Relationships of seven N6-methyladenosine regulators with the tumor microenvironment and tumor immune infiltrating cells

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Research Article

Keywords: N6-methyladenosine methylation, tumor microenvironment, tumor mutation burden, tumor immune infiltrating cells, The Cancer Genome Atlas (TCGA), pan-cancer analysis.

Posted Date: February 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1323400/v1>

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Abstract

Background: N6-methyladenosine (m6A) modification plays key roles in tumor cell proliferation, invasion and immune escape, but the function of m6A modification in the tumor immune microenvironment and immunotherapy remain poorly understood.

Methods: We researched the expression of seven m6A regulators (METTL3, WTAP, FTO, ALKBH5, ZC3H13, YTHDF1, YTHDF2) and their associations with prognosis using The Cancer Genome Atlas pan-cancer dataset. Further, we explored the relationships between expression of these m6A regulators and tumor mutation burden (TMB), microsatellite instability (MSI), stemness score, drug sensitivity, and immune subtype. Additionally, the association of these m6A regulators with the tumor microenvironment, immune-related gene expression, and tumor immune cell infiltration characteristics were systematically analyzed in pan-gynecological cancer.

Results: The evaluated m6A regulators exhibited striking prognostic heterogeneity in the pan-cancer dataset. Moreover, expression levels of the m6A regulators were correlated with stemness score, MSI, and TMB in various types of cancer. METTL3, ZC3H13, FTO, and YTHDF1 were highly expressed in immune subtype C5 (immunologically quiet). In pan-gynecological cancers, m6A regulator expression was clearly correlated with stromal score, immune score, immune gene expression, and tumor immune infiltrating cells. Further, ZC3H13 expression was strongly correlated with tumor immune cell infiltration, and positively correlated with selumetinib, trametinib, hypothemycin, vemurafenib, dabrafenib, and cobimetinib drug sensitivity.

Conclusions: In gynecological cancers, there are striking relationships between m6A regulator expression and the tumor microenvironment, immune genes, and tumor immune cell infiltration. These findings provide prospects for further investigation of m6A regulators as potential cancer therapeutic targets.

Introduction

N6-Methyladenosine (m6A) is the most common epigenetic modification in all RNA molecules, including eukaryotic mRNA and non-coding RNA [1], and is controlled by m6A methyltransferases and demethylases, and discerned by m6A reading proteins. m6A modification is enriched in the consensus sequence, RRACH (where R indicates A or G, and H A, C, or U), located in 3' untranslated regions (3' UTRs), stop codons, and long internal exon regions [2]. The fate of RNA molecules and cell function are affected by m6A, which can alter the splicing, export, translation, and stability of RNA, and influence RNA-protein interactions during biological processes [3-5]. Further, m6A modification has a key role in various biological processes in vivo, particularly cancer [6].

The tumor microenvironment (TME) is the interior environment in which tumor cells grow and metastasize. Besides tumor cells, the TME also contains various other cellular components, for example fibroblasts, immune cells, and inflammatory cells, as well as surrounding intercellular substances, new blood vessels, and various infiltrating biological factors. The TME has a crucial role in tumor progression

and is considered a prospective target for effective novel anticancer therapy [7, 8]. Immunotherapy is an emerging treatment for cancer that differs from chemotherapy and surgery, and has achieved satisfactory results for the treatment of some solid tumors [9, 10]. The TME plays a crucial role in tumor immune escape, leading to tumor progression [11]; hence, the TME remains an important bottleneck that restricts the success of immunotherapy. How m6A regulators influence the TME and cancer metastasis remains unexplored to date.

Multi-omics data from The Cancer Genome Atlas (TCGA) enables pan-cancer research across multiple aspects of cancer biology [12, 13], including the TME. Using data from TCGA database, Zhang et al. classified patients with gastric cancer according to m6A score, and systematically analyzed the associations between m6A modification patterns and TME characteristics [14]. Further, Li et al. explored correlations between m6A regulatory factors and TME in lung adenocarcinoma [15].

In this study, using data from TCGA, we comprehensively analyzed the expression characteristics and association with prognosis of 7 m6A regulators (METTL3, WTAP, ZC3H13, FTO, ALKBH5, YTHDF1, and YTHDF2) in different types of cancer. Subsequently, we analyzed associations of these factors with the TME, immune subtype, and tumor immune cell infiltration in the context of pan-gynecological cancer. The aim of our investigation was to provide a more detailed picture of the associations between alterations in m6A regulator expression and the TME, to better understand the heterogeneity in response to various tumor immunotherapies.

Materials And Methods

Data sources

The original data analyzed in our study are publicly available from TCGA (<https://portal.gdc.cancer.gov/>). Ethics committee approval was not needed, because the data are open access. We obtained fragments per kilobase of transcript per million mapped reads (FPKM) RNA-seq expression data (level 3) and somatic mutation data with corresponding clinical information from TCGA Pan-cancer database via the UCSC Xena Browser (January 2021, <https://xenabrowser.net/>). The study included 11057 tumor samples from TCGA cohort. RNA sequencing data were all normalized [$\log_2$ (FPKM + 1)].

Stemness indices

Tumor cells share certain similarities with stem cells and stemness indices can be used to assess the similarity of tumor cells to stem cells [16]. Stemness indices can be used to generate RNA and DNA stemness scores (RNAss and DNAss), based on transcriptome and epigenetic data, respectively. TCGA Project Stemness group used DNA methylation data based on experiments using 216 stem cell signature probes to obtain DNAss for 9,603 pan-cancer samples. Further, they used RNA expression data to derive RNAss for 10,876 pan-cancer patients. These data can be downloaded from the Pan-cancer atlas hub (<https://pancanatlas.xenahubs.net>). Stemness score values range from 0 to 1, with larger scores

indicating stronger tumor cell stemness and a lower degree of differentiation, which also indicates a higher similarity to stem cells.

Tumor microenvironment estimation

The TME is the cellular environment in which tumor cells are located, and its composition includes soluble molecules, extracellular matrix, and tumor stromal cells. Tumor purity refers to the proportion of cancer cells in a tumor sample. Stromal cells and immune cells are the two main types of non-tumor components in the TME. The ESTIMATE algorithm in the R package “ESTIMATE” was used to calculate immune score, stromal score, and tumor purity from gene expression data, to generate immune and matrix scores for each patient in the pan-cancer data set. Immune score, stromal score, and tumor purity ranged from 0 to 1. CIBERSORT is an R/web version tool that deconvolves human immune cell subtype expression matrix data based on the principle of linear support vector regression. This approach uses a known reference set, including gene expression features of 22 immune cell subtypes [17]. Using the CIBERSORT algorithm, we obtained the scale of tumor-infiltrating immune cells (TIC) for each sample in the pan-cancer cohort from TCGA.

Immune subtypes and immune genes

Several scholars have used TCGA to perform immune genomic analyses, including more than 10,000 patients from 33 different cancer types, and have identified six immune subtypes, including wound healing, IFN- γ dominant, inflammatory, lymphocyte depleted, immunologically quiet, and TGF- β dominant [18]. Pan-cancer-specific immune typing of data based on TCGA can be acquired through the UCSC Xena Browser; a gene set including 47 immune-related genes was downloaded from the import website (<https://www.immport.org/home>) for this study.

Tumor mutation burden and microsatellite instability

Tumor mutation burden (TMB), a biomarker for immunotherapy prognosis, refers to the total number of mutations per million bases in tumor tissue. Microsatellites are short tandem repeats, comprising 10–50 copies of single, double, or higher numbers of nucleotides, present throughout the human genome. Compared with normal cells, microsatellites change in length due in tumor cells to the insertion or deletion of repeat units, which is referred to as microsatellite instability (MSI). MSI is caused by defects in mismatch repair genes and closely associated with tumor occurrence. Clinically, MSI is a significant biomarker for molecular subtype, prognosis, and adjuvant treatment of solid tumors, such as colorectal [19-21] and endometrial [22, 23] cancers. TMB is also associated with MSI. Here, TMB was calculated for each patient in the pan-cancer dataset, relative to somatic mutation data. MSI data for pan-cancer samples in TCGA were obtained based on previous studies [24]. Then, correlations between the expression levels of the seven m6A regulators in pan-cancer with TMB and MSI were calculated. The results are presented as radar charts drawn using the R package “fmsb”.

CellMiner and Gene Set Enrichment Analysis

The CellMiner web application (<http://discover.nci.nih.gov/cellminer/>) provides extensive genomics and drug sensitivity data, based on experiments in the NCI-60 cell line, which is currently the cancer cell sample type most widely used for anti-cancer pharmacologic testing [25]. This online tool provides a quick access to more than 20,000 gene catalogs and calculates correlations between genes and drug activity, to identify potential anti-cancer drugs.

Gene set enrichment analysis (GSEA) was also applied to research the biological functions of the seven m6A regulators in pan-gynecological cancers. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) signatures were accessed via the GSEA online website (<https://www.gsea-msigdb.org/GSEA/downloads.jsp>).

Statistical analysis

Gene expression data from cancer and adjacent normal tissues in TCGA were analyzed by Student's t test. Kaplan-Meier (K-M) survival curves were evaluated using the log-rank test. Hazard ratio (HR) values were calculated using Cox proportional hazard regression models. Correlation was tested using Spearman's method. $P < 0.05$ was considered statistically significant. All analyses were performed using packages in R software (<http://www.r-project.org>, version 3.5.2), including: "ggpubr", "colorspace", "enrichplot", "org.Hs.eg.db", "clusterProfiler", "DOSE", "limma", "survival", "survminer", "forestplot", "ggplot2", and "ggExtra".

Results

Pan-cancer analysis of the expression of m6A regulators

m6A RNA modification is dynamically regulated by methylases and demethylases, whereas readers mediate m6A-dependent functions. In this study, we selected seven mRNA m6A regulators including three "writers" (METTL3, WTAP, and ZC3H13), two "erasers" (FTO and ALKBH5) and two "readers" (YTHDF1 and YTHDF2). The overall expression levels of these seven m6A regulators in pan-cancer are shown in Figure 1A. Our results indicate that ALKBH5 had the highest expression in pan-cancers, while FTO expression was lowest. Further analysis revealed that YTHDF1 had the highest expression in CHOL and READ, while ZC3H13 had the highest expression in STAD, WTAP was expressed at the highest levels in GBM, and FTO was most strongly expressed in CHOL (Fig. 1B). Correlations among the seven m6a regulators are presented in Figure 1C.

We also analyzed the differential expression of these m6A regulators between tumor and adjacent normal tissues in 18 TCGA cancer datasets (cancers with > 5 normal tissue samples were selected for this analysis). Our results show that the expression of ALKBH5 did not differ in ESCA, KIPK, READ, or STAD compared with adjacent normal tissue, but was clearly expressed at higher levels in CHOL, HNSC, COAD, KIRC, LIHC, BLCA, and BRCA, and expressed at significantly lower levels in GBM, KICH, LUAD, LUSC, PRAD, THCA, UCEC (Fig. 2D). The expression levels of the other six m6A regulators in pan-cancer are presented in Figure 2E–J.

Prognostic value of m6A regulators in pan-cancer

Subsequently, we evaluated the prognostic value for overall survival (OS) of the seven m6A regulators in pan-cancer. K-M curves showed that m6A regulators exhibited striking prognostic heterogeneity among cancers. Compared with the low expression group, the ALKBH5 high expression group had worse OS in BLCA and LAMA, while in ESCA, OV, PAAD, high ALKBH5 expression was related to good OS. High FTO expression was associated with worse OS in BLCA and THCA, while it was related to better OS in KIRC and LGG. The prognostic data for all seven m6A regulators in pan-cancer are presented in Figure 2. Overall, these data suggest that m6A regulators are key factors affecting the prognosis of patients with cancer, with differing roles across various cancers, depending on the type of tumor.

Correlations of m6A regulator expression with TMB and MSI in various cancers

We calculated TMB and MSI values for each pan-cancer patient and analyzed correlations of these values with the expression levels of the seven m6A regulators. Our data showed that ALKBH5 levels are positively correlated with TMB in COAD, UCEC, SARC, and LGG, and negatively correlated with TMB in KIRC, BRCA, THYM, THCA, PRAD, PAAD, ESCA, and DLBC (Fig. 3A). Correlations of the other six m6A regulators with TMB in pan-cancer are presented in Figure 3B–G.

ALKBH5 levels were positively correlated with MSI in COAD, UCS, UCEC, STAD, SARC, PCPG, and LUAD and negatively correlated with MSI in THCA and LAML (Fig. 3H). Correlations between the other six m6A regulators and MSI in pan-cancer are shown in Figure 3I–N.

These results demonstrate that the correlations of m6A regulators with TMB and MSI across various cancers are heterogeneous.

Correlations of m6A regulators with tumor stemness

Stemness indices are used to describe the proportions of tumor cells resembling stem cells. Here, we evaluated the associations of seven m6A regulators with stemness scores (RNAss and DNAss) (Fig. 4A–B). Expression levels of YTHDF1 and YTHDF2 were positively correlated with RNAss and DNAss in the majority of 33 TCGA tumor types tested, with higher expression levels associated with greater tumor stemness scores. In contrast, FTO and ZC3H13 expression levels were negatively correlated with tumor stemness scores in most TCGA tumors.

Correlation of m6A regulator expression with immune subtype and immune genes

Correlation coefficients between immune characteristics have been applied to cluster non-hematological tumors in TCGA into six immune subtypes (C1–C6). Here, we analyzed potential correlations between the expression levels of seven m6A regulators and immune subtypes in pan-cancer. The expression levels of the seven m6A regulators in pan-cancer data C1–C6 immune subtypes are shown in Figure 4C, and demonstrate that expression levels of the m6A regulators differed significantly among immune subtypes.

METTL3, ZC3H13, FTO, and YTHDF1 were highly expressed in C5 subtype tumors; however, ALKBH5, YTHDF2, and WTAP were expressed at lower levels in C5 subtype.

Immune genes, particularly immune checkpoint genes, have a major impact on tumor immune infiltration, immune escape, and response to immunotherapy [26, 27]. Exploration of associations between expression of the seven m6A regulators and that of immune genes was conducted to better comprehend the potential influence of m6A regulators on immunotherapy. We evaluated the associations of seven m6A regulators with 47 immune genes in pan-gynecologic cancers. The results showed that expression levels of m6A regulators were mostly positively correlated with those of immune genes (Fig. 4D). In particular, in UCEC 31 of 47 immune genes were associated with WTAP expression. These findings suggest that these m6A regulators may interact with immune genes in various signal transduction pathways, and may represent ideal future immunotherapy targets.

Correlations of expression of m6A regulators with immune, stromal, and ESTIMATE scores

The ESTIMATE algorithm can use expression profile data to estimate the proportions of stromal and immune cells in malignant tumor tissues, and calculate corresponding stromal and immune scores, as well as an estimate score representing tumor purity. We calculated immune, stromal, and Estimate scores for each sample in pan-gynecological cancer using the Estimate algorithm, and analyzed correlations of expression levels of the seven m6A regulators with each score in gynecological pan-cancer. The results are presented in Figure 5 and show that expression levels of the seven m6A regulators were significantly positively or negatively correlated with stromal and immune scores in pan-gynecological cancers.

Correlations of m6A regulator expression and immune infiltration in pan-gynecological cancer

Tumor-infiltrating immune cells are closely associated with tumor metastasis and cancer progression, and may serve as therapeutic targets for improving patient survival [28, 29]. Here, we determined the percentages of immune cells in each pan-gynecological cancer (including CESC, OV, UCEC, etc.) and explored the correlations between levels of the seven m6A regulators and immune infiltration level using the CIBERSORT algorithm. We found that ZC3H13 expression was clearly related to B cells memory, Macrophages M2, T cells CD4 memory resting in CESC, B cells memory, B cells naïve, T cells CD4 memory resting in OV, and NK cells activated, Plasma cells, T cells CD4 memory resting in UCEC. Further FTO expression was with T cells CD4 memory resting in CESC, B cells memory in OV, NK cells activated, B cells memory, T cells CD4 memory resting in UCEC. The relationships between the other five m6A regulators and immune infiltration cells are illustrated in Figure 6. These results reveal that, compared with the other six m6A regulators, ZC3H13 expression has a strong correlation with the level of immune infiltration in pan-gynecological cancers.

Enrichment analysis of m6A regulator expression

We conducted single-gene GSEA analysis for each m6a regulator in pan-gynecological cancers, to explore which regulatory pathways and biological processes are enriched for these molecules. In KEGG pathway

GSEA, among immune-related pathways in OV, YTHDF2 and WTAP associated genes were enriched for antigen processing and presentation, the TOLL-like receptor signaling pathway, and natural killer cell mediated cytotoxicity. Genes negatively correlated with ALKBH5 in UCEC were enriched in pathways including autoimmune thyroid disease and antigen processing and presentation, among others. YTHDF2 is a positive regulator of the autophagy pathway in OV, while in UCEC, ALKBH5 was a negative regulator of this pathway (figure 7). The results of GO analysis of m6a in pan-gynecological cancer are presented in Figure 8. FTO was enriched in olfactory receptor activity and sensory perception of smell. In OV, WTAP expression was positively correlated with immune response pathways, such as immune response regulating cell surface receptor signaling and regulation of lymphocyte activation. In CESC, YTHDF2 expression was positively correlated with response to interleukin 12.

CellMiner

We next explored potential therapeutic methods using the Cellminer database, with m6A regulators as targets. Correlations between NCI-60 drug z score and expression of the corresponding NCI-60 cell line RNA-seq/composite were analyzed, where higher z score for a cell line indicated that it was more sensitive to the corresponding drug. Here, we selected drugs approved by the US Food and Drug Agency and drugs undergoing clinical trials. Drugs sensitive to the seven m6A regulators are listed in Figure 9. ZC3H13 expression was positively correlated with sensitivity to the drugs selumetinib, trametinib, hypothemycin, vemurafenib, dabrafenib, and cobimetinib. Further, YTHDF2 expression was negatively correlated with drug sensitivity to dasatinib.

Discussion

As the TME influences tumor treatment response, dynamic changes that affect the microenvironment are particularly important for immunotherapy; however, very little is known about the functions of m6A modification in the TME and immunotherapy. Some researchers have proposed that expression of m6A regulators in tumor cells is beneficial to immunotherapy outcomes. Li et al. reported that ALKBH5 is a potential therapeutic target, which can improve the efficacy of immunotherapy for melanoma and colorectal cancer by regulating lactic acid and inhibiting Immune cell accumulation in the TME [30]. Wang et al. discovered that inhibition of m6A mRNA modification by the methyltransferases, METTL3 and METTL14, can enhance the response of mismatch-repair-proficient or MSI-low colorectal cancer and melanoma to PD-1 treatment [31].

In tumors, m6A modification can regulate cell proliferation, invasion, drug resistance, and immune evasion to promote or suppress tumors. For example, METTL3 is highly expressed in hepatocellular carcinoma and its downregulation can enhance the expression of the tumor suppressor gene, *SOCS2*, thereby inhibiting tumor progression [32]. In contrast, m6A modification inhibits tumorigenesis and self-renewal ability of tumor stem cells in glioma, whereas down-regulation of METTL3 or METTL14 could up-regulate a series of proto-oncogenes [33]. m6A modification is extremely heterogeneous during the development of tumors, and whether it acts to promote or suppress cancer is related to the function of

m6A-modified genes. Therefore, it is necessary to study the influence of m6A regulation on prognosis in different tumors, as well as its effects on immune genes, the TME, and immune cell infiltration.

Here, we studied the gene expression levels of seven m6A regulators (METTL3, WTAP, ZC3H13, ALKBH5, FTO, YTHDF1, and YTHDF2) and their associations with prognosis in TCGA pan-cancer dataset. The results revealed that m6A regulators have heterogeneous relationships with prognosis across cancers. This finding will assist in comprehension of the prognostic roles of the seven m6A regulators in pan-cancer at a global level. Our results are consistent with the findings of previous investigations. For example, high ALKBH5 expression levels are significantly associated with improved OS in ESCA, OV, PAAD, while low expression of this molecule is significantly associated with superior OS in BLCA and LAML. A previous study found that the RNA demethylase, ALKBH5, promotes ovarian cancer progression via the NF- κ B pathway in a simulated TME [34]. Tang et al. reported that ALKBH5 inhibits pancreatic cancer tumorigenesis by reducing WIF-1 RNA methylation and regulating Wnt signaling [35]. Based on TCGA ESCA cohort data, Wang et al. constructed and verified a prognostic model comprising two factors, HNRNPC and ALKBH5, which could predict the prognosis of patients with esophageal cancer [36]. In acute myeloid leukemia, ALKBH5 has a tumor-promoting role through post-transcriptional regulation of its key target, TACC3 [37]. The carcinogenic effect of ALKBH5 in bladder urothelial carcinoma indicated by our findings has not been previously reported.

We also analyzed correlations between the expression levels of the seven m6A regulators with TMB and MSI across 33 cancer types. MSI and TMB have attracted widespread attention as predictive biomarkers of immunotherapy efficacy, particularly in lung and colorectal cancers [38, 39]. Our results show that expression levels of the seven m6A regulators are significantly associated with MSI and TMB in most types of cancer, implying that m6A regulator expression may affect the responses of patients with cancer to immune checkpoint therapy, providing new insights with potential to improve immunotherapy efficacy. Moreover, we found that the expression levels of METL3, ZC3H13 and FTO were higher in immune infiltrating C3 and C5 subtypes than in other immune subtypes, indicating that expression of these factors is related to good immune composition, since these two immune subtypes are associated with significantly improved patient survival rates. In contrast, YTHDF2 is highly expressed in immune infiltrating C1, C2, and C6 subtypes, which are related to poor prognosis. Correlation between higher levels of METL3, ZC3H13, and FTO and C3 and C5 infiltration subtypes indicate that these molecules may have tumor suppressor effects, since patients in these categories have better survival, associated with enriched immune silencing and high levels of inflammatory factors.

Analysis of correlations of m6A regulator levels with stemness scores and TME were conducted in this study. TME is important in tumorigenesis and progression [11, 40, 41]. Therefore, research on the immune microenvironment will help attempts to reshape the TME and facilitate its transformation from a cancer promoting to a cancer suppressing environment. Transcriptome analysis of pan-gynecological cancer data based on TCGA showed that the immune components of the TME are significantly related to m6A regulators that influence patient prognosis. In ovarian cancer, the expression of ALKBH5 and WTAP was clearly related to immune score in the TME, while in cervical cancer, ZC3H13 levels were also

significantly correlated with immune score. Further analysis using the CIBERSORT algorithm showed that ZC3H13 expression in cervical cancer was markedly associated with the infiltration of immune cells including B cells memory, Macrophages M2, and T cells CD4 memory resting. In ovarian cancer, WTAP was associated with T cells CD4 memory activated immune infiltration. These results emphasize the interaction between immune cells and m6A regulators, and illuminate the relationship between the immune microenvironment and m6A, providing new insights to inform the development of more effective treatment options in the future. Nevertheless, additional genome data set analysis and further in vivo and in vitro laboratory investigations are required to verify these findings.

Furthermore, we analyzed the potential correlations between expression of the seven m6A regulator genes and drug sensitivity, based on data from the CellMiner™ database. The results showed most m6A methylation regulators are significantly positively correlated with drug responses, consistent with the findings of a previous report [42]. More than 20,000 confirmed genes and more than 20,000 compounds tested in the NCI-60 cell line can be queried through the Cellminer database, and the NCI-60 cancer cell line is currently the most widely-used cancer cell sample used for anti-cancer drug testing [43, 44]. The expression of ZC3H13 is positively correlated with the drug sensitivity to selumetinib, trametinib, hypothemycin, vemurafenib, dabrafenib, and cobimetinib. Selumetinib and trametinib are both mitogen-activated protein kinase (MEK) inhibitors. Combined with other targeted therapies, MEK inhibitors have shown good results for the treatment of a series of malignant tumors, including melanoma, non-small cell lung cancer, and low-grade glioma [45, 46]. Additionally, MEK inhibitors have been approved by the FDA for the treatment of BRAF V600E/K-mutated melanoma, and combined BRAF inhibitor plus MEK inhibitor treatment can promote gasdermin E (GSDME) cleavage and release of HMGB1, thereby interfering with tumor-associated T cell and dendritic cell infiltration, influencing melanoma growth [47]. Selumetinib and trametinib can induce T cell activation; after cyclical pulsatile MEK inhibitor treatment, CTLA-4 expression on T cells increases and that of PD-1 decreases in vivo [48]. Moreover, combined treatment with MEK inhibitors and immunomodulatory antibodies targeting PD-1 and PD-L1 can also extend the survival of patients with Kras/p53-driven lung cancer [49]. Whether or not these drugs are effective requires verification in additional clinical trials.

In this study, we analyzed the influence of seven m6A regulators on prognosis in a pan-cancer dataset, as well as their associations with TMB, MSI, immune subtypes, and tumor stemness. At the level of pan-gynecological cancer, we explored the correlations of the seven m6A regulators with immune genes and the TME. Further, we found that the expression of ZC3H13 in cancer cells is associated with sensitivity to specific drugs. These findings provide perspectives to inform further research into the potential for m6A regulators as therapeutic targets in cancer.

Abbreviations

TCGA: The Cancer Genome Atlas; **FPKM:** Fragments per kilobase of transcript per million mapped reads; **OS:** Overall survival; **HR:** Hazard ratio; **TMB:** Tumor mutation burden; **m6A:** N6-methyladenosine; **MSI:** microsatellite instability; **TME:** tumor microenvironment; **TIC:** Tumor-infiltrating immune cells; **GSEA:** Gene

set enrichment analysis; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; UCEC: Uterine Corpus Endometrial Carcinoma; OV: Ovarian serous cystadenocarcinoma; ESCA: Esophageal carcinoma; PAAD: Pancreatic adenocarcinoma; CHOL: Cholangiocarcinoma; READ: Rectum adenocarcinoma; STAD: Stomach adenocarcinoma; GBM: Glioblastoma multiforme; KIRP: Kidney renal papillary cell carcinoma; HNSC: Head and Neck squamous cell carcinoma; COAD: Colon adenocarcinoma; KIRC: Kidney renal clear cell carcinoma; LIHC: Liver hepatocellular carcinoma; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; KICH: Kidney Chromophobe; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PRAD: Prostate adenocarcinoma; THCA: Thyroid carcinoma.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are openly available in the

Pan-cancer cohort from the TCGA database (January 2021, <https://portal.gdc.cancer.gov/>) and somatic mutation data with corresponding clinical information from TCGA Pan-cancer

database via the UCSC Xena Browser (January 2021, <https://xenabrowser.net/>).

Competing interests

The authors declare no conflicts of interest.

Funding

This work was not funded

Authors' contributions

Conception and design: DO. Development of methodology: DO and

LL. Acquisition of data (provided animals, acquired and managed patients,

provided facilities, etc.): DO, LL. Analysis and interpretation of data (e.g.,

statistical analysis, biostatistics, computational analysis): DO, YZ. Writing,

review, and/or revision of the manuscript: DO, LL. Administrative, technical, or

material support (i.e., reporting or organizing data, constructing databases):

DO. All authors read and approved the final manuscript.

Acknowledgements

We thank the members of our laboratory for their help and support.

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Figures

Figure 1

(A) Boxplot of 7 m6A regulators expression in 18 TCGA cancer tissues. (B) The heatmap of 17 m6A regulators differential expression in 18 TCGA cancers. The red and green boxes indicate that the expression of m6A regulators is high and low in correspondence cancer, respectively. (C) Correlations between 7 m6A regulators. (D-J) Boxplot of 7 m6A regulators differential expression between cancer and adjacent normal tissues. The blue boxplots indicate the normal tissues. The red boxplots indicate the cancer tissues.

Figure 2

Survival analysis of m6A-related regulators across multiple cancer types and the forest map of hazard ratio of 7 m6A regulators across 33 TCGA cancer types.

Figure 3

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(A-J) Radar map of correlation between the expression of 7 m6A regulators and TMB H-N Radar map of correlation between the SRI expression and MSI.

Figure 4

(A-B) The expression of 7 m6A regulators associated with DNAss and RNAss in different cancers; (C) Association of the expression of 7 m6A regulators with immune subtypes across all the cancer types; (D) Each grid represents a pairing of seven m6A regulators and immune genes in pan-gynecologic cancers. For each pair, the upper-left triangle represents the p-value, and the lower-right triangle represents the correlation coefficient between the two.

Figure 5

Correlation of the expression of 7 m6A regulators with Immune Score, Stromal Score, and ESTIMATE Score in pan-gynecologic cancers.

Figure 6

Correlation of the expression of 7 m6A regulators with immune infiltration cell in pan-gynecologic cancers.

Figure 7

Single-gene Kyoto encyclopedia of genes and genomes (KEGG) Gene Set Enrichment Analysis (GSEA) of seven m6A regulons in pan-gynecologic cancers.

Figure 8

Single-gene Gene Ontology (GO) Gene Set Enrichment Analysis (GSEA) of seven m6A regulons in pan-gynecologic cancers.

Scatter plots to show the association between the expression of 7 m6A regulators and drug sensitivity (Z-score from CellMiner interface).