Development of a novel colon adenocarcinoma m6A-related lncRNA pair prognostic model

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Research Article

Keywords: colon adenocarcinoma, M6A, lncRNA pairs, TCGA, prognosis signature

Posted Date: June 27th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3044227/v1

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Abstract

**Background:** Colon adenocarcinoma (COAD) is among the most prevalent malignancies. N6-methyladenosine (m6A) alterations, the most prevalent RNA modification, can influence COAD progress. In addition, long noncoding RNA (lncRNA) plays an important role in COAD and is closely related to m6A modification. However, the prognostic value of lncRNAs associated to m6A in COAD is unknown.

**Methods:** In present study, the information from The Cancer Genome Atlas (TCGA) was employed to examine the predictive relevance of m6A-related IncRNA pair signatures in COAD. M6A-related IncRNAs was identified based on co-expression analysis utilizing the Pearson correlation. Then, the IncRNAs paired related to prognosis were identified, followed by univariate Cox regression analysis. The receiver operating characteristic (ROC) curves for predicting overall survival (OS) were conducted by using the least absolute shrinkage and selection operator (LASSO) penalized Cox analysis to identify and construct a risk score prognostic model. After determining if it was an independent prognostic factor, relationships between the risk score model and clinical traits, immune-related factors, and medication sensitivity analysis were analysed.

**Results:** A total of 319 m6A-related IncRNA pairs were found, and 35 of which were connected to a predictive pattern for risk scores. The risk score model was proven to be an independent predictive factor and was notably superior to the clinicopathological features. Correlation analyses revealed differences between high- and low-risk groups in clinicopathological characteristics, immune-related factors, and drug sensitivity analysis. **Conclusions:** The novel COAD prognostic model based on paired differentially expressed m6A-related IncRNAs showed promising clinical predictive value.

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I. INTRODUCTION

Colorectal cancer (CRC) is the third most frequent diagnosed and the second most lethal malignancy in the world.\(^1\) The majority of CRC cases are colon adenocarcinomas (COADs), accounting for 80–90% of all pathological subtypes.\(^2\)\(^,\)\(^3\) Despite the fact that screening techniques are meant to increase early CRC identification, 25% of patients are already in an advanced state when they are discovered.\(^4\) A higher cancer stage is connected with a higher risk of death in colon cancer, compared to Stage I patient, Stage IV patient only has an 8.86 times greater risk of death.\(^5\)

Long non-coding RNAs (lncRNAs), a subset of RNAs longer than 200 nucleotides, regulate a variety of biological activities, including the growth of tumors and the infiltration of immune cells.\(^6\)\(^,\)\(^7\) The dysregulation of lncRNAs is important in many malignancies, such as colon cancer, gastric cancer, breast cancer, pancreatic cancer, etc.\(^8\)–\(^11\) For instance, THAP7-AS1, a lncRNA with carcinogenic characteristics is transcriptionally activated by SP1 and post-transcriptionally stabilized by METTL3-mediated m6A
alteration. The IncRNA ITGB8-AS1 promotes colorectal cancer growth and migration via integrin-mediated focal adhesion signaling.

However, the mechanisms regulating IncRNA expression remain largely unknown. Several studies have shown that IncRNAs are regulated by N6-methyladenosine (m6A) alteration. The most frequent epigenetic methylation of mRNAs and non-coding RNAs (ncRNAs), N6-methyladenosine (m6A), has a significant effect on RNA translation, splicing, transportation, and stability. M6A regulators, which are made up of methyltransferases (writers), RNA-binding proteins (readers), and demethylases (erasers), control invertible and dynamic RNA epigenetic modification. Writers are made up of METTL3, METTL14, KIAA1429, RBM15, WTAP, and ZC3H13. Readers are made up of the proteins YTHDF1/2/3, YTHDC1/2, IGF2BP1/2/3, HNRNPC, and HNRNPA2B1. Erasers are made composed of ALKBH3, ALKBH5, and FTO to perform demethylation activity.

M6A enzymes and IncRNAs are both excellent prognostic and diagnostic biomarkers. In previous studies, m6A-related mRNAs and IncRNAs were found to be useful in predicting the prognosis for multiple cancers. For instance, Wang et al. created a signature to predict the prognosis of gastric cancer (GC) that contained 11 m6A-related IncRNAs with variably elevated levels. The AUC for 5-year overall survival (OS) was 0.94, indicating that the IncRNA signature has good predictive accuracy for GC. Additionally, Zhang et al. created the m6A-related IncRNA prognostic score (m6A-LRS), which predicted a poor prognosis for bladder cancer patients. The ROC curves of m6A-LRS for 5-year overall survival prediction was 0.67, showing that the risk model was effective. LncRNA signature showed promising prognostic potential, but it was not perfect. To lessen batch effects between various testing platforms, specific levels of IncRNAs should be standardized prior to clinical use of the signature. Additionally, Tang et al. developed a ferroptosis-related IncRNA pair prognostic signature in pancreatic ductal cancer using a novel modeling technique, pairing, and iteration.

In the current research, we created a brand-new m6A-related IncRNA pair predictive model for COAD. The model's relationships with clinicopathological traits, immune-related variables, and medication sensitivity analysis were exhibited.

II. Materials and Methods

A. Data collection

The TCGA website (https://portal.gdc.cancer.gov) was used to download all data for the TCGA-COAD cohort, including mRNA sequencing data, IncRNA sequencing data and clinical characteristics of patients. The training cohort and validation cohort were split up into 8:2 ratios based on cases having survival data. Using information from a recent study, we were able to derive the expression matrix for 23 m6A RNA methylation regulators, including writers (METTL3, METTL14, METTL16, WTAP, VIRMA, RBM15, RBM15B, and ZC3H13), erasers (FTO and ALKBH5), and readers (YTHDC1, YTHDC2, IGF2BP1, IGF2BP2, IGF2BP3, YTHDF1, YTHDF2, YTHDF3, HNRNPC, LRPPRC, HNRNPA2B1, FMR1, and RBMX).
B. Identification of m6A-related IncRNAs and pairing differentially expressed m6A-related IncRNAs

The association between the m6A-related genes and long non-coding RNAs was examined using Pearson correlation to identify the IncRNAs connected to m6A. The absolute value of correlation coefficient >0.4 and P<0.001 were deemed to have statistical significance. The significant thresholds of differentially expressed m6A-related IncRNAs were set as |\log_{2}\text{FC}| >1.0 and false discovery rate (FDR) <0.05. After that, we established m6A-related IncRNA pairs based on these differentially expressed m6A-related IncRNAs, as previously described. All differentially expressed m6A-related IncRNAs were paired cyclically and given a value based on pairwise comparisons: suppose that IncRNA A and IncRNA B were paired as IncRNA pair C, which was given a value of 1 if IncRNA A's expression level was higher than IncRNA B's and a value of 0 otherwise. A IncRNA pair was filtered if it had a 0 or 1 ratio of less than 20% or more than 80% across all samples. The prognostic significance of m6A-related IncRNA pairings was assessed using Cox regression analysis (P<0.01).

C. Building and evaluating a predictive signature for m6A-related IncRNA pairs

In the training cohort, the least absolute shrinkage and selection operator, regression was constructed using prognostically associated m6A-related IncRNA pairs. We then constructed a risk score model for these m6A-related IncRNA pairs, and calculated the risk score for each patient as follows: Risk score = coefficient IncRNA pair\(^1\) × expression IncRNA pair\(^1\) + coefficient IncRNA pair\(^2\) × expression IncRNA pair\(^2\) + coefficient IncRNA pair\(^3\) × expression IncRNA pair\(^3\) +……+ coefficient IncRNA pair\(^n\) × expression IncRNA pair\(^n\). The risk score model was used to create the 1-year ROC curve for predicting the OS. Patients in the training cohort and validation cohort were divided into high- and low-risk groups based on the median risk ratings. The Kaplan-Meier method was used to calculate survival curves. In order to evaluate the stability of this model, a cross test was conducted using the validation cohort. We built the final model using data from the full cohort in order to get an accurate model with a bigger sample size. The ROC curves for 1, 3, and 5 years were calculated. As the ideal cut-off point for classifying various risk categories, the maximum inflection point of the 1-year ROC curve was chosen. Survival results and risk scores for each patient were anticipated using a risk assessment model of prognosis prediction. To determine if the model was a standalone prognostic factor for OS in COAD patients, univariate and multivariate regression analyses were also utilized.

D. Clinical correlation analysis of the risk score model and development of a nomogram

A heatmap displaying relationships between risk score model and clinicopathological variables was constructed using chi-squared testing. The variations in risk scores among various groups of these clinicopathological variables were determined using the Wilcoxon signed-rank test and shown using box diagrams. Following are the labels for the p values: P0.001 = ***, P0.01 = **, and P0.05 = *. A nomogram was created using the multivariate logistic model, which included the risk score model and clinicopathological features, to predict the survival probability of COAD patients. The nomogram's endpoints were the 1-, 3-, and 5-year OS rates.
E. Correlations between the risk score model and immune-related factors

Differential gene expression between the two risk score groups was examined using the Wilcoxon signed-rank test. To assess the relationships between the risk score and tumor-infiltrating immune cells (TIICs), we used a few widely used algorithms, including XCELL, TIMER, QUANTISEQ, MCPCOUNTER, EPIC, CIBERSORTABS, and CIBERSORT. The statistical significance was set at P<0.05.

F. Chemosensitivity Prediction

The Cancer Drug Sensitivity Genomics (GDSC) database (https://cancerrxgene.org) can be utilized to do large-scale drug screening. Combining genetic analysis with chemotherapy, medication responses can be systematically discovered. Based on the GDSC database, the half-maximum inhibitory concentration (IC50) of 30 commonly used chemotherapy drugs for gastrointestinal tumors was calculated, and the clinical application of this model in the treatment of COAD was evaluated. The R package "pRRophetic" was used in the preceding procedure. The Wilcoxon signed-rank test was then used to assess the difference in IC50 between the high-risk group and low-risk group. To illustrate the data, box drawings were generated in R using "pRRophetic" and "ggplot2".

G. Statistical Analysis

The HTSeq FPKM and simple nucleotide variation data were extracted and structured using Perl software (version 5.32). Based on the log fold change and FDR, the differentially expressed IncRNAs were found using the Benjamini-Hochberg method. The Kaplan-Meier method was used to evaluate the survival analyses of COAD patients based on the risk score model. The Cox regression model was used for multivariate analysis. The studies were carried out using R software 4.0.5 and Bioconductor packages.

III. Results

A. Data characteristics

The current study includes 473 colon adenocarcinoma and 41 adjacent normal tissues with expression data. The clinical data of the patients (n=452), including age, gender, stage, T status, N status, and M status, are displayed in Table 1. 23 m6A-related genes in total were obtained from one earlier article.16 579 IncRNAs were identified as m6A-related IncRNAs in total. Following that, 243 (232 upregulated and 11 downregulated) of these were identified as differentially expressed m6A-related IncRNAs, which were displayed using a heatmap (Figure 1(a)) and a volcano plot (Figure 1(b)).

B. Establishment of a m6A-related IncRNA pair risk score prognostic model

A 0-or-1 matrix of 14980 m6A-related IncRNA pairs was built to investigate a more objective prognostic evaluation model that did not require normalization of individual expression values. Univariate Cox
proportional hazards regression analyses indicated that 318 m6A-related lncRNA pairs were prognostic-associated lncRNA pairs. After performing LASSO regression analysis on the training cohort, a predictive signature containing 26 m6A-related lncRNA pairs was established. The risk score model's AUC for the 1-year survival rate in the training and validation cohort were 0.926 and 0.979, correspondingly (Supplementary Figure 1(a), 1(b)). In both the training cohort and the validation cohort, survival analyses revealed a substantial difference between the high- and low-risk groups (Supplementary Figure 1(c), 1(d)). Following that, we built a risk score model using data from the complete cohort. A prognostic signature with 35 m6A-related lncRNA pairs was created after LASSO regression analysis (Figure 2(a), 2(b), 2(c)). Table 2 lists the 35 m6A-related pairs along with the appropriate calculation coefficients. The AUCs were 0.938, 0.930, and 0.916 for the 1-, 3-, and 5-year survival rates, respectively (Figure 3(a)). Additionally, we determined that the ideal cut-off point on the 1-year receiver operator characteristic (ROC) curve was the greatest inflection point of 20.296 (Figure 3(b)). Furthermore, the risk score model outperformed standard clinicopathological factors like age, gender, and stage in predicting the OS of COAD patients, according to our findings (Figure 3(c)).

C. Predictive assessment and clinical correlation of the prognostic model

Using the previously established cut-off point, 344 patients were assigned to the low-risk group and 82 to the high-risk group. According to the risk assessment model for prognosis prediction, there were more deaths as the risk score rose (Figure 4(a), 4(b)). Analysis of survival data showed that the high-risk group had significantly lower OS than the low-risk group (Figure 4(c)). In the univariate analysis, age, T status, N status, M status, and risk score model were found to be significant risk factors (all P<0.01) (Figure 4(d)). Multivariate analysis supported the risk score model, T status, and M status as independent predictive variables (all P<0.01) (Figure 4(e)). Additionally, the risk score model was substantially correlated with the T status, N status, M status, and stage, according to our findings (Figure 5). Furthermore, a precise predictive nomogram using the risk score model and typical clinicopathological traits was developed for predicting 1-, 3-, and 5-year OS probabilities. This nomogram could be useful in the clinical assessment of COAD patients (Figure 6).

D. Correlations between the risk score model and immune-related factors

In consideration of the increasing evidence on the correlation between immunological features and survival in malignant tumors, the correlation between risk score model and tumor-infiltrating immune cells (TIICs) was investigated. The results showed that the high-risk group was associated with more TIICs, such as CD8+ T cells, CD4+ T cells, and macrophage, whereas the low-risk group was associated with more TIICs, such as neutrophils, B cells, and NK cells (Figure7).

E. Correlations between the risk score model and sensitivity to anticancer drugs

In order to determine potential treatment modalities for COAD, the sensitivity to 30 common anticancer drugs between the high- and low-risk groups were compared. According to the findings, patients in high-risk groups had lower IC50 values for the food and drug administration (FDA)-approved antitumor
medicines rapamycin, lenalidomide, embelin, and DMOG. This shows that increasing prognostic model risk was accompanied by increased sensitivity to these treatments (Figure 8). In this context, these drugs have the potential to be applied in the treatment of high-risk groups COAD in the future.

IV. Discussion

M6A is an RNA modification that interacts with mRNAs and lncRNAs, and affects almost all biological functions of tumor cells. For example, METTL14, an m6A writer, is relevant to colorectal cancer progression by modulating SOX4 expression. Recent study found that LINC00460 improved HMGA1 mRNA stability and protein expression by directly interacting with IGF2BP2 and DHX9 to bind the 3' untranslated region (UTR) of HMGA1 mRNA. M6A modification of HMGA1 mRNA by METTL3 enhanced HMGA1 expression in CRC. Lu and his coworkers revealed that the m6A reader IMP2 stabilizes ZFAS1, and that these two factors work together to induce CRC by boosting mitochondrial energy metabolism. It was reported that YTHDF1, an m6A reader, effectively synergizes with cisplatin by inhibiting protein synthesis of GLS1 to induce colon cancer cell death. These investigations shown that m6A is essential for regulating the development and division of colon cancer cells and that it works in synergy with drugs to cause colon cancer cell death. Additionally, researches have demonstrated that m6A-related lncRNA signatures can be utilized to predict prognosis, improve survival risk assessment, and enable tailored treatment in colon cancer. There are certain practical issues with these models. These prognostic models were developed using the particular expression levels of the discovered lncRNAs. Before applying the measurements in a clinical setting, the measured values must be standardized to eliminate batch effects between various testing platforms. In the current work, we created a brand-new m6A-related lncRNA pair predictive signature for colon cancer. For 1-, 3-, and 5-year survival rates, the AUCs showing excellent predictive value were 0.938, 0.930, and 0.916, respectively. Our prognostic signature, which includes 35 m6A-related lncRNA pairs, was verified by Cox regression analysis to be an independent predictive factor. Notably, it was better at predicting the OS for COAD than frequent clinicopathological factors such age, sex, T status, N status, and M status. More crucially, the signature was created by pairing, iteration, and a novel modeling technique; as a result, it may be used more effectively in clinical settings.

The immune microenvironment is crucial in carcinogenesis. Infiltrating immune cells may work to antagonize tumors or to promote tumors. Cancer cells have evolved multiple mechanisms to escape immune surveillance, resulting in cancer development. In recent years, immunotherapy has innovated in cancer treatment and has demonstrated remarkable success. Furthermore, m6A and immunity are intimately related. M6A can regulate a wide range of immune cells and has a number of regulatory modes and processes, including influencing T cell development, regulatory T cell status, and dendritic cell maturation. In this work, we looked at the relationship between the risk score model and immune-related variables. The findings demonstrated that the high-risk group was connected to more TIICs, such as CD8+ T cells, CD4+ T cells, and macrophages, whereas the low-risk group was related to more TIICs, such as neutrophils, B cells, and NK cells. The discovery that m6A-related lncRNAs were connected to
immune cell infiltration in COAD may help us identify new treatment targets. Despite recent advances in tumor immunotherapy, the overall therapeutic impact of COAD is unsatisfactory. Therefore, creating multimode therapy and biointegration targets is required. The relationship between COAD and m6A-related IncRNAs requires more research.

High-risk patients with COAD are often treated with a combination of chemotherapy and operation. We discovered using the GDSC database that high-risk individuals were more sensitive to routinely used chemotherapeutic medicines (such as lenalidomide, embelin, DMOG, and rapamycin) than low-risk patients. This finding may open up new treatment options for COAD patients. Although lenalidomide's anticancer mechanism is still not fully understood, it appears to cause angiogenesis inhibition and immunomodulation. In the tumor environment, embelin boosted the infiltration of CD8+ T cells, NK cells, and mature dendritic cells while decreasing the number of regulatory T cells. Rapamycin, the first naturally occurring mTOR inhibitor, prevented the growth of colorectal cancer cells that were susceptible to rapamycin. More clinical experiments are required to investigate the efficacy of these chemotherapeutic drugs in COAD patients.

However, there are still some restrictions to consider. First, additional experimental confirmation is needed as we can only draw inferences from bioinformatics research. Second, more samples should be included in the future.

V. Conclusions

In conclusion, the m6A-related IncRNA pairs in COAD was systematically identified and analyzed for the first time in this study. We uncover m6A-related IncRNA pairs of prognostic value and develop a novel risk model with good prognostic and survival status predicting ability. The risk score is a new and potential biomarker since it has a strong correlation with the malignant clinicopathological characteristics of COAD. Moreover, our findings offer crucial support for additional research on the role of m6A-related IncRNA pair in COAD, which may offer fresh perspectives on how to direct an efficient immunotherapy regimen for COAD.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in TCGA (https://portal.gdc.cancer.gov/).
Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Guangxi Traditional Chinese Medicine Appropriate Technology Development and Promotion Project (GZSY21-56) and National Natural Science Foundation of China (No. 82260579).

Authors’ contributions

SL and XQ are co-first authors: Validation, writing—original draft, writing—review & editing, DC and LC: Methodology, software, formal analysis, FW: Resources, data curation, visualization, SL and JH: Conceptualization, supervision, project administration. All authors read and approved the final manuscript.

Acknowledgements

None.

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9. Liu HT, Zou YX, Zhu WJ, et al., “lncRNA THAP7-AS1, transcriptionally activated by SP1 and post-transcriptionally stabilized by METTL3-mediated m6A modification, exerts oncogenic properties by


Tables

Table 1: The clinical characteristics of COAD patients in the TCGA dataset.
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Table 2: The list of lncRNA pairs and corresponding calculation coefficients.
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Figures

(a) Differentially expressed m6A-related lncRNAs in COAD visualized by a heatmap. (b) Differentially expressed m6A-related lncRNAs in COAD represented by a volcano plot.

Figure 1

(a) Differentially expressed m6A-related lncRNAs in COAD visualized by a heatmap. (b) Differentially expressed m6A-related lncRNAs in COAD represented by a volcano plot.
Figure 2

Establishment of a prognostic model based on (a, b) LASSO regression analysis; (c) Univariate Cox regression analysis.

Figure 3

(a) The ROC curves for predicting the 1-, 3-, and 5-year OS; (b) identification of the maximum inflection point as the optimal cut-off value on the 1-year ROC curve; (c) comparison of the risk score model and clinicopathological characteristics in predicting the 1-year OS.
Figure 4

Risk scores (a) and survival outcomes (b) of each patient; (c) survival curves of high-risk group and low-risk group patients; (d) univariate and (e) multivariate Cox regression analyses of the risk score model and clinicopathological characteristics.
Figure 5

Correlations between the risk score model and clinicopathological characteristics, represented by a heatmap (a), and box diagrams (b).
**Figure 6**

Prognostic nomogram incorporating the risk score model and clinicopathological characteristics.

**Figure 7**

Correlation coefficient plot showing the relationship between different immune cells and software tools.
Correlations between the risk score model and tumor-infiltrating immune cells.

Figure 8
Estimated drug sensitivity in patients with high- and low- risk groups.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.png