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.

Contents

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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 CH_3- 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 CH_3- 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	Pentaspan 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 *et al*, 2019	METTL3	M6a writer	METTL3 promotes PDAC proliferation and invasion	Upregulated	Oncogene	(73)
Zhang *et al*, 2019	METTL3	M6a writer	METTL3-dependent excessive miR-25-3p maturation promotes PDAC	Upregulated	Oncogene	(74)
Tang *et al*, 2022	METTL3	M6a writer	METTL3 promotes PDAC by regulating stability of E2F5	Upregulated	Oncogene	(75)
Guo *et al*, 2022	METTL3	M6a writer	METTL3-IGF2BP3-axis regulates spermine synthase m6a modification	Upregulated	Oncogene	(76)
Li *et al*, 2022	METTL3	M6a writer	Increased expression of METTL3 in PDAC associates with poor survival of he patients	Upregulated	Oncogene	(77)
Song *et al*, 2022	METTL3	M6a writer	lncRNA MALAT1 regulates METTL3-mediated PD-L1 expression in PDAC	Upregulated	Presumably Oncogene	(78)
Taketo *et al*, 2018	METTL3	M6a writer	METTL3 promotes chemo- and radioresistance in PDAC	Upregulated	Oncogene	(79)
Jiang *et al*, 2022	METTL3, METTL14	M6a writer	M6a-mediated miR-380-3p maturation and upregulation promotes PDAC	Upregulated	Oncogene	(80)
Chen *et al*, 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 *et al*, 2023	METTL3	M6a writer	Linc-UROD stabilized by IGF2BP3/METTL3 contributes to glycolysis and malignant phenotype of PDAC	Upregulated	Presumably Oncogene	(82)
Tatekawa *et al*, 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 *et al*, 2022	METTL3	M6a writer	Increased m6a modification of lncRNA DBH-AS1 suppresses PDAC via miR-3163/USP44 axis	Downregulated	Tumor suppressive	(84)
Huang *et al*, 2022	ZC3H13	M6a writer	ZC3Hl3 mediates m6a modification of PHF10, which induces DNA damage repair as a barrier	Upregulated	Oncogene	(85)
Hou *et al*, 2020	KIAA1429	M6a writer	A promising prognostic biomarker	Upregulated	Presumably Oncogene	(86)
Chijimatsu, *et al*, 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 *et al*, 2022	FTO	M6a eraser	FTO promotes the progression of PDAC through reducing m6a/ YTHDF1 mediated TFPI-2 mRNA stability	Upregulated	Oncogene	(87)
Garg, *et al*, 2022	ALKBH5	M6a eraser	ALKBH5 deficiency reduces the mRNA stability of key pancreatic transcription factors	Upregulated	Oncogene	(49)
Huang, *et al*, 2021	ALKBH5	M6a eraser	ALKBH5 protects against PDAC	Downregulated	Tumor suppressive	(88)
Hou *et al*, 2020	HNRNPC	M6a reader	A promising prognostic biomarker	Upregulated	Presumably Oncogene	(86)
Hou *et al*, 2020	IGF2BP2	M6a reader	A promising prognostic biomarker	Upregulated Oncogene	Presumably	(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

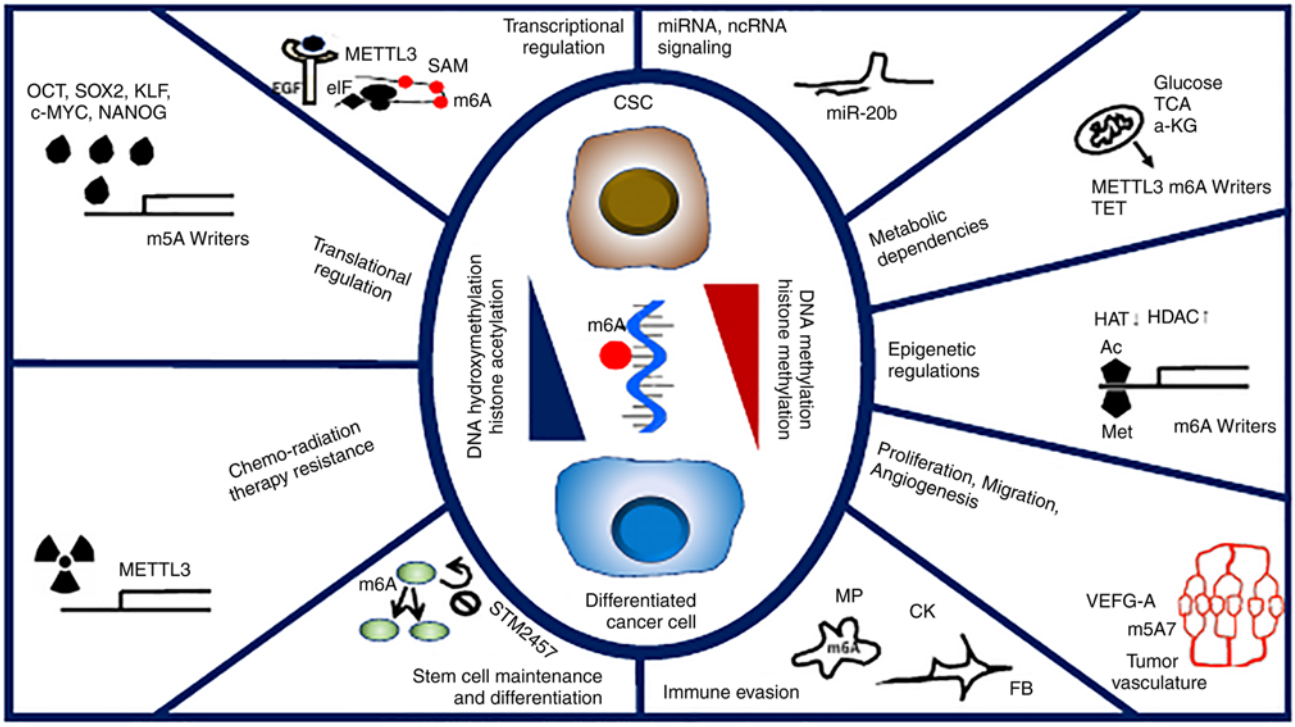

Figure 1. Roles of CSC in the regulation of PDAC. Earlier studies suggested that RNA methylation contributed to PDAC and other elements of tumor heterogeneity (25). RNA modification reportedly exerted a critical function in transcriptional regulation (25), translational regulation (25), A-to-I editing (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 methylosystem) 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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References

1. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG and McCain RS: Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol 24: 4846-4861, 2018.
2. Zeng S, Pöttler M, Lan B, Grützmann R, Pilarsky C and Yang H: Chemoresistance in pancreatic cancer. Int J Mol Sci 20: 4504, 2019.
3. Zhang L, Sanagapalli S and Stoita A: Challenges in diagnosis of pancreatic cancer. World J Gastroenterol 24: 2047-2060, 2018.
4. Ansari D, Tingstedt B, Andersson B, Holmquist F, Sturesson C, Williamsson C, Sasor A, Borg D, Bauden M and Andersson R: Pancreatic cancer: Yesterday, today and tomorrow. Future Oncol 12: 1929-1946, 2016.
5. Fialkow PJ, Singer JW, Raskind WH, Adamson JW, Jacobson RJ, Bernstein ID, Dow LW, Najfeld V and Veith R: Clonal development, stem-cell differentiation, and clinical remissions in acute nonlymphocytic leukemia. N Engl J Med 317: 468-473, 1987.
6. McCulloch EA, Howatson AF, Buick RN, Minden MD and Izaguirre CA: Acute myeloblastic leukemia considered as a clonal hemopathy. Blood Cells 5: 261-282, 1979.
7. Vogelstein B, Fearon ER, Hamilton SR and Feinberg AP: Use of restriction fragment length polymorphisms to determine the clonal origin of human tumors. Science 227: 642-645, 1985.
8. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, *et al*: Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 506: 328-333, 2014.
9. Reya T, Morrison SJ, Clarke MF and Weissman IL: Stem cells, cancer, and cancer stem cells. Nature 414: 105-111, 2001.

10. O'Brien CA, Pollett A, Gallinger S and Dick JE: A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445: 106-110, 2007.
11. Haraguchi N, Ohkuma M, Sakashita H, Matsuzaki S, Tanaka F, Mimori K, Kamohara Y, Inoue H and Mori M: CD133+CD44+ population efficiently enriches colon cancer initiating cells. Ann Surg Oncol 15: 2927-2933, 2008.
12. Haraguchi N, Ishii H, Mimori K, Tanaka F, Ohkuma M, Kim HM, Akita H, Takiuchi D, Hatano H, Nagano H, *et al*: CD13 is a therapeutic target in human liver cancer stem cells. J Clin Invest 120: 3326-3339, 2010.
13. Ishiwata T, Matsuda Y, Yoshimura H, Sasaki N, Ishiwata S, Ishikawa N, Takubo K, Arai T and Aida J: Pancreatic cancer stem cells: Features and detection methods. Pathol Oncol Res 24: 797-805, 2018.
14. Li H, Wang C, Lan L, Yan L, Li W, Evans I, Ruiz EJ, Su Q, Zhao G, Wu W, *et al*: METTL3 promotes oxaliplatin resistance of gastric cancer CD133+ stem cells by promoting PARP1 mRNA stability. Cell Mol Life Sci 79: 135, 2022.
15. Li B, Jiang J, Assaraf YG, Xiao H, Chen ZS and Huang C: Surmounting cancer drug resistance: New insights from the perspective of N6-methyladenosine RNA modification. Drug Resist Updat 53: 100720, 2020.
16. Gao Q, Zheng J, Ni Z, Sun P, Yang C, Cheng M, Wu M, Zhang X, Yuan L, Zhang Y and Li Y: The m6A methylation-regulated AFF4 promotes self-renewal of bladder cancer stem cells. Stem Cells Int 2020: 8849218, 2020.
17. Ziegenhain C, Vieth B, Parekh S, Reinius B, Guillaumet-Adkins A, Smets M, Leonhardt H, Heyn H, Hellmann I and Enard W: Comparative analysis of single-cell RNA sequencing methods. Mol Cell 65: 631-643.e4, 2017.
18. Lei Y, Tang R, Xu J, Wang W, Zhang B, Liu J, Yu X and Shi S: Applications of single-cell sequencing in cancer research: Progress and perspectives. J Hematol Oncol 14: 91, 2021.
19. Ren X, Zhou C, Lu Y, Ma F, Fan Y and Wang C: Single-cell RNA-seq reveals invasive trajectory and determines cancer stem cell-related prognostic genes in pancreatic cancer. Bioengineered 12: 5056-5068, 2021.
20. Karmakar S, Rauth S, Nallasamy P, Perumal N, Nimmakayala RK, Leon F, Gupta R, Barkeer S, Venkata RC, Raman V, *et al*: RNA polymerase II-associated factor 1 regulates stem cell features of pancreatic cancer cells, independently of the PAF1 complex, via interactions with PHF5A and DDX3. Gastroenterology 159: 1898-1915.e6, 2020.
21. Wood A, Schneider J, Dover J, Johnston M and Shilatifard A: The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J Biol Chem 278: 34739-34742, 2003.
22. Chaudhary K, Deb S, Moniaux N, Ponnusamy MP and Batra SK: Human RNA polymerase II-associated factor complex: Dysregulation in cancer. Oncogene 26: 7499-7507, 2007.
23. Cao Y, Wang Z, Yan Y, Ji L, He J, Xuan B, Shen C, Ma Y, Jiang S, Ma D, *et al*: Enterotoxigenic bacteroidesfragilis promotes intestinal inflammation and malignancy by inhibiting exosome-packaged miR-149-3p. Gastroenterology 161: 1552-1566.e12, 2021.
24. Sato H, Sasaki K, Hara T, Kobayashi S, Doki Y, Eguchi H, Satoh T and Ishii H: Targeting the regulation of aberrant protein production pathway in gastrointestinal cancer treatment. Front Oncol 12: 1018333, 2022.
25. Konno M, Taniguchi M and Ishii H: Significant epitranscriptomes in heterogeneous cancer. Cancer Sci 110: 2318-2327, 2019.
26. Desrosiers R, Friderici K and Rottman F: Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Porc Natl Acad Sci USA 71: 3971-3975, 1974.
27. Perry RP and Kelley DE: Existence of methylated messenger RNA in mouse L cells. Cell 1: 37-42, 1974.
28. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, *et al*: Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485: 201-206, 2012.
29. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE and Jaffrey SR: Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell 149: 1635-1646, 2012.
30. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG and He C: N6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 7: 885-887, 2011.
31. Meyer KD and Jaffrey SR: Rethinking m6A readers, writers, and erasers. Annu Rev Cell Dev Biol 33: 319-342, 2017.
32. Wang L, Zhang S, Li H, Xu Y, Wu Q, Shen J, Li T and Xu Y: Quantification of m6A RNA methylation modulators pattern was a potential biomarker for prognosis and associated with tumor immune microenvironment of pancreatic adenocarcinoma. BMC Cancer 21: 876, 2021.
33. Xu F, Zhang Z, Yuan M, Zhao Y, Zhou Y, Pei H and Bai L: M6A regulatory genes play an important role in the prognosis, progression and immune microenvironment of pancreatic adenocarcinoma. Cancer Invest 39: 39-54, 2021.
34. Li F, He C, Yao H, Zhao Y, Ye X, Zhou S, Zou J, Li Y, Li J, Chen S, *et al*: Glutamate from nerve cells promotes perineural invasion in pancreatic cancer by regulating tumor glycolysis through HK2 mRNA-m6A modification. Pharmacol Res 187: 106555, 2023.
35. Jiang X, Liu B, Nie Z, Duan L, Xiong Q, Jin Z, Yang C and Chen Y: The role of m6A modification in the biological functions and diseases. Signal Transduct Target Ther 6: 74, 2021.
36. Li J, Wang F, Liu Y, Wang H and Ni B: N6-methyladenosine (m6A) in pancreatic cancer: Regulatory mechanisms and future direction. Int J Biol Sci 17: 2323-2335, 2021.
37. Guo X, Li K, Jiang W, Hu Y, Xiao W, Huang Y, Feng Y, Pan Q and Wan R: RNA demethylase ALKBH5 prevents pancreatic cancer progression by posttranscriptional activation of PER1 in an m6A-YTHDF2-dependent manner. Mol Cancer 19: 91, 2020.
38. Ma Z and Ji J: N6-methyladenosine (m6A) RNA modification in cancer stem cells. Stem Cells 38: 1511-1519, 2020.
39. Shen C, Sheng Y, Zhu AC, Robinson S, Jiang X, Dong L, Chen H, Su R, Yin Z, Li W, *et al*: RNA demethylase ALKBH5 selectively promotes tumorigenesis and cancer stem cell self-renewal in acute myeloid leukemia. Cell Stem Cell 27: 64-80.e9, 2020.
40. Paris J, Morgan M, Campos J, Spencer GJ, Shmakova A, Ivanova I, Mapperley C, Lawson H, Wotherspoon DA, Sepulveda C, *et al*: Targeting the RNA m6A reader YTHDF2 selectively compromises cancer stem cells in acute myeloid leukemia. Cell Stem Cell 25: 137-148.e6, 2019.
41. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, He X and Semenza GL: Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m6A-demethylation of NANOG mRNA. Proc Natl Acad Sci USA 113: E2047-E2056, 2016.
42. Zhu P, He F, Hou Y, Tu G, Li Q, Jin T, Zeng H, Qin Y, Wan X, Qiao Y, *et al*: A novel hypoxic long noncoding RNA KB-1980E6.3 maintains breast cancer stem cell stemness via interacting with IGF2BP1 to facilitate c-Myc mRNA stability. Oncogene 40: 1609-1627, 2021.
43. Dixit D, Prager BC, Gimple RC, Poh HX, Wang Y, Wu Q, Qiu Z, Kidwell RL, Kim LJY, Xie Q, *et al*: The RNA m6A reader YTHDF2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells. Cancer Discov 11: 480-499, 2021.
44. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, Chen Y, Sulman EP, Xie K, Bögler O, *et al*: m6A Demethylase ALKBH5 maintains tumorigenicity of glioblastoma Stem-like Cells by sustaining FOXM1 expression and cell proliferation program. Cancer Cell 31: 591-606.e6, 2017.
45. Cui Q, Shi H, Ye P, Li L, Qu Q, Sun G, Sun G, Lu Z, Huang Y, Yang CG, *et al*: m6A RNA methylation regulates the self-renewal and tumorigenesis of glioblastoma stem cells. Cell Rep 18: 2622-2634, 2017.
46. Ma S, Chen C, Ji X, Liu J, Zhou Q, Wang G, Yuan W, Kan Q and Sun Z: The interplay between m6A RNA methylation and noncoding RNA in cancer. J Hematol Oncol 12: 121, 2019.
47. Dai D, Wang H, Zhu L, Jin H and Wang X: N6-methyladenosine links RNA metabolism to cancer progression. Cell Death Dis 9: 124, 2018.
48. Ma X, Cao J, Zhou Z, Lu Y, Li Q, Jin Y, Chen G, Wang W, Ge W, Chen X, *et al*: N6-methyladenosine modification-mediated mRNA metabolism is essential for human pancreatic lineage specification and islet organogenesis. Nat Commun 13: 4148, 2022.
49. Garg R, Melstrom L, Chen J, He C and Goel A: Targeting FTO suppresses pancreatic carcinogenesis via regulating stem cell maintenance and EMT pathway. Cancers (Basel) 14: 5919, 2022.
50. Chijimatsu R, Kobayashi S, Takeda Y, Kitakaze M, Tatekawa S, Arao Y, Nakayama M, Tachibana N, Saito T, Ennishi D, *et al*: Establishment of a reference single-cell RNA sequencing dataset for human pancreatic adenocarcinoma. iScience 25: 104659, 2022.

51. Ishii H, Iwatsuki M, Ieta K, Ohta D, Haraguchi N, Mimori K and Mori M: Cancer stem cells and chemoradiation resistance. Cancer Science 99: 1871-1877, 2008.
52. Mehdi A and Rabbani SA: Role of methylation in pro- and anti-cancer immunity. Cancers (Basel) 13: 545, 2021.
53. Tatekawa S, Ofusa K, Chijimatsu R, Vecchione A, Tamari K, Ogawa K and Ishii H: Methylosystem for cancer sieging strategy. Cancers (Basel) 13: 5088, 2021.
54. Monné M, Marobbio CMT, Agrimi G, Palmieri L and Palmieri F: Mitochondrial transport and metabolism of the major methyl donor and versatile cofactor S-adenosylmethionine, and related diseases: A review. IUBMB Life 74: 573-591, 2022.
55. Wu X and Zhang Y: TET-mediated active DNA demethylation: Mechanism, function and beyond. Nat Rev Genet 18: 517-534, 2017.
56. He L, Li H, Wu A, Peng Y, Shu G and Yin G: Functions of N6-methyladenosine and its role in cancer. Mol Cancer 18: 176, 2019.
57. Lan Q, Liu PY, Haase J, Bell JL, Hüttelmaier S and Liu T: The critical role of RNA m6A methylation in cancer. Cancer Res 79: 1285-1292, 2019.
58. Ryall JG, Cliff T, Dalton S and Sartorelli V: Metabolic reprogramming of stem cell epigenetics. Cell Stem Cell 17: 651-662, 2015.
59. Nuñez JK, Chen J, Pommier GC, Cogan JZ, Replogle JM, Adriaens C, Ramadoss GN, Shi Q, Hung KL, Samelson AJ, *et al*: Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. Cell 184: 2503-2519.e17, 2021.
60. Yankova E, Blackaby W, Albertella M, Rak J, De Braekeleer E, Tsagkogeorga G, Pilka ES, Aspris D, Leggate D, Hendrick AG, *et al*: Small molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature 593: 597-601, 2021.
61. Huff S, Tiwari SK, Gonzalez GM, Wang Y and Rana TM: m6A-RNA demethylase FTO inhibitors impair self-renewal in glioblastoma stem cells. ACS Chem Biol 16: 324-333, 2021.
62. Wang JN, Wang F, Ke J, Li Z, Xu CH, Yang Q, Chen X, He XY, He Y, Suo XG, *et al*: Inhibition of METTL3 attenuates renal injury and inflammation by alleviating TAB3 m6A modifications via IGF2BP2-dependent mechanisms. Sci Transl Med 14: eabk2709, 2022.
63. Huang B, Liu C, Wu Q, Zhang J, Min Q, Sheng T, Wang X and Zou Y: Long non-coding RNA NEAT1 facilitates pancreatic cancer progression through negative modulation of miR-506-3p. Biochem Biophys Res Commun 482: 828-834, 2017.
64. Gupta VK and Banerjee S: Isolation of lipid raft proteins from CD133+ cancer stem cells. Methods Mol Biol 1609: 25-31, 2017.
65. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF and Simeone DM: Identification of pancreatic cancer stem cells. Cancer Res 67: 1030-1037, 2007.
66. Zhou T, Liu J, Xie Y, Yuan S, Guo Y, Bai W, Zhao K, Jiang W, Wang H, Wang H, *et al*: ESE3/EHF, a promising target of rosiglitazone, suppresses pancreatic cancer stemness by down-regulating CXCR4. Gut 71: 357-371, 2022.
67. Hamada S, Satoh K, Hirota M, Kanno A, Umino J, Ito H, Masamune A, Kikuta K, Kume K and Shimosegawa T: The homeobox gene MSX2 determines chemosensitivity of pancreatic cancer cells via the regulation of transporter gene ABCG2. J Cell Physiol 227: 729-738, 2012.
68. Ling X, Wu W, Fan C, Xu C, Liao J, Rich LJ, Huang RY, Repasky EA, Wang X and Li F: An ABCG2 non-substrate anticancer agent FL118 targets drug-resistant cancer stem-like cells and overcomes treatment resistance of human pancreatic cancer. J Exp Clin Cancer Res 37: 240, 2018.
69. Li C, Wu JJ, Hynes M, Dosch J, Sarkar B, Welling TH, Pasca di Magliano M and Simeone DM: c-Met is a marker of pancreatic cancer stem cells and therapeutic target. Gastroenterology 141: 2218-2227.e5, 2011.
70. Aliebrahimi S, Kouhsari SM, Arab SS, Shadboorestan A and Ostad SN: Phytochemicals, withaferin A and carnosol, overcome pancreatic cancer stem cells as c-Met inhibitors. Biomed Pharmacother 106: 1527-1536, 2018.
71. Nimmakayala RK, Leon F, Rachagani S, Rauth S, Nallasamy P, Marimuthu S, Shailendra GK, Chhonker YS, Chugh S, Chirravuri R, *et al*: Metabolic programming of distinct cancer stem cells promotes metastasis of pancreatic ductal adenocarcinoma. Oncogene 40: 215-231, 2021.
72. Matsuda Y, Tanaka M, Sawabe M, Mori S, Muramatsu M, Mieno MN, Ishiwata T and Arai T: The stem cell-specific intermediate filament nestin missense variation p.A1199P is associated with pancreatic cancer. Oncol Lett 17: 4647-4654, 2019.
73. Xia T, Wu X, Cao M, Zhang P, Shi G, Zhang J, Lu Z, Wu P, Cai B, Miao Y and Jiang K: The RNA m6A methyltransferase METTL3 promotes pancreatic cancer cell proliferation and invasion. Pathol Res Pract 215: 152666, 2019.
74. Zhang J, Bai R, Li M, Ye H, Wu C, Wang C, Li S, Tan L, Mai D, Li G, *et al*: Excessive miR-25-3p maturation via N6-methyladenosine stimulated by cigarette smoke promotes pancreatic cancer progression. Nat Commun 10: 1858, 2019.
75. Tang Y, Gao G, Xia WW and Wang JB: METTL3 promotes the growth and metastasis of pancreatic cancer by regulating the m6A modification and stability of E2F5. Cell Signal 99: 110440, 2022.
76. Guo Z, Zhang X, Lin C, Huang Y, Zhong Y, Guo H, Zheng Z and Weng S: METTL3-IGF2BP3-axis mediates the proliferation and migration of pancreatic cancer by regulating spermine synthase m6A modification. Front Oncol 12: 962204, 2022.
77. Li Y, Huang H, Zhu Y, Xu B, Chen J, Liu Y, Zheng X and Chen L: Increased expression of METTL3 in pancreatic cancer tissues associates with poor survival of the patients. World J Surg Oncol 20: 283, 2022.
78. Song Z, Wang X, Chen F, Chen Q, Liu W, Yang X, Zhu X, Liu X and Wang P: LncRNA MALAT1 regulates METTL3-mediated PD-L1 expression and immune infiltrates in pancreatic cancer. Front Oncol 12: 1004212, 2022.
79. Taketo K, Konno M, Asai A, Koseki J, Toratani M, Satoh T, Doki Y, Mori M, Ishii H and Ogawa K: The epitranscriptome m6A writer METTL3 promotes chemo- and radioresistance in pancreatic cancer cells. Int J Oncol 52: 621-629, 2018.
80. Jiang Z, Song X, Wei Y, Li Y, Kong D and Sun J: N(6)-methyladenosine-mediated miR-380-3p maturation and upregulation promotes cancer aggressiveness in pancreatic cancer. Bioengineered 13: 14460-14471, 2022.
81. Chen JQ, Tao YP, Hong YG, Li HF, Huang ZP, Xu XF, Zheng H and Hu LK: M6A-mediated up-regulation of LncRNA LIFR-AS1 enhances the progression of pancreatic cancer via miRNA-150-5p/VEGFA/Akt signaling. Cell Cycle 20: 2507-2518, 2021.
82. He Y, Liu Y, Wu D, Chen L, Luo Z, Shi X, Li K, Hu H, Qu G, Zhao Q and Lian C: Linc-UROD stabilizes ENO1 and PKM to strengthen glycolysis, proliferation and migration of pancreatic cancer cells. Transl Oncol 27: 101583, 2023.
83. Tatekawa S, Tamari K, Chijimatsu R, Konno M, Motooka D, Mitsufuji S, Akita H, Kobayashi S, Murakumo Y, Doki Y, *et al*: N(6)-methyladenosine methylation-regulated polo-like kinase 1 cell cycle homeostasis as a potential target of radiotherapy in pancreatic adenocarcinoma. Sci Rep 12: 11074, 2022.
84. Ye X, Wang LP, Han C, Hu H, Ni CM, Qiao GL, Ouyang L and Ni JS: Increased m6A modification of lncRNA DBH-AS1 suppresses pancreatic cancer growth and gemcitabine resistance via the miR-3163/USP44 axis. Ann Transl Med 10: 304, 2022.
85. Huang C, Zhou S, Zhang C, Jin Y, Xu G, Zhou L, Ding G, Pang T, Jia S and Cao L: ZC3H13-mediated N6-methyladenosine modification of PHF10 is impaired by fisetin which inhibits the DNA damage response in pancreatic cancer. Cancer Lett 530: 16-28, 2022.
86. Hou J, Wang Z, Li H, Zhang H and Luo L: Gene signature and identification of clinical trait-related m6 A regulators in pancreatic cancer. Front Genet 11: 522, 2020.
87. Wang W, He Y, Zhai LL, Chen LJ, Yao LC, Wu L, Tang ZG and Ning JZ: m6A RNA demethylase FTO promotes the growth, migration and invasion of pancreatic cancer cells through inhibiting TFPI-2. Epigenetics 17: 1738-1752, 2022.
88. Huang R, Yang L, Zhang Z, Liu X, Fei Y, Tong WM, Niu Y and Liang Z: RNA m6A demethylase ALKBH5 protects against pancreatic ductal adenocarcinoma via targeting regulators of iron metabolism. Front Cell Dev Biol 9: 724282, 2021.
89. Cui L, Ma R, Cai J, Guo C, Chen Z, Yao L, Wang Y, Fan R, Wang X and Shi Y: RNA modifications: Importance in immune cell biology and related diseases. Signal Transduct Target Ther 7: 334, 2022.
90. Sato H, Hara T, Tatekawa S, Sasaki K, Kobayashi S, Kitagawa T, Doki Y, Eguchi H, Ogawa K, Uchida S and Ishii H: Emerging roles of long noncoding and circular RNAs in pancreatic ductal adenocarcinoma. Front Physiol 13: 1025923, 2022.

91. Takeda Y, Chijimatsu R, Vecchione A, Arai T, Kitagawa T, Ofusa K, Yabumoto M, Hirotsu T, Eguchi H, Doki Y and Ishii H: Impact of one-carbon metabolism-driving epitranscriptome as a therapeutic target for gastrointestinal cancer. Int J Mol Sci 22: 7278, 2021.
92. Takeda Y, Chijimatsu R, Ofusa K, Kobayashi S, Doki Y, Eguchi H and Ishii H: Cancer metabolism challenges genomic instability and clonal evolution as therapeutic targets. Cancer Sci 113: 1097-1104, 2022.
93. Zagorac S, Garcia-Bermejo L and Sainz B Jr: The epigenetic landscape of pancreatic cancer stem cells. Epigenomes 2: 10, 2018.
94. Liu Y, Tang G and Li J: Long non-coding RNA NEAT1 participates in ventilator-induced lung injury by regulating miR-20b expression. Mol Med Rep 25: 66, 2022.
95. Xia L, Li F, Qiu J, Feng Z, Xu Z, Chen Z and Sun J: Oncogenic miR-20b-5p contributes to malignant behaviors of breast cancer stem cells by bidirectionally regulating CCND1 and E2F1. BMC Cancer 20: 949, 2020.
96. Kroeze LI, van der Reijden BA and Jansen JH: 5-Hydroxymethylcytosine: An epigenetic mark frequently deregulated in cancer. Biochim Biophys Acta 1855: 144-154, 2015.