Construction and validation of m6A RNA methylation regulators associated prognostic model for gastrointestinal cancer

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Abstract
N6-methyladenosine (m6A) RNA methylation is correlated with carcinogenesis and dynamically possessed through the m6A RNA methylation regulators. This paper aimed to explore 13 m6A RNA methylation regulators’ role in gastrointestinal cancer (GIC) and determine the risk model and prognosis value of m6A RNA methylation regulators in GIC. We used several bioinformatics methods to identify the differential expression of m6A RNA methylation regulators in GIC, constructed a prognostic model, and carried out functional enrichment analysis. Eleven of 13 m6A RNA methylation regulators were differentially expressed in different clinicopathological characteristics of GIC, and m6A RNA methylation regulators were nearly associated with GIC. We constructed a risk model based on five m6A RNA methylation regulators (METTL3, FTO, YTHDF1, ZC3H13, and WTAP); the risk score is an independent prognosis biomarker. Moreover, the five m6A RNA methylation regulators can also forecast the 1-, 3- and 5-year overall survival through a nomogram. Furthermore, four hallmarks of oxidative phosphorylation, glycolysis, fatty acid metabolism, and cholesterol homeostasis gene sets were significantly enriched in GIC. m6A RNA methylation regulators were related to the malignant clinicopathological characteristics of GIC and may be used for prognostic stratification and development of therapeutic strategies.

KEYWORDS
bioinformatic analysis, epigenetics, gastrointestinal carcinoma, m6A methylation, RNA modification

1 | INTRODUCTION

It is generally accepted that epigenetics mainly involves DNA methylation, nucleosome remodelling and histone modification, and non-coding RNAs are essential to the genesis of cancer [1]. In the past decade, RNA sequencing (RNA-seq) has become an essential tool for transcriptional genome-wide analysis of differential gene expression [2]. Many RNA modifications have been recognized in various RNAs, such as mRNAs, tRNAs, micro-RNAs, small nuclear RNAs, rRNAs, and long non-coding RNA [3–6]. It is reported that these RNA modifications have several modes, including 5-methylcytosine, N1-methyladenosine, N6,2’-O-demethyladenosine (m6A), N7-methyladenosine, and 2’-O-methylation [7, 8]. The m6A modification is the first determined and most widespread pattern of mRNA methylation in eukaryotes [9–12].

RNA modification is mediated through ‘writers’-methyltransferases, ‘readers’-binding proteins, and ‘erasers’-demethylases [13]. m6A modification regulators made up of ‘writers’, such as methyltransferase like 3 (METTL3), KIAA1429, RNA binding motif protein 15 (RBM15), methyltransferase like 4 (METTL4), zinc finger CCCH domain-containing protein 13 (ZC3H13), and WT1-related protein (WTAP). ‘Readers’ such as YTH N6-methyladenosine RNA binding protein 1 (YTHDF1), heterogeneous nuclear ribonucleoprotein C (HNRNPC), YTH...
domain-containing 1 (YTHDC1), YTH domain-containing 2 (YTHDC2), and YTH N6-methyladenosine RNA binding protein 2 (YTHDF2). ‘Erasers’ such as α-ketoglutarate-dependent dioxygenase alkB homologue 5 (ALKBH5) and fat mass-and obesity-associated protein (FTO) [7, 13]. m6A RNA methylation displayed an invertible and crucial biological process by regulation of ‘readers’, ‘writers’, and ‘erasers’. The authentication of m6A RNA methylation regulators has remarkably enhanced the character of m6A modification in the gene expression regulation. The crucial function of m6A RNA methylation regulators is also observed in various cancers, including ovarian cancer, acute myeloid leukemia, renal clear cell carcinoma, glioma, and glioblastoma [14–18].

Emerging evidence demonstrated that m6A methylation was closely associated with the occurrence, development, metastasis, and angiogenesis of gastrointestinal cancer (GIC) [19]. METTL3 promotes tumour angiogenesis in gastric cancer and facilitates CRC growth by preventing SOX2 mRNA degradation [20, 21]. Oncogene c-Myc promotes YTHDF1 expression in CRC [22]. Besides, YTHDF1 inhibited the Wnt/β-catenin pathway activity of CRC cells [23]. Nevertheless, literature was deficient in comprehensively analysing the expression of m6A RNA methylation regulators in GIC with various clinicopathological features and their role and prognosis value in the malignant development of GIC.

In the present research, we comprehensively analysed 13 extensively reported m6A RNA regulators [16, 17, 24, 25] using mRNA sequencing data of the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets in GIC. The data of TCGA is the training cohort, and the data of GEO is the validation cohort. We obtained the expression data of each m6A modification regulator concerning various clinicopathological features. Then, a specific prognostic model was constructed and filtered out by five key m6A RNA methylation regulators, and a risk score was used to classify and predict the GIC patients' prognosis. We also discovered a conspicuously increased expression of FTO and ZC3H13 in GIC with malignant clinicopathological characteristics and compared with the previous research. Our work provided a novel risk signature as an independent prognostic biomarker for GIC patients. The flow chart of our study is manifested in Figure 1.

2 | MATERIALS AND METHODS

2.1 | Data sources

Both the RNA-sequencing (RNA-seq) and clinicopathological data of gastrointestinal tumours, including stomach adenocarcinoma (STAD), colon adenocarcinoma (COAD), rectosigmoid junction cancer, and rectum adenocarcinoma (READ), were downloaded from the TCGA database (Data release 23.0 - April 7, 2020, https://tcga-data.nci.nih.gov/tcga/). mRNA expression was selected from the matrix file obtained from theRNA-seq data. GSE39582 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39582), and GSE87211, (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87211), obtained from GEO (GEO, https://www.ncbi.nlm.nih.gov/geo/) for the validation studies [26, 27]. Gene chips' original data were normalized using the Robust Multi-Array Average (RMA) algorithm provided by R-package ‘limma’ [28]. We used the Perl script and R-package ‘sva’ to merge the different microarray data from GSE39582 and GSE87211 [29] and reduce the heterogeneity. The m6A RNA methylation regulators are obtained from previously published literature [13, 15, 17]. 13 m6A RNA methylation regulators were obtained. Data annotation and extracting gene expression of m6A RNA methylation regulators from the TCGA and GEO data were done through the R software (version 3.6.2). The data are obtained from TCGA and GEO databases, strictly following the publication guidelines approved by TCGA and GEO. Therefore, there is no requirement for ethics committee approval.

2.2 | Identification m6A RNA methylation regulators’ expression in GIC

Perl script was used for data extraction, and integration and the Wilcoxon test were utilised to screen the expression of m6A RNA methylation regulators in GIC with disparate clinicopathological features. $p < 0.05$ are set as the cut-offs. $p < 0.05$ annotated ‘∗’, $p < 0.01$ annotated ‘∗∗’ and $p < 0.001$ annotated ‘∗∗∗’. Bidirectional hierarchical clustering analysis and a drawing heat map were performed by R-package ‘pheatmap’. R-package ‘vioplot’ was used to draw the violin plot.

2.3 | Accordance clustering of m6A RNA methylation regulators to determine two clusters in GIC and perform survival analysis

To explore the function of m6A RNA methylation regulators in GIC, we divided GIC patients into two groups through the R-package ‘ConsensusClusterPlus’ (50 iterations, Pearson correlation resample rate is 0.8, http://www.bioconductor.org/) based on the m6A RNA methylation regulators’ expression. Principal component analysis analysis is implemented through the R-package ‘limma’ using R software to research the gene expression modes in different GIC clusters. The survival analysis was performed by the Kaplan-Meier method and log-rank test ($p < 0.05$).

2.4 | Exploring the prognostic value and constructing a prognosis model of m6A RNA methylation regulators in GIC

We performed a univariate Cox analysis to evaluate the prognostic value of m6A RNA methylation regulators in GIC. The least absolute shrinkage and selection operator (Lasso) Cox regression analysis was utilised to construct the optimal model of m6A RNA methylation regulators. Each patient’s risk score was computed by the following formula: Risk
The median risk score is chosen as a cut-off value of the bisection GIC cohort.

2.5 | Clinical correlation analysis of the risk score

The R-package ‘survival ROC’ was utilised to draw the Receiver Operating Characteristic (ROC) curve, which was used to test the sensitivity and specificity of the risk score to predict survival. The prognostic accuracy is evaluated by the area under the curve (AUC) value. Depending on the different patients’ risk scores, we drew the survival state diagram, risk curve, and heat map. Univariate and multivariate Cox proportional regression analyses are used to perform independent prognostic analysis of m^6^A RNA methylation regulators. R-package ‘beeswarm’ is used to conduct clinical correlation analysis. The prognostic m^6^A RNA methylation regulators have verified in the GSE87211 and GSE39582 cohorts.

2.6 | Drawing the nomogram

The expression of FTO, METTL3, WTAP, YTHDF1, and ZC3H13 was used to construct a nomogram via R-package ‘Formula’, ‘foreign’, ‘ggplot2’, ‘Hmisc’, ‘lattice’, and ‘rms’. Furthermore, a calibration curve was utilised to evaluate the consistency between actual and predicted survival. Besides, we evaluated the performance of the model in predicting prognosis through the consistency index (c-index). The C-index value of 0.5 and 1.0 represents a random possibility and an
exceptional capacity to predict survival with the model, respectively.

2.7 Correlation and functional enrichment analysis of m^6A RNA methylation regulators in GIC

The correlation analysis of m^6A RNA methylation regulators is performed by the R-package 'corplot'. Functional annotation and enrichment analysis of five m^6A RNA methylation regulators are based on R-package 'clusterProfiler', 'ggplot2', 'enrichplot', and 'org.Hs.eg.db', which classified Gene Ontology (GO) to the Biological Processes (BP), false discovery rate (FDR) <0.05 is used as the cut-off. The Gene Set Enrichment Analysis (GSEA) is used to execute the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and hallmark of GIC. Permutation number is set to 1,000, and FDR <0.25 was recognized as statistically significant [31].

3 RESULTS

3.1 Patients' features

TCGA GIC cohort consisted of 1067 patients. 821 patients were included in our analysis and patients with incomplete clinical information and those with survival time of less than 90 days, were excluded. The GSE39582 cohort included 585 patients, 19 colon mucosa, and 566 stage I-IV COAD patients [26]. The GSE87211 cohort consisted of 363 patients, 160 rectal mucosa, and 203 rectal tumour patients [27]. Nine hundred forty-eight patients were obtained by merging these two cohorts. Using the same exclusion criteria as the TCGA GIC cohort, 701 CRC patients were included in our validation analysis. The clinical features of the patients are listed in Table S1.

3.2 The expression of m^6A RNA methylation regulators was correlative with clinicopathological traits in GIC

Because m^6A RNA methylation regulators play a significant role in the cancer occurrence and progression, we investigated the expression level of 13 m^6A RNA methylation regulators in various pathological features of GIC, containing tumour status (normal and tumour), pathological stage (early stage- stage I and II; advanced stage-stage III and IV), and regional lymph node metastasis (N0 and N1). A total of 1099 RNA-seq was obtained; 83 (7.5%) samples were normal and 1016 samples (92.5%) were tumours. 11 m^6A RNA methylation regulators are abnormally expressed in GIC tissues patients (Figure 2a).

Compared with the normal tissue, expressions of METTL3, YTHDF1, YTHDF2, KIAA1429, ZC3H13, WTAP, FTO, HNRNPC, RBM15, and YTHDC1 were up-regulated, while ALKBH5 expression was down-regulated in GIC tissue samples (Figure 2b).

Then, we investigated the relationship between the expression of 13 m^6A RNA methylation regulators and pathological stage, regional lymph node metastasis in GIC, respectively. Six m^6A RNA methylation regulators were differentially expressed between the advanced and early stages of GIC (Figure 2c). The expressions of ZC3H13, FTO, and RBM15 are up-regulated in the advanced stage, while the expression of METTL3, WTAP, HNRNPC are up-regulated in the early stage (Figure 2d). Similarly, five m^6A RNA methylation regulators are differentially expressed between the N0 and N1 subgroup of GIC (Figure 2e). ZC3H13 and FTO were highly expressed in the N1 subgroup, while METTL3, KIAA1429, and HNRNPC were highly expressed in the N0 subgroup (Figure 2f).

3.3 Accordance clustering of m^6A RNA methylation regulators identified two gastrointestinal cancer clusters

Depending on the expression correspondence of m^6A RNA methylation regulators, with clustering stability-enhancing from k = 2 to 10 in the TCGA dataset, k = 4 seemed to be the right choice (Figure 3a, b). However, we noticed that the inter-group correlation is significantly higher in the k = 4 group than in the k = 2 group (Figure S1, S2). So, we divided the expression of m^6A RNA methylation regulators into two groups according to k = 2 and contrasted the clinicopathological characteristics of the two subgroups by k = 2, named cluster 1 and cluster 2, then used Principal component analysis to contrast the transcriptional profile between cluster 1 and cluster 2 subgroups. There are apparent differences between them (Figure 3c). Cluster 2 subgroup contains more lymph node metastasis patients than cluster 1 (Figure 3d, Table S2, p < 0.001). Furthermore, we observed a shorter overall survival (OS) in the cluster 2 subgroup than the cluster 1 subgroup (p < 0.05, Figure 3e).

3.4 Prognostic value of m^6A RNA methylation regulators and construction of the prognosis model

A univariate Cox analysis on the expression of the TCGA dataset was implemented to investigate the prognostic value of m^6A RNA methylation regulators in GICs. The results demonstrated that nine out of 13 m^6A RNA methylation-related genes are conspicuously related to OS (p < 0.05). FTO and ZC3H13 are high-risk prognosis-related genes (Hazard ratio [HR] > 1, p < 0.05), while METTL14, METTL3, WTAP, YTHDC1, YTHDF2, YTHDF1, and HNRNPC are protective prognosis-related genes (HR < 1, p < 0.05). The nine prognosis-related m^6A RNA methylation genes showed in Figure 4a, Table S3.

We utilised the LASSO Cox analysis method to better forecast GICs' clinical outcomes to analyse the nine prognosis-associated m^6A RNA methylation in the TCGA dataset. Five prognosis-related m^6A RNA methylation regulators were filtered out through the minimum criteria, and the LASSO algorithm acquired the coefficient (Figure 4b–d). The coefficient of each
gene in GIC is utilised to compute the risk score of training (TCGA) and validation dataset (GEO). The prognosis model is constructed according to five m\textsuperscript{6}A RNA methylation genes, FTO, METTL3, ZC3H13, YTHDF1, and WTAP. The full name, function, and coefficient of these genes are shown in Table S4. The expression level of the m\textsuperscript{6}A RNA methylation regulator and the risk coefficient of every gene, the risk score of each patient is calculated. Risk score = 0.030 × expression of WTAP + (−0.013) × expression of YTHDF1 + 0.012 × expression of ZC3H13 + (−0.103) × expression of METTL3 + 0.148 × expression of FTO. According to the median risk score, the risk score was used to predict the prognosis and divide patients into high-risk and low-risk subgroups. The OS of the low-risk group is higher than the high-risk group in both the TCGA (Figure 4c) and GEO datasets (Figure 4f, \( p < 0.05 \)).

### 3.5 The risk score had a significantly association with the clinicopathological features of GIC

A heatmap was used to show the expression of the five m\textsuperscript{6}A RNA methylation related-genes in high- and low-risk patients from the TCGA GICs dataset (Figure 5a). Distributions of the risk score in GIC patients and the relationship between risk
score and survival time are visualised in Figure 5b. As the risk score enhanced, the survival time of the patients decreased, and death numbers increased. High-risk genes (FTO and ZC3H13) were more probably expressed in the high-risk group, while protective genes (YTHDF1, METTL3, and WTAP) tended to express in the low-risk group (Figure 5a). FTO expression was higher in the advanced stage group and N1–3 (Figure 5c, 5d, \( \rho < 0.001 \)). Similarly, ZC3H13 showed a high expression in the advanced stage group, T3–4, and N1–3 (Figure 5e–g, \( \rho < 0.05 \)). On the contrary, WTAP was high expressed in the early stage, and \( N_0 \) (Figure 5i, 5k, \( \rho < 0.05 \)) METTL3 expression was also higher in the \( N_0 \) subgroup (Figure 5l, \( \rho < 0.001 \)). The risk score was higher in the advanced stage group and N1–3 (Figure 5h, 5i, \( \rho < 0.05 \)). 1-year, 3-year, and 5-year AUC value of ROC for risk score was 0.668, 0.658, and 0.687, respectively; prognostic accuracy of N-stage and pathological stage was higher than other clinical characters (Figure 5m–o). These results indicate that the risk scores and FTO and ZC3H13 are closely related to the malignant clinicopathological features and can accurately predict the GICs patients’ outcomes.

Furthermore, univariate and multivariate Cox analyses were performed on the TCGA dataset to identify whether the risk score was an independent prognostic factor. Then we validated in the GEO dataset. The older patients, \( N_1–3, M_1 \) and high risk-scores were associated with the poor OS both in the univariate and multivariate independent prognostic Cox analysis \( (\rho < 0.01, \text{Figure 5p, 5q, Table 1}) \). Similar results are discovered in the GEO dataset (Figure 5r, Table 2). Overall, these were found to validate the risk score, based on five m\(^6\)A RNA methylation regulators, and could independently predict prognosis and closely associated with \( t \) GIC, patients’ malignancy.

### 3.6 The prognostic prediction models

Nomogram is an effective tool that has been used to quantitatively identify the individuals’ risk in the clinical setting through combining multiple risk elements [32]. We designed a nomogram to predict 1-, 3- and 5-year OS probability by combining five m\(^6\)A RNA methylation regulators’ signatures (Figure 6a). We computed every patient’s total point by summing up the number of points for five m\(^6\)A RNA methylation regulators, which might aid to appropriate practitioners to make clinical decisions for GIC patients. The calibration curve demonstrated that the actual
survival rates in the TCGA data set matched well with the predicted 1-, 3- and 5- year survival rates; the C-index is 0.728 (Figure 6b, 6d, 6f). The nomogram is also verified in the GEO cohorts, the C-index is 0.734, and calibration curves of 1-, 3- and 5- years are shown in Figure 6c, 6e, 6g.

3.7 Correlation and function enrichment analysis of m6A RNA methylation regulators

We also analysed these regulators' relationship to better appreciate the 13 m6A RNA methylation regulators (Figure 7a). The expressions of YTHDC1, YTHDF1, and YTHDF2 are significantly associated with each other. HNRNPC had a significant correlation with YTHDC1, YTHDC2, and YTHDF2 in GIC. WTAP expression was also prominently correlated with the ‘writers’ of KIAA1429, RBM15, METTL14, and METTL3 in GIC. Moreover, the expressions of FTO and ZC3H13 are positively related to each other; FTO was not correlated with WTAP, YTHDF1, YTHDF2, and negatively correlated with METTL3 and HNRNPC; ZC3H13 was not correlated with WTAP, METTL3, and negatively correlated with HNRNPC. These findings were consistent with that of the FTO and ZC3H1 which were risky genes, while the WTAP, YTHDF1, YTHDF2, METTL3, and HNRNPC were the protective genes.

We further used functional GO enrichment analysis to understand the previous five m6A RNA methylation regulators' pathways. A total of 80 GO terms of BP are identified to be significant (FDR <0.05). These genes are categorised into several essential processes, mainly containing RNA modification, mRNA methylation, RNA methylation, and mRNA metabolic process regulation. Top-20 GO terms of BP are shown in Figure 7b. GSEA analysis was performed that altered the genes associated with several common KEGG pathways, including focal adhesion (NES = 2.17, p = 0.002), Extracellular matrix-receptor interaction (NES = 2.14, p = 0.002), regulation of actin cytoskeleton (NES = 2.13, p = 0.000), and dilated cardiomyopathy (NES = 2.02, p = 0.004; Figure 7c). Moreover, GSEA revealed that the malignant hallmarks of tumours,
FIGURE 5 Association between the risk score and clinicopathological characteristics in gastrointestinal carcinoma. (a) The heatmap manifested the expression levels of the five m^6^A RNA methylation regulators in high and low-risk groups. The distribution of clinicopathological characteristics is compared between the high- and low-risk groups, *p < 0.05, ***p < 0.001. (b) The distribution of the risk score and survival time of the patient, as well as the status of the gastrointestinal cancer (GIC). The black dotted line was the optimum cutoff dividing patients into high-risk and low-risk groups. (c–l) The association between the clinicopathological characteristics and expression of m^6^A RNA methylation regulators. The expression level of FTO in different stages (c) and N subgroups (d). The ZC3H13 expression in distinct stages (e) and N subgroup (f). The risk score in different stages (h) and N subgroups (i). The expression level of WTAP in diverse stages (j) and N subgroups (k). (l) The MTTL3 expression in different N subgroups. (m–o) Receiver Operating Characteristic (ROC) analysis of the specificity and sensitivity of the overall survival (OS) for the combination of the risk score and clinical features in the the Cancer Genome Atlas (TCGA) gastrointestinal carcinoma. (p) Univariate and (q) Multivariate independent prognostic Cox regression analysis in the TCGA database. (r) Univariate and (s) Multivariate independent prognostic Cox regression analysis in the Gene Expression Omnibus (GEO) database. TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus.
In this study, we illustrated that the expression of group prediction, progression, prognosis, and response to treatment coding RNAs has diverse functions in early cancer detection, classifications, nucleosome remodelling, DNA methylation, and non-conventional epigenetics concentrated on histone modifications. [33, 34]. The previous report has found that carbonic anhydrase IV targeting the WT1 axis to inhibit Wnt signalling pathways, and GIC’s malignant hallmarks.

This paragraph thoroughly analysed the expression of 13 m^6^A RNA methylation regulators in GIC with different clinicopathological traits. Previous research has reported that the eraser FTO was influential in promoting gastric cancer and FTO polymorphisms associated with colorectal adenomas in African-Americans [35, 36]; ALKBH5 was down-regulated in colorectal cancer FTO which positively correlated with poor prognosis in colorectal cancer patients [39]. ALKBH5 altered cell adhesion of bladder cancer cells by regulating the ITGA6 activity towards N (6)-methyladenosine in RNA [9]; these results indicate that ALKBH5 and FTO may adjust demethylation of disparate methylation targets in GIC, and it deserves to be explored in future research studies. The expression of ZC3H13 and RBM15 was significantly increased in the advanced stage, indicating potential functions of ZC3H13 and RBM15 in GIC malignancy. METTL3 promotes the translation of many sub-gene networks of oncogenic mRNAs and acts as an oncogene, sustains SOX2 expression of CRC cells by an m^6^A-IGF2BP2-dependent mechanism, and facilitates tumour progression [21, 38]. The previous report has found that carbonic anhydrase IV targeting the WTPA-WT1-TBL1 axis to inhibit Wnt signalling pathway [39] and ZC3H13 may suppress invasion.

**Table 1** The prognostic value of different clinical characters in the training group

| Variables          | Univariate prognostic analysis | Multivariate prognostic analysis |
|--------------------|-------------------------------|----------------------------------|
|                    | HR   | 95% CI | p-value | HR   | 95% CI | p-value |
| Age (≤65 years VS>65 years) | 1.065 | 1.174–2.194 | 2.99E–4 | 2.207 | 1.589–3.065 | 2.35E–06 |
| Gender (female vs. male) | 1.280 | 0.942–1.735 | 0.115 | 1.218 | 0.893–1.662 | 0.213 |
| Stage (I/II vs. III/IV) | 2.877 | 2.092–3.956 | 7.87E–11 | 1.153 | 0.610–2.177 | 0.662 |
| T (1–2 vs. 3–4) | 1.835 | 1.193–2.824 | 0.006 | 1.411 | 0.873–2.278 | 0.159 |
| N (0 vs. 1–3) | 3.100 | 2.215–4.339 | 4.27E–11 | 1.987 | 1.065–3.706 | 0.031 |
| M (0 vs. 1) | 2.876 | 2.039–4.058 | 1.79E–09 | 2.457 | 1.680–3.595 | 3.64E–06 |
| Risk score | 4.282 | 2.721–6.736 | 3.18E–10 | 7.113 | 4.049–12.495 | 8.80E–12 |

**Table 2** The prognostic value of different clinical characters in the validation group

| Variables          | Univariate prognostic analysis | Multivariate prognostic analysis |
|--------------------|-------------------------------|----------------------------------|
|                    | HR   | 95% CI | p-value | HR   | 95% CI | p-value |
| Age (≤65 years VS>65 years) | 1.289 | 0.954–1.742 | 0.098 | 1.461 | 1.076–1.984 | 0.015 |
| Gender (female vs. male) | 1.167 | 0.863–1.578 | 0.317 | 1.223 | 0.901–1.659 | 0.197 |
| Stage (I/II vs. III/IV) | 1.481 | 1.096–2.001 | 0.011 | 1.136 | 0.452–2.854 | 0.787 |
| T (1–2 vs. 3–4) | 1.805 | 0.888–3.670 | 0.103 | 1.459 | 0.714–2.982 | 0.301 |
| N (0 vs. 1–3) | 1.297 | 0.962–1.747 | 0.088 | 1.012 | 0.427–2.396 | 0.979 |
| M (0 vs. 1) | 5.031 | 3.417–7.409 | 2.77E–11 | 4.589 | 2.911–7.234 | 5.38E–11 |
| Risk score | 3.808 | 1.841–7.875 | 3.10E–04 | 2.992 | 1.424–6.289 | 0.004 |

Abbreviation: HR, hazard ratio.

4 | DISCUSSION

Conventional epigenetics concentrated on histone modifications, nucleosome remodelling, DNA methylation, and non-coding RNAs has diverse functions in early cancer detection, prediction, progression, prognosis, and response to treatment [33, 34]. In this study, we illustrated that the expression of m^6^A RNA methylation regulators, another field of epigenetics, is also closely related to the prognosis and malignancy of GIC. Firstly, 10 of 13 m^6^A RNA methylation regulators are substantially up-regulated in the GIC patients. We discovered that ZC3H13 and FTO were both positively associated with the group of advanced stage and N1-3. Then, we identified that the cluster 1/2 subgroups influenced the prognosis and clinicopathological features of GIC. We also designed a prognostic risk model according to five m^6^A RNA methylation regulators and classified the GIC patients into high- and low-risk subgroups. We further identified risk scores and high-risk genes (FTO and ZC3H13) closely associated with the malignant clinicopathological feature and can accurately predict the GICs patients' outcomes. The five m^6^A RNA methylation regulators can predict the probability of 1-, 3- and 5-year OS through a nomogram and are approximately associated with BP, hub signalling pathways, and GIC's malignant hallmarks.

This paragraph thoroughly analysed the expression of 13 m^6^A RNA methylation regulators in GIC with different clinicopathological traits. Previous research has reported that the eraser FTO was influential in promoting gastric cancer and FTO polymorphisms associated with colorectal adenomas in African-Americans [35, 36]; ALKBH5 was down-regulated in colorectal cancer FTO which positively correlated with poor prognosis in colorectal cancer patients [39]. ALKBH5 altered cell adhesion of bladder cancer cells by regulating the ITGA6 expression [37]. FTO and ALKBH5 showed demethylase activity towards N (6)-methyladenosine in RNA [9]; these results indicate that ALKBH5 and FTO may adjust demethylation of disparate methylation targets in GIC, and it deserves to be explored in future research studies. The expression of ZC3H13 and RBM15 was significantly increased in the advanced stage, indicating potential functions of ZC3H13 and RBM15 in GIC malignancy. METTL3 promotes the translation of many sub-groups of oncogenic mRNAs and acts as an oncogene, sustains SOX2 expression of CRC cells by an m^6^A-IGF2BP2-dependent mechanism, and facilitates tumour progression [21, 38]. The previous report has found that carbonic anhydrase IV targeting the WTPA-WT1-TBL1 axis to inhibit Wnt signalling pathway [39] and ZC3H13 may suppress invasion.
FIGURE 6 The nomogram to anticipate prognostic possibilities in gastrointestinal carcinoma. (a) The nomogram for predicting 1-, 3-, and 5-year overall survival (OS) of gastrointestinal carcinoma by expression of five m^6^A RNA methylation regulators. (b–g) Calibration curves assess consistency between actual and predicted OS for 1, 3, and 5 years. (b, d, f) The Cancer Genome Atlas (TCGA) dataset, (c, e, g) Gene Expression Omnibus (GEO) dataset. TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; OS, overall survival.
and proliferation of CRC inactivating regulator of the Ras-ERK signalling pathway [40]. RBM15 mutations also may be crucial in the pathogenesis of borderline/malignant phyllodes tumours [41]. These findings are also advantageous to investigate novel therapeutic methods of GIC by distinguishing the expression of each m6A methylation regulator.

In the present research, the prognostic model had a moderate prognostic value in GIC. The patients in the high-risk group had an obviously shorter survival time than patients in the low-risk group. Expression of FTO and ZC3H13 increased in GIC with malignant clinico-pathological features, such as advanced stage, sizeable primary tumour, and lymph node metastasis. The risk score was also obviously related to the malignant clinico-pathological features in GIC. These results were consistent with the previous studies [37]. 1-year, 3-year, and 5-year AUC values of ROC for the risk score are consistent with a previous study that the AUC of the ROC curve is 0.68 for the ability of YKL-40 to predict GIC [42]. Both univariate and multivariate COX regression analyses confirmed that malignant clinico-pathological traits were related to poor prognosis in GIC patients from TCGA and GEO databases.

Subsequently, we established a nomogram to forecast the individual patient’s clinical outcomes. The nomogram was a
Our research comprehensively clarified the expression, molecular function, and prognostic value of five m^6^A RNA methylation regulators in GIC. The expression of the m^6^A-RNA methylation regulatory factor was significantly associated with the clinicopathological traits of GIC, and it is also significantly associated with the biological process of promoting the malignant progress of GIC and the increase of gene expression level in the signal pathway. Our research also highlights the essential role of the risk score as an independent prognostic marker for GIC. These findings provide an extensive viewpoint for further research on the role of m^6^A RNA methylation regulators in the GIC’s pathogenesis and as potential markers for GIC diagnosis and treatment.

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CONFLICT OF INTEREST STATEMENT
The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT
The authors certify that all the original data in this research could be obtained from a public database. Both the RNA-sequencing (RNA-seq) and clinicopathology of the gastrointestinal tumor, including STAD, COAD, rectosigmoid junction cancer, and READ, were obtained from the TCGA database (Data release 22.0 - 16 January 2020, https://tcga-data.nci.nih.gov/). The expression of mRNA extracted from the matrix file was obtained from the RNA-seq data. The data access GSE39582 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39582) and GSE87211, (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87211), were obtained from GEO (GEO, https://www.ncbi.nlm.nih.gov/geo/). Other data used to support the findings of this study are included within the supplementary information files.

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