA demethylase is a dioxygenase

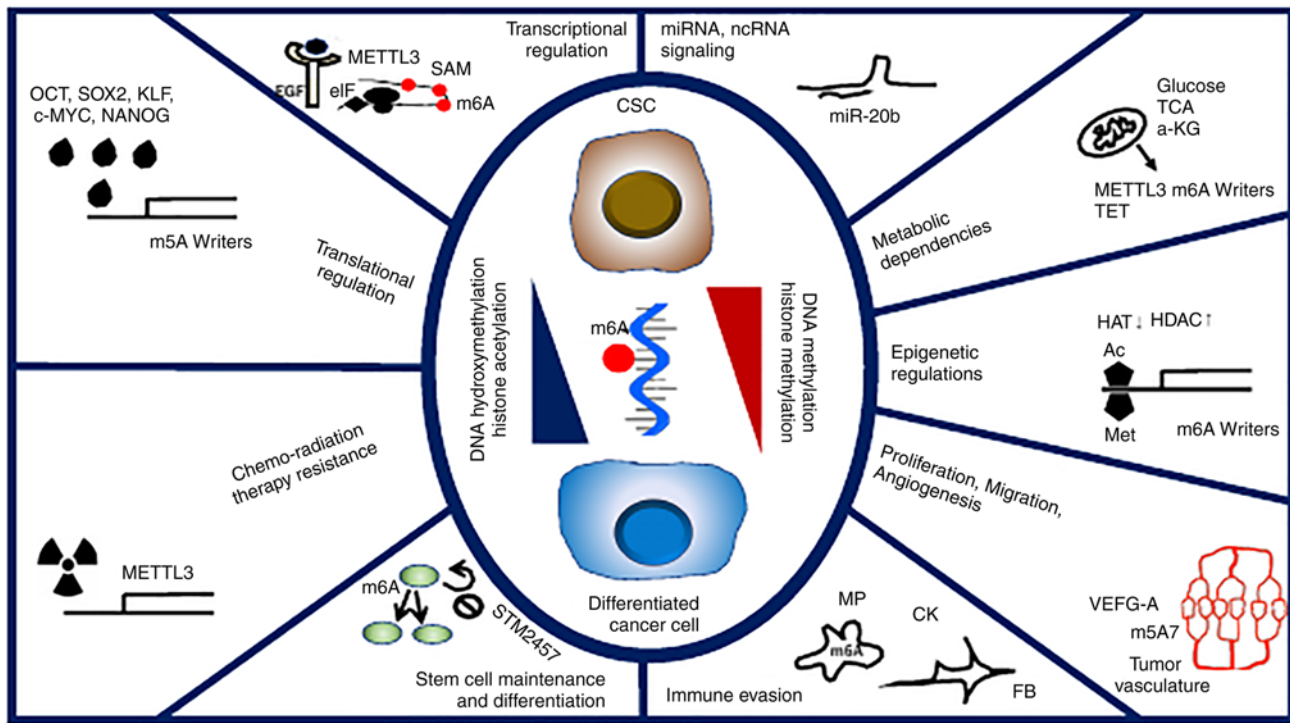


Figure 1. Roles of CSC in the regulation of PDAC. Earlier studies suggested that RNA methylation contributed to PDAC and other elements of tumor heterogeneity (25). RNA modification reportedly exerted a critical function in transcriptional regulation (25), translational regulation (25), A-to-I editing (89), chemoradiation therapy resistance (53,77), miRNA and ncRNA signaling (90), metabolic dependencies (91), and epigenetic regulation (92). These mechanisms are responsible for the orchestration of the control of proliferation, migration, and angiogenesis of CSC-like cells, for stem cell maintenance and differentiation, as well as for evasion of antitumor immunogenic cells (93). Therefore, the aforementioned mechanisms are involved in CSC-mediated regulation of PDAC. The involvement of ncRNAs, such as miR-20b (94), has been reported. It was shown that miR-20b enhanced both cyclin D1 and E2F transcription factor 1 target via direct regulation (95). It was also suggested that miR-20b played a role in the regulation of the cell cycle of CSCs (95). The active DNA demethylation process includes an intermediate step known as TET, which catalyzes the conversion of 5mC into 5hmC (96). TCA, tricarboxylic acid cycle; α -KG, α -ketoglutarate; VEGF-A, vascular endothelial growth factor A; HDAC, histone deacetylase; HAT, histone acetyltransferase; MP, macrophage; FB, fibroblast; CK, cytokine; METTL3, N6-adenosine-methyltransferase catalytic subunit; Oct3/4, POU class 5 homeobox 1; SOX2, SRY-box transcription factor 2; KLF, Kruppel-like factor transcription factor; c-MYC, V-MYC avian myelocytomatosis viral oncogene homolog; SAM, S-adenosylmethionine; eIF, eukaryotic translation initiation factor; TET, ten-eleven translocation; 5mc, 5-methylcytosine; 5hmc, 5-hydroxymethylcytosine.

working in the nucleus with mitochondrial α -ketoglutarate, a cofactor involved in cancer aggressiveness, and could be a therapeutic target (52-54). The RNA methylation process, (that is, the methylome) may be a target of several cancers as a cancer-sieging strategy (53). DNA demethylase is also catalyzed by dioxygenases that belong to the ten-eleven translocation (TET) family (55). The TET proteins are important players in dioxygenase for DNA hydroxymethylation, which regulates gene expression in tumor cells, though its roles in PDAC remain to be fully investigated (55). ii) RNA methylation is involved in splicing and translation and plays an essential role in cell differentiation (56,57). The hierarchy of differentiation in CSCs is hypothesized to develop through abnormal RNA processing rather than through the inclusion of new DNA mutations (53,56,57); this is due to the fact that cellular reprogramming occurs during the process of epigenetic mechanism in iPS cells (58,59). Beyond the occurrence of DNA mutations in cancer, RNA methylation is involved in splicing and translation, but also in RNA editing via posttranscriptional regulation, although the significance of this in PDAC remains to be fully understood (Fig. 1). iii) By clarifying RNA methylation at the single-cell level, precision medicine can be established (50). Although RNA-methylation writer proteins are generally oncogenic in all pancreatic

cancers, the role of erasers in cancer varies considerably (25). The significance and role of RNA methylation at the molecular level are expected to be clarified by examining information at the cellular and gene levels. Although certain studies have already been conducted to assess the whole status of RNA methylation in tumors (25), the development and practical application of a single-cell technique for RNA methylation analysis is warranted. Although standard single-cell analysis allows for the identification of sequence information of mRNAs with a poly(A) tail, recent studies indicate the possible involvement of non-coding RNAs (Fig. 1). iv) Treatments targeting RNA methylation and the associated pathways using low-molecular-weight drugs and related strategies have been assessed in clinical studies (60-62). It has already been shown that pharmacological inhibition of METTL3 *in vivo* leads to impaired engraftment and prolonged survival in animal models of hematopoietic malignancies, specifically targeting key stem cell subpopulations of leukemia (60). If the targets for diagnosis and therapy can be clarified and similarly narrowed down, it can potentially reveal hitherto unknown combinations of CSC-targeting strategies for the management of PDAC. Whether targeting RNA methylation in PDAC is a suitable approach remains to be determined, the beneficial effects can be expected in the therapeutic targeting of RNA methylation

for hematopoietic malignancies as shown in the studies of STM2457, which exhibited pronounced anti-tumor efficacy in animal models (60). In the case of possible application for the management of PDAC or other types of cancers, adverse effects may serve to limit the viability of this approach. To the best of our knowledge, there have been no reports on adverse events from small animal tests, that have led to the discontinuation of the entire development process. However, careful considerations and precautions should be taken such as testing medium-sized animals, before moving on to human studies. The efficacy and cytotoxicity of these treatments remain to be investigated fully in humans. Therefore, extra experimental and clinical data are warranted for the development of this approach as novel anti-tumor therapeutic reagents. However, further investigation regarding RNA modifications may highlight novel frontiers for the field of cancer research and treatment, whilst also providing further insight into the molecular mechanisms underlying the development and progression of PDAC.

Finally, the mechanisms underlying the development and the functions of CSCs in PDACs are not fully understood. For example, the involvement of long non-coding RNAs, such as NEAT1, which is regulated by short non-coding RNAs has been hypothesized, though its significance in a CSC population of PDAC remains to be elucidated fully (63). Further mechanistic studies will contribute to the elucidation of the molecular mechanisms, drug discovery, and the promotion of precision medicine of PDAC, by targeting a CSC population in PDAC. Strategies designed to elucidate the mechanism and the profile of CSCs in PDAC, both in the lab and in clinical practice will be required to ensure they allow for more personalized treatments based on a patient's specific profile.

This review article summarizes the available body of knowledge on targeting RNA methylation in the management of PDAC and discusses potential future approaches, with the aim of improving the survival and the quality of life of patients with PDAC.

6. Conclusions

The present study reviewed the functional roles and mechanisms of RNA methylation in the regulation of CSCs in PDAC. Given that the existence of CSCs in PDAC tissues and CSCs plays a role in response to chemotherapy and radiation therapy, and cancer invasion and metastasis, the understanding of the metastatic cascade of CTCs will give rise to tremendous potential for the identification of therapeutic targets and the development of novel approaches. Further investigation will be necessary for precision medicine against the deleterious cancer PDAC.

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Authors' contributions

HI conceived the study. YT, TH, SM, HS, YA, KO, and HI contributed to creating the figure, reviewing the literature, and writing the manuscript. TH and HI were involved in drafting in the manuscript. 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

Not applicable.

Competing interests

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