

Identification of a m6A RNA methylation regulators-based signature for predicting the prognosis of esophageal adenocarcinoma

Yue Zhou

Fudan University Shanghai Cancer Center

Shuyan Li

Fudan University Shanghai Cancer Center

Liqing Zou

Fudan University Shanghai Cancer Center

Tiantian Guo

Fudan University Shanghai Cancer Center

Xi Yang (✉ ntgeorge@qq.com)

Fudan University <https://orcid.org/0000-0001-6125-7641>

Zhengfei Zhu (✉ fuscczzf@163.com)

Fudan University Shanghai Cancer Center

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Abstract

Background

N6-methyladenosine (m6A) is an abundant modification in RNAs that affects RNA metabolism, and it is reported to be closely related to cancer occurrence and metastasis. The aim of this study was to identify novel prognostic biomarkers by using m6A RNA methylation regulators capable of improving the risk-stratification criteria of survival for esophageal adenocarcinoma patients.

Methods

The gene expression data of 16 m6A methylation regulators and its relevant clinical information were extracted from The Cancer Genome Atlas (TCGA) database. The expression pattern of these m6A methylation regulators was evaluated. Consensus clustering analysis was conducted to identify clusters of esophageal adenocarcinoma patients with different prognosis. Univariate, least absolute shrinkage and selection operator (LASSO), and multivariate Cox regression analysis were performed to construct multiple-gene risk signature. A survival analysis was carried out to determine the prognosis significance.

Results

Ten m6A methylation regulators (HNRNPA2B1, HNRNPC, YTHDF1, METTL3, YTHDF2, RBM15, YTHDC1, WTAP, KIAA1429 and YTHDF3) showed significant up-regulation in tumor tissue. Consensus clustering analysis identified three clusters of esophageal adenocarcinoma patients with different overall survival. A five-gene signature, HNRNPA2B1, KIAA1429, WTAP, METTL16 and ALKBH5, was constructed to serve as a prognostic indicator for distinguish esophageal adenocarcinoma patients with different prognosis. The receiver operator characteristic (ROC) curve which indicated the area under the curve (AUC) were 0.803, demonstrated that the prognostic signature had preferable prediction efficiency.

Conclusions

m6A methylation regulators exert as potential biomarkers for prognostic stratification of esophageal adenocarcinoma patients and might help clinicians make individualized therapy for this patient population.

Background

Esophageal cancer, one of the most aggressive malignant tumors, has been reported to be the ninth most common cancer and the sixth most common cause of cancer-related deaths globally [1, 2]. The incidence of esophageal cancer has been rising, with 17,650 new cases and 16,080 deaths estimated by the latest cancer statistic report in the United States [3, 4]. Despite the considerable progress made in multimodal therapies of esophageal cancer, including surgery, chemotherapy, radiotherapy, and chemoradiotherapy, the prognosis remains poor; the overall 5-year survival ranges from 15% to 25% among all patients and approximately 40% among those who undergo curative surgery [5-7]. The two main histological subtypes

of esophageal cancer, esophageal adenocarcinoma (EAC) and esophageal squamous-cell carcinoma (ESCC), differ greatly in terms of epidemiology and risk factors. EAC is three to four times as common in men as it is in women whereas the gender distribution is relatively equal in ESCC [4]. Although EAC accounted for only about 10% of cases of esophageal cancer worldwide, its incidence has increased rapidly and even surpassed that of ESCC in several regions in North America and Europe [8]. In addition to the well-known risk factors in EAC like symptomatic gastroesophageal reflux disease, Barrett's esophagus, obesity and tobacco using, genetic variants have been associated with risk for EAC [9]. Over the last decade, genome-wide association studies (GWAS) have identified about 20 genetic risk loci for EAC, indicating that single nucleotide polymorphisms (SNPs) play a pivotal role in EAC susceptibility [10-13]. Identifying novel biomarkers for predicting EAC patients' long term survival is urgently to be addressed.

Previously, epigenetic research mainly focused on DNA and histone modifications whereas mRNA was believed to only contribute to information transmission. However, with great progress made in high-throughput sequencing technology, it is agreed that there also exist various modifications in mRNA, such as N¹-methyladenosine (m¹A), N⁶-methyladenosine (m⁶A), 5-methylcytosine (m⁵C) and pseudouridine methylation during the process of exon splicing, 5'-capping and 3'-tailing [14-17]. Among 171 different RNA modifications that have been identified by the end of 2017 [18], N⁶-methyladenosine (m⁶A) modification was firstly identified and was the most abundant internal modification of RNA in eukaryotic cells [19]. In addition to mRNA, the m⁶A methylation can also be detected in transfer RNA (tRNA), ribosome (rRNA), microRNA and long non-coding RNAs (lncRNAs) [20-22]. Similar to DNA and protein modification, the regulatory effects of m⁶A methylation is a dynamic and reversible process modulated by methyltransferases called "writers" (KIAA1429, METTL3, METTL14, METTL16, RBM15, WTAP and ZC3H13), binding proteins called "readers" (ALKBH5 and FTO), and demethylases called "erasers" (HNRNPA2B1, HNRNPC, YTHDC1, YTHDC2, YTHDF1, YTHDF2 and YTHDF3) [23, 24]. Accumulative evidence has indicated that m⁶A methylation is closely related to the tumor development and m⁶A related regulators play different roles in different types of cancer [25-27]. For instance, FTO has been reported to promote cell proliferation and migration in esophageal cancer through regulation of MMP13 [28]. Highlighting these bases, we hypothesized that m⁶A RNA methylation related regulator genes play a role in EAC. However, a comprehensive analysis of the expression pattern of m⁶A RNA methylation regulators in EAC is still lacking and the prognostic value of such regulators remains to be explored.

In this study, we systematically analyzed the expression pattern of sixteen widely studied m⁶A RNA methylation regulators in EAC. Then analysis by means of bioinformatics and statistical analysis were performed in order to explore the potential value of m⁶A methylation regulators in the prognosis of patients with EAC.

Methods

Dataset acquisition

The available RNA-seq transcriptome data and clinicopathological information from 78 esophageal adenocarcinoma samples and 9 normal samples were downloaded from the TCGA data (<https://portal.gdc.cancer.gov/>).

Selection, differential expression analysis and correlation analysis of m⁶A methylation regulators

According to latest published review on m⁶A RNA Methylation in human cancer, sixteen m⁶A methylation regulators (METTL3, METTL14, WTAP, KIAA1429, RBM15, ZC3H13, METTL16, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, HNRNPC, HNRNPA2B1, FTO and ALKBH5) with available expression data in the TCGA datasets. We compared the expression level of those m⁶A related genes between tumor and normal samples by t-tests with a threshold of $p < 0.05$. We also compared the expression level of those genes in esophageal adenocarcinoma patients with different clinical characteristics. In order to demonstrate the different expression patterns of m⁶A related genes, heatmaps and violin plot were made by “pheatmap” and “vioplot” R package. After that, the co-expression correlation analysis was performed to investigate the association among those m⁶A methylation regulators by means of “corrplot” R package.

Consensus clustering analysis

To investigate the expression characteristics of m⁶A methylation regulators in esophageal adenocarcinoma, we classified the tumor samples into different groups by “ConsensusClusterPlus” R package. A principal component analysis (PCA) was conducted to verify the different gene expression patterns in different groups. Subsequently, the OS of patients in different groups was analyzed by “survival” R package. The different expression pattern of m⁶A related genes and clinicopathological features in different groups were visualized by “pheatmap” R package. Chi square test was performed to compare the distribution of gender and AJCC stage between the groups.

Construction of prognostic signatures

We performed univariate Cox regression analysis of the expression of m⁶A RNA methylation regulators. Genes with $p < 0.1$ were considered associated with esophageal adenocarcinoma patients’ survival and further selected for performing LASSO Cox regression analysis. After that, a five-gene risk signature and their corresponding coefficient were determined. By multiplying the gene’s expression value and its corresponding coefficient, the risk score for each patient was calculated as the sum of each gene’s score. Then patients were divided into high-risk and low-risk groups based on the median value of the risk score. The Kaplan-Meier method was made to analyze the OS difference between the high-risk and low-risk groups. The Receiver operating characteristic (ROC) analysis was conducted to evaluate the prediction efficiency of the five-gene risk signature. Heatmaps was utilized to visualize the different expression pattern of those five genes between high-risk and low-risk groups with “pheatmap” R package.

Statistical analysis

All data were analyzed using the R statistical package (R version 4.0.0). All assessments were considered statistically significant when the two-sided p -value is less than 0.05.

Results

The expression pattern of m⁶A RNA methylation regulators in esophageal adenocarcinoma

We extracted the sequencing data of sixteen m⁶A related genes from the TCGA esophageal adenocarcinoma cohort in order to investigate the expression pattern of m⁶A RNA methylation regulators in esophageal adenocarcinoma. Ten out of sixteen genes (HNRNPA2B1, HNRNPC, YTHDF1, METTL3, YTHDF2, RBM15, YTHDC1, WTAP, KIAA1429 and YTHDF3) represented significantly higher expression level ($p < 0.05$) in 78 EAC tissues than in 9 normal tissues. Among these ten genes, the expression of HNRNPA2B1 was in the highest level in EAC tissues, which almost doubled the expression level in normal tissues (Fig. 1b). Five genes (METTL16, ALKBH5, ZC3H13, YTHDC2 and METTL14) showed relatively high expression in tumor tissues, while FTO represented relatively high expression in normal tissues; however, no significant difference was seen ($p > 0.05$) (Fig. 1a and b).

The correlation among the m⁶A RNA methylation regulators in esophageal adenocarcinoma

The correlation analysis was performed to analyze the correlation of the m⁶A RNA methylation regulators (Fig. 2). Among the chosen sixteen regulators, none of the four genes (ALKBH5, METTL16, YTHDC2 and WTAP) had significant correlation with any other 15 regulators ($p < 0.05$). KIAA1429 had the strongest correlation with YTHDF3 ($r = 0.67$). A relatively strong correlation was observed between HNRNPA2B1 and HNRNPC ($r = 0.62$), METTL3 and KIAA1429 ($r = 0.59$). A negative correlation value was seen in METTL16 and WTAP ($r = -0.23$); however, there was no significant difference ($p > 0.05$).

Consensus clustering of m⁶A RNA methylation regulators identified three clusters of esophageal adenocarcinoma with distinct clinical outcomes

Using the ConsensusClusterPlus package, we classified the tumor samples into different groups according to the similarity of the expression of the 16 m⁶A RNA methylation regulators. As shown in Fig. 3a and b, it seemed to be the most appropriate to divide the EAC cohort into three groups, namely cluster 1, cluster 2 and cluster 3. The principal component analysis (PCA) displayed the distinction among the three cluster subgroups (Fig. 3c).

After that, we compared the overall survival (OS) of EAC patients among the three cluster subgroups in order to investigate the association between the clustering result and clinical outcome. The survival analysis demonstrated that the patients in cluster 3 had a significantly shorter OS than the patients in cluster 1 or 2 ($p < 0.05$) (Fig. 3d). Taken together, these results indicated that the clustering result was closely related to the clinical outcome in EAC.

Construction of a five-gene risk signature with distinct prognostic value

In order to investigate the prognostic role of m⁶A RNA methylation regulators in EAC, univariate Cox regression analysis was performed to identify regulators associated with OS in TCGA EAC cohort (n=88). The results exhibited that 5 out of 16 regulators were associated with OS ($p < 0.1$), among which ALKBH5 played a role as a protective gene with HR=0.952, whereas the other four (HNRNPA2B1, KIAA1429, WTAP and METTL16) were risky genes with HR>1 (Fig. 4). HR for these four genes were 1.023 (95% CI 1.011-1.035), 1.280 (95% CI 1.116-1.468), 1.178 (95% CI 1.046-1.326), and 1.345 (95% CI 0.958-1.887), respectively. Subsequently, these five genes were included in the LASSO Cox regression analysis so as to construct a better predicting model of m⁶A RNA methylation regulators on the clinical outcomes of EAC. Based on the minimum criteria, all the five genes were retained (Fig. 5a and b), thus constructing a five-gene risk signature. Coefficients generated from the LASSO Cox regression analysis were applied to calculate each patient's risk score using the following formula: risk score = (0.145) × WTAP + (0.257) × KIAA1429 + (0.108) × METTL16 + (0.019) × HNRNPA2B1 + (-0.043) × ALKBH5.

To evaluate the prognostic role of the five-gene risk signature, the EAC patients were divided into high-risk group and low-risk group according to the median risk score. After that, we compared the OS between the two groups. Compared with patients in low-risk group, those in high-risk group had a significantly shorter OS ($p < 0.05$) (Fig. 5c). The ROC curve displayed the acceptable prediction efficiency of the prognostic signature with the AUC value of 0.681 (Fig. 5d). These results, taken together, suggested that the five-gene risk signature could effectively screen out high-risk EAC patients with relatively worse clinical outcome.

Discussion

RNA epigenetics has become a hot topic in recent years. Among different modifications, the m⁶A is the most abundant RNA modification modulated by methyltransferases, demethylases and binding proteins, which regulates almost each step of mRNA metabolism and multiple biological processes[29]. Emerging data from recent studies have indicated that m⁶A RNA methylation regulators play a crucial role in most of cancers, contributing to the self-renewal of cancer stem cell, promotion of cancer cell proliferation, and so on[30]. For instance, METTL3, a methyltransferase of m⁶A, has been reported to be significantly up-regulated in human hepatocellular carcinoma (HCC) and the overexpression of METTL3 is associated with poor prognosis in patients with HCC[31]. WTAP, a methyltransferase of m⁶A, has been found to regulate migration and invasion of glioblastoma cells[32]. The m⁶A demethylase ALKBH5 has been reported to demonstrate a high expression in glioblastoma stem-like cells (GSCs) and to maintain tumorigenicity of GSCs by sustaining FOXM₁ expression and cell proliferation program[33]. Esophageal cancer is one of the most aggressive malignant tumors with two main histological subtypes, EAC and ESCC. Several studies have explored the association between m⁶A modifications and ESCC[28, 34, 35]; however, relevant data on EAC remains rare. Given the rising role of m⁶A modifications in multiple tumors, its exact role in esophageal cancer, especially EAC, needs to be further investigated.

In this present study, we proved that 10 out of 16 m⁶A RNA methylation regulators were highly expressed in EAC. This is consistent with the results from previous studies focusing on gastric carcinoma, lung

cancer, HCC and other malignant tumors[36-38]. Compared with the expression level in normal tissues, the expression of three genes, HNRNPA2B1, HNRNPC, and YTHDF1, were significantly up-regulated in EAC tissues with $p < 0.001$. There was a similar phenomenon in a study on melanoma, where YTHDF1 and HNRNPA2B1 affected modification by genes related to p53-signaling, further up-regulating their expression and facilitated their roles in inhibiting p53 to suppress tumorigenesis[39]. The m⁶A demethylase FTO represented a relatively higher expression in normal tissues than in EAC tissues in this study (Fig. 1b). Interestingly, FTO has been reported to be highly expressed in ESCC and other malignant tumors such as melanoma and gastric cancer, implying its distinct role in EAC[28, 40, 41]. In the correlation analysis, KIAA1429 was found to present the strongest correlation with YTHDF3. A previous study found that YTHDF3 could enhance the mRNA stability of Zeb1, the downstream target of KIAA1429, thus leading to cell metastasis in HCC[42]. This may contribute to the strong correlation between the two genes. Furthermore, through consensus clustering, three clusters of EAC subgroups were identified based on the expression pattern of m⁶A RNA methylation regulators. A significant difference in OS was observed among the three subgroups, which indicated that the expression pattern of m⁶A RNA methylation regulators had a close relationship to the prognosis of patients with EAC. Taken together, these results unveiled the vital role of m⁶A RNA methylation in EAC.

In our study, we performed univariate, LASSO Cox regression analyses to develop a prognostic related risk signature with 5 genes, HNRNPA2B1, KIAA1429, WTAP, METTL16 and ALKBH5, and then divided the EAC patients into high- and low-risk group. Among the 5 genes, ALKBH5 was the only protective gene for the prognosis of EAC. Nonetheless, a previous study investigated the association between ALKBH5 and prognosis in 177 patients with ESCC, and identified ALKBH5 as the first demethylase that accelerated cell cycle progression and promoted cell proliferation of ESCC cells, suggesting that the up-regulation of ALKBH5 was associated with poor prognosis in ESCC[35]. Conversely, ALKBH5 has been reported to inhibit pancreatic cancer motility by demethylating lncRNA KCN15-AS1[43], which agrees with the result in our study to some extent. Considering the opposite effect of ALKBH5 in different types of malignant tumors, more preclinical and clinical evidences are required to find out the exact role of ALKBH5 in EAC. HNRNPA2B1, KIAA1429, WTAP, METTL16 were identified as protective genes, which was consistent with the results in many studies[44-47]. At present, the prognosis assessment mainly relies on TNM stage which includes limited risk factors[2]. Establishing an effective prognosis model with molecular biology risk factors becomes an issue of increasing importance. In the present study, a five-gene risk score model was built (Risk score = $(0.145) \times \text{WTAP} + (0.257) \times \text{KIAA1429} + (0.108) \times \text{METTL16} + (0.019) \times \text{HNRNPA2B1} + (-0.043) \times \text{ALKBH5}$) and used to stratify the survival of EAC patients into high and low risk categories, which exhibited significant difference in OS ($p=0.0016$), showing a potential value for EAC treatment.

To our best knowledge, this is the first study to compare the expression pattern of m⁶A RNA methylation regulators in EAC and normal tissues. Our analyses revealed the predictive value of five identified m⁶A RNA methylation regulators in prognosis of EAC patients and built a risky signature. However, there are some limitations in our study. First, the sample size of normal tissues is limited, which may lead to some

biases during the statistical analysis. Second, further molecular biology experiments are required to investigate the mechanisms by which the five identified genes influence the progression of EAC. In addition, the prognostic efficacy of the five-gene signature constructed in the present study still needs further verification in a large-scale population of EAC patients.

Conclusions

In conclusion, our study demonstrates a dysregulated expression of m⁶A RNA methylation regulators between EAC and normal controls. 10 of 16 m⁶A RNA methylation regulators represented high expression in EAC. Furthermore, a five-gene risky signature was constructed to distinguish EAC patients with different prognosis, indicating its prognostic value as a promising molecular biomarker.

Declarations

Acknowledgements

Not applicable.

Author's contributions

Xi Yang, Zhengfei Zhu conceived and designed the present study. Liqing Zou, Tiantian Guo, Yue Zhou, Shuyan Li analyzed the data. Yue Zhou, Shuyan Li interpreted the data and wrote the manuscript.

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Availability of data and materials

All data are available from the sources listed in the manuscript—the TCGA data portal.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Pennathur A, Gibson MK, Jobe BA, Luketich JD: **Oesophageal carcinoma**. *Lancet* 2013, **381**(9864):400-412.
2. Lagergren J, Smyth E, Cunningham D, Lagergren P: **Oesophageal cancer**. *Lancet* 2017, **390**(10110):2383-2396.
3. Siegel RL, Miller KD, Jemal A: **Cancer statistics, 2019**. *CA: a cancer journal for clinicians* 2019, **69**(1).
4. Rustgi AK, El-Serag HB: **Esophageal carcinoma**. *The New England journal of medicine* 2014, **371**(26):2499-2509.
5. Saeki H, Tsutsumi S, Yukaya T, Tajiri H, Tsutsumi R, Nishimura S, Nakaji Y, Kudou K, Akiyama S, Kasagi Y *et al*: **Clinicopathological Features of Cervical Esophageal Cancer: Retrospective Analysis of 63 Consecutive Patients Who Underwent Surgical Resection**. *Ann Surg* 2017, **265**(1):130-136.
6. Mariette C, Dahan L, Mornex F, Maillard E, Thomas P-A, Meunier B, Boige V, Pezet D, Robb WB, Le Brun-Ly V *et al*: **Surgery alone versus chemoradiotherapy followed by surgery for stage I and II esophageal cancer: final analysis of randomized controlled phase III trial FFCO 9901**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2014, **32**(23):2416-2422.
7. van Hagen P, Hulshof MCCM, van Lanschot JJB, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BPL, Richel DJ, Nieuwenhuijzen GAP, Hospers GAP, Bonenkamp JJ *et al*: **Preoperative chemoradiotherapy for esophageal or junctional cancer**. *The New England journal of medicine* 2012, **366**(22):2074-2084.
8. Hur C, Miller M, Kong CY, Dowling EC, Nattinger KJ, Dunn M, Feuer EJ: **Trends in esophageal adenocarcinoma incidence and mortality**. *Cancer* 2013, **119**(6):1149-1158.
9. Coleman HG, Xie S-H, Lagergren J: **The Epidemiology of Esophageal Adenocarcinoma**. *Gastroenterology* 2018, **154**(2):390-405.
10. Gharahkhani P, Fitzgerald RC, Vaughan TL, Palles C, Gockel I, Tomlinson I, Buas MF, May A, Gerges C, Anders M *et al*: **Genome-wide association studies in oesophageal adenocarcinoma and Barrett's oesophagus: a large-scale meta-analysis**. *The Lancet Oncology* 2016, **17**(10):1363-1373.
11. Contino G, Vaughan TL, Whiteman D, Fitzgerald RC: **The Evolving Genomic Landscape of Barrett's Esophagus and Esophageal Adenocarcinoma**. *Gastroenterology* 2017, **153**(3).
12. Schröder J, Schüller V, May A, Gerges C, Anders M, Becker J, Hess T, Kreuser N, Thieme R, Ludwig KU *et al*: **Identification of loci of functional relevance to Barrett's esophagus and esophageal adenocarcinoma: Cross-referencing of expression quantitative trait loci data from disease-relevant tissues with genetic association data**. *PLoS ONE* 2019, **14**(12):e0227072.
13. Lee E, Stram DO, Ek WE, Onstad LE, MacGregor S, Gharahkhani P, Ye W, Lagergren J, Shaheen NJ, Murray LJ *et al*: **Pleiotropic analysis of cancer risk loci on esophageal adenocarcinoma risk**. *Cancer Epidemiol Biomarkers Prev* 2015, **24**(11):1801-1803.
14. 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* 2012, **485**(7397):201-206.

15. Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S, Peer E, Kol N, Ben-Haim MS, Dai Q, Di Segni A, Salmon-Divon M, Clark WC *et al*: **The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA.** *Nature* 2016, **530**(7591):441-446.
16. Spenkuch F, Motorin Y, Helm M: **Pseudouridine: still mysterious, but never a fake (uridine)!** *RNA Biol* 2014, **11**(12):1540-1554.
17. Squires JE, Patel HR, Nousch M, Sibbritt T, Humphreys DT, Parker BJ, Suter CM, Preiss T: **Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA.** *Nucleic Acids Res* 2012, **40**(11):5023-5033.
18. Boccaletto P, Machnicka MA, Purta E, Piatkowski P, Baginski B, Wirecki TK, de Crécy-Lagard V, Ross R, Limbach PA, Kotter A *et al*: **MODOMICS: a database of RNA modification pathways. 2017 update.** *Nucleic Acids Res* 2018, **46**(D1):D303-D307.
19. Yue Y, Liu J, He C: **RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation.** *Genes Dev* 2015, **29**(13):1343-1355.
20. Visvanathan A, Somasundaram K: **mRNA Traffic Control Reviewed: N6-Methyladenosine (m A) Takes the Driver's Seat.** *Bioessays* 2018, **40**(1).
21. Alarcón CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF: **N6-methyladenosine marks primary microRNAs for processing.** *Nature* 2015, **519**(7544):482-485.
22. Ma S, Chen C, Ji X, Liu J, Zhou Q, Wang G, Yuan W, Kan Q, Sun Z: **The interplay between m6A RNA methylation and noncoding RNA in cancer.** *J Hematol Oncol* 2019, **12**(1):121.
23. Yang Y, Hsu PJ, Chen Y-S, Yang Y-G: **Dynamic transcriptomic mA decoration: writers, erasers, readers and functions in RNA metabolism.** *Cell Res* 2018, **28**(6):616-624.
24. Meyer KD, Jaffrey SR: **Rethinking mA Readers, Writers, and Erasers.** *Annu Rev Cell Dev Biol* 2017, **33**:319-342.
25. Sun T, Wu R, Ming L: **The role of m6A RNA methylation in cancer.** *Biomed Pharmacother* 2019, **112**:108613.
26. Deng X, Su R, Feng X, Wei M, Chen J: **Role of N-methyladenosine modification in cancer.** *Curr Opin Genet Dev* 2018, **48**:1-7.
27. He L, Li H, Wu A, Peng Y, Shu G, Yin G: **Functions of N6-methyladenosine and its role in cancer.** *Mol Cancer* 2019, **18**(1):176.
28. Liu S, Huang M, Chen Z, Chen J, Chao Q, Yin X, Quan M: **FTO promotes cell proliferation and migration in esophageal squamous cell carcinoma through up-regulation of MMP13.** *Exp Cell Res* 2020, **389**(1):111894.
29. Liu Z-X, Li L-M, Sun H-L, Liu S-M: **Link Between m6A Modification and Cancers.** *Front Bioeng Biotechnol* 2018, **6**:89.
30. Dai D, Wang H, Zhu L, Jin H, Wang X: **N6-methyladenosine links RNA metabolism to cancer progression.** *Cell Death Dis* 2018, **9**(2):124.

31. Chen M, Wei L, Law C-T, Tsang FH-C, Shen J, Cheng CL-H, Tsang L-H, Ho DW-H, Chiu DK-C, Lee JM-F *et al*: **RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2**. *Hepatology* 2018, **67**(6):2254-2270.
32. Jin D-I, Lee SW, Han M-E, Kim H-J, Seo S-A, Hur G-Y, Jung S, Kim B-S, Oh S-O: **Expression and roles of Wilms' tumor 1-associating protein in glioblastoma**. *Cancer Sci* 2012, **103**(12):2102-2109.
33. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, Chen Y, Sulman EP, Xie K, Bögler O *et al*: **mA Demethylase ALKBH5 Maintains Tumorigenicity of Glioblastoma Stem-like Cells by Sustaining FOXM1 Expression and Cell Proliferation Program**. *Cancer Cell* 2017, **31**(4).
34. Yang N, Ying P, Tian J, Wang X, Mei S, Zou D, Peng X, Gong Y, Yang Y, Zhu Y *et al*: **Genetic variants in m6A modification genes are associated with esophageal squamous-cell carcinoma in the Chinese population**. *Carcinogenesis* 2020, **41**(6):761-768.
35. Nagaki Y, Motoyama S, Yamaguchi T, Hoshizaki M, Sato Y, Sato T, Koizumi Y, Wakita A, Kawakita Y, Imai K *et al*: **m A demethylase ALKBH5 promotes proliferation of esophageal squamous cell carcinoma associated with poor prognosis**. *Genes Cells* 2020.
36. Zhu Z, Qian Q, Zhao X, Ma L, Chen P: **N-methyladenosine ALKBH5 promotes non-small cell lung cancer progress by regulating TIMP3 stability**. *Gene* 2020, **731**:144348.
37. Chen Y, Peng C, Chen J, Chen D, Yang B, He B, Hu W, Zhang Y, Liu H, Dai L *et al*: **WTAP facilitates progression of hepatocellular carcinoma via m6A-HuR-dependent epigenetic silencing of ETS1**. *Mol Cancer* 2019, **18**(1):127.
38. Yue B, Song C, Yang L, Cui R, Cheng X, Zhang Z, Zhao G: **METTTL3-mediated N6-methyladenosine modification is critical for epithelial-mesenchymal transition and metastasis of gastric cancer**. *Mol Cancer* 2019, **18**(1):142.
39. Li T, Gu M, Deng A, Qian C: **Increased expression of YTHDF1 and HNRNPA2B1 as potent biomarkers for melanoma: a systematic analysis**. *Cancer Cell Int* 2020, **20**:239.
40. Yang S, Wei J, Cui Y-H, Park G, Shah P, Deng Y, Aplin AE, Lu Z, Hwang S, He C *et al*: **mA mRNA demethylase FTO regulates melanoma tumorigenicity and response to anti-PD-1 blockade**. *Nat Commun* 2019, **10**(1):2782.
41. Li Y, Zheng D, Wang F, Xu Y, Yu H, Zhang H: **Expression of Demethylase Genes, FTO and ALKBH1, Is Associated with Prognosis of Gastric Cancer**. *Dig Dis Sci* 2019, **64**(6):1503-1513.
42. Wang M, Yang Y, Yang J, Yang J, Han S: **circ_KIAA1429 accelerates hepatocellular carcinoma advancement through the mechanism of mA-YTHDF3-Zeb1**. *Life Sci* 2020, **257**:118082.
43. He Y, Hu H, Wang Y, Yuan H, Lu Z, Wu P, Liu D, Tian L, Yin J, Jiang K *et al*: **ALKBH5 Inhibits Pancreatic Cancer Motility by Decreasing Long Non-Coding RNA KCN15-AS1 Methylation**. *Cell Physiol Biochem* 2018, **48**(2):838-846.
44. Yang Y, Wei Q, Tang Y, Yuanyuan W, Luo Q, Zhao H, He M, Wang H, Zeng Q, Lu W *et al*: **Loss of hnRNPA2B1 inhibits malignant capability and promotes apoptosis via down-regulating Lin28B expression in ovarian cancer**. *Cancer letters* 2020, **475**:43-52.

45. Lan T, Li H, Zhang D, Xu L, Liu H, Hao X, Yan X, Liao H, Chen X, Xie K *et al*: **KIAA1429 contributes to liver cancer progression through N6-methyladenosine-dependent post-transcriptional modification of GATA3.** *Mol Cancer* 2019, **18**(1):186.
46. Tang J, Wang F, Cheng G, Si S, Sun X, Han J, Yu H, Zhang W, Lv Q, Wei J-F *et al*: **Wilms' tumor 1-associating protein promotes renal cell carcinoma proliferation by regulating CDK2 mRNA stability.** *J Exp Clin Cancer Res* 2018, **37**(1):40.
47. Liu X, Liu L, Dong Z, Li J, Yu Y, Chen X, Ren F, Cui G, Sun R: **Expression patterns and prognostic value of mA-related genes in colorectal cancer.** *Am J Transl Res* 2019, **11**(7):3972-3991.

Figures

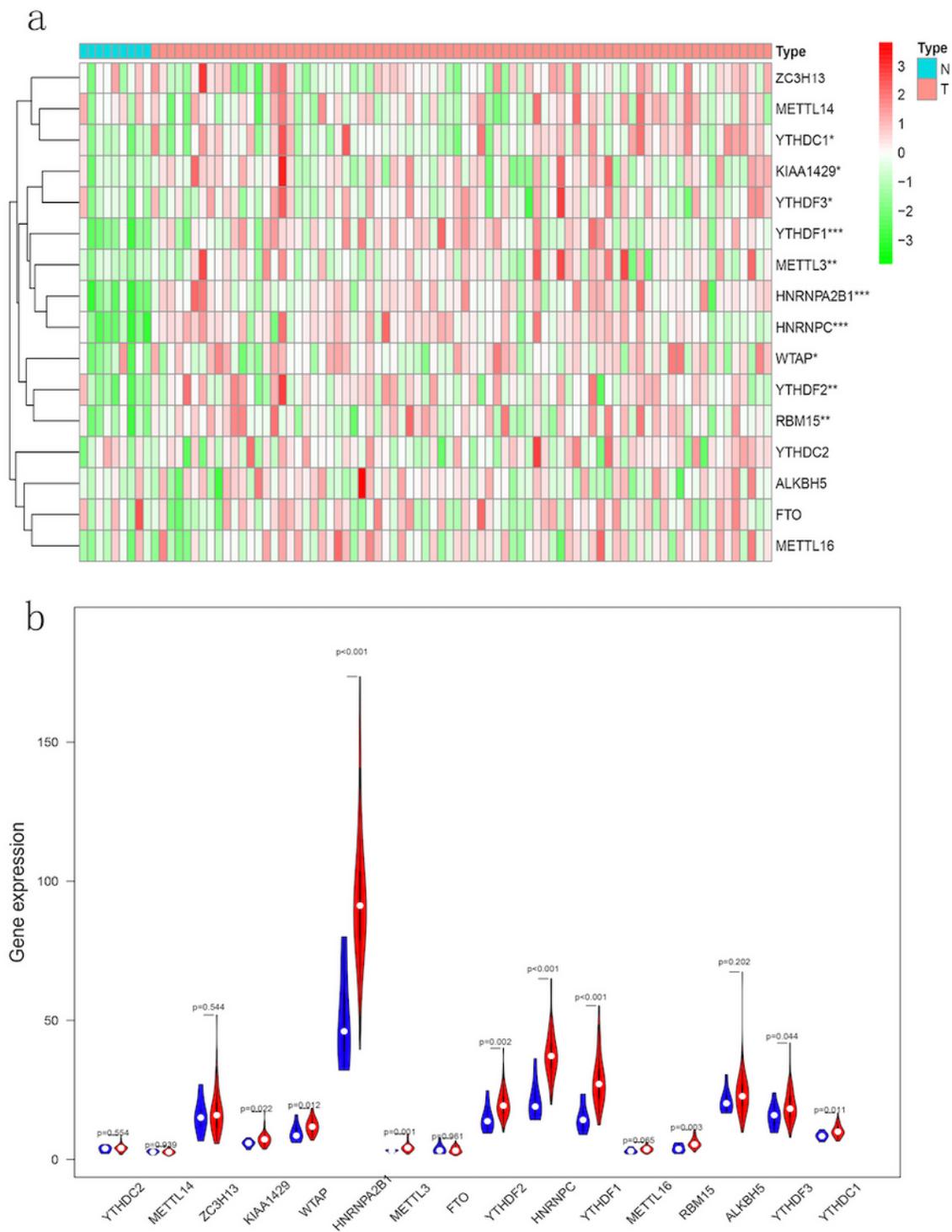


Figure 1

The expression pattern of 16 m6A RNA methylation regulators in TCGA EAC cohort. a Heatmap visualizing the expression levels of m6A RNA methylation regulators in tumor samples and normal samples. (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$) b Violin plot visualizing the differentially expressed m6A RNA methylation regulators in EAC.

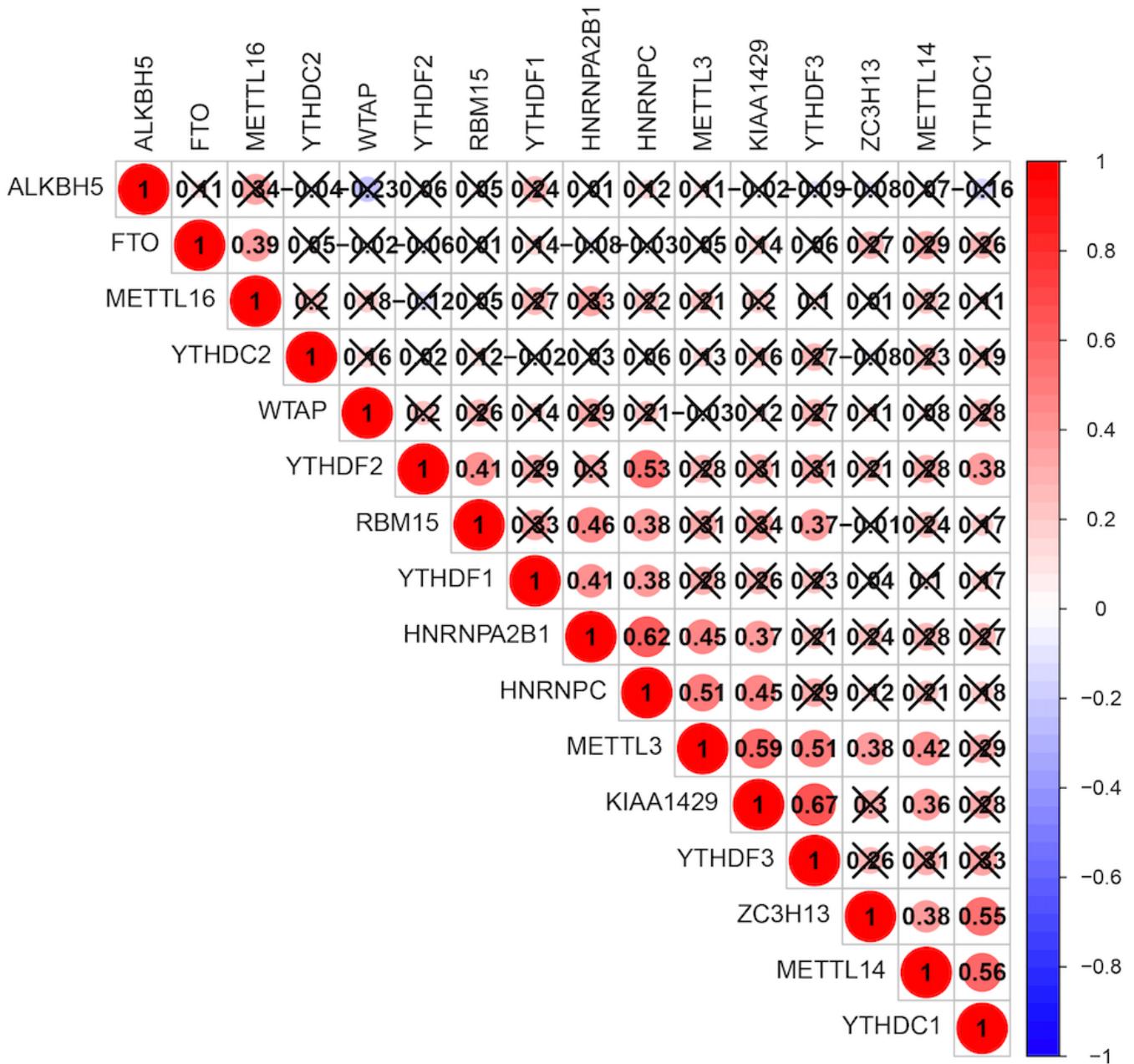


Figure 2

The Pearson correlation analysis of the 16 m6A RNA methylation regulators in TCGA EAC cohort. (“x” p>0.05, Colour bar: Spearman R)

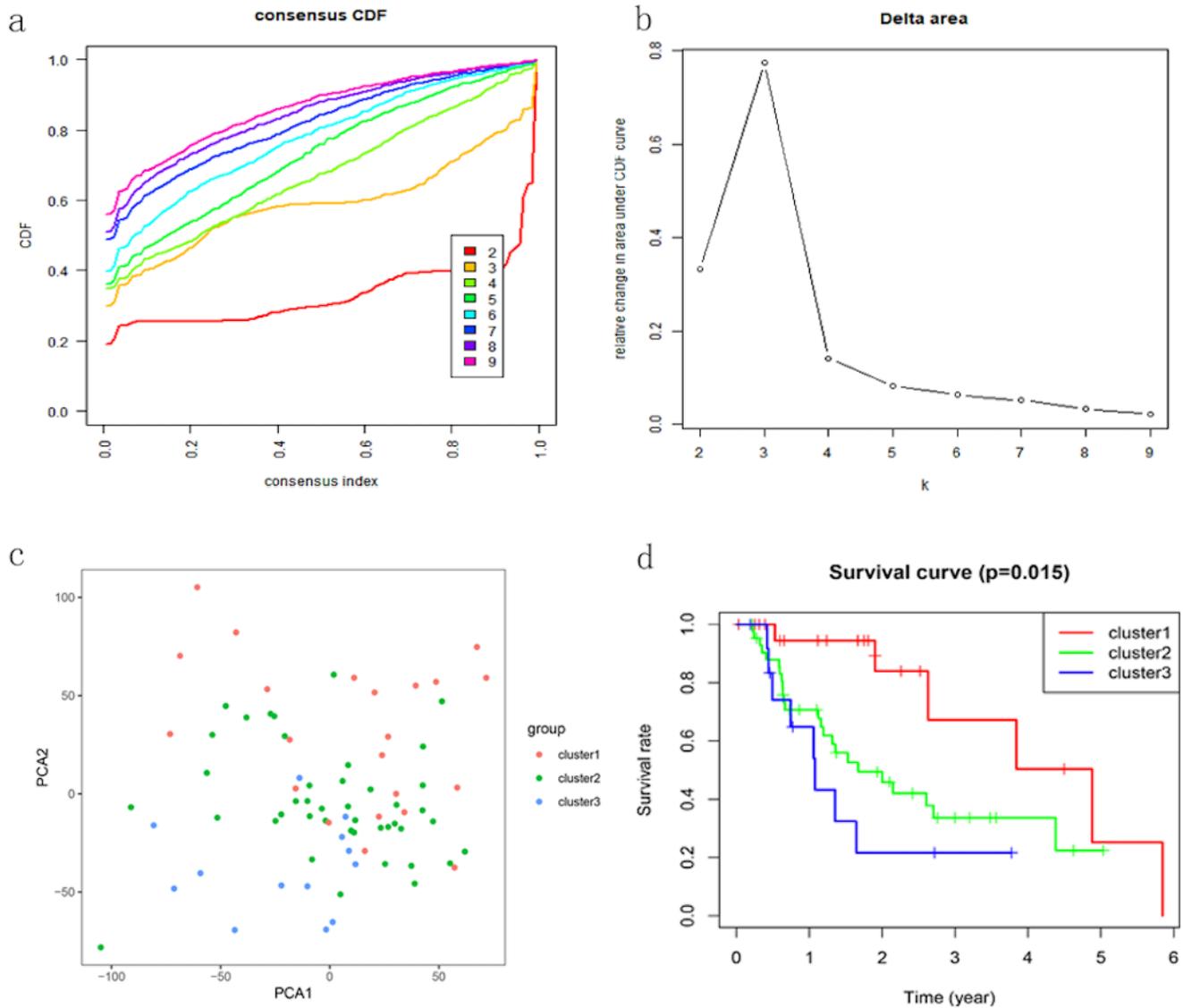


Figure 3

Differential expression pattern and clinical outcome of TCGA EAC patients in the three different clusters of 16 m6A RNA methylation regulators in cohort. a Consensus clustering cumulative distribution function (CDF) for k=2-9. b relative change in area under CDF curve for k=2-9. c Principal component analysis of the total RNA expression profile in the TCGA EAC cohort. d The survival analysis for the three clusters by Kaplan-Meier method.

	pvalue	Hazard ratio
YTHDC2	0.323	1.157(0.866–1.545)
METTL14	0.596	1.148(0.690–1.909)
ZC3H13	0.728	1.007(0.969–1.046)
KIAA1429	<0.001	1.280(1.116–1.468)
WTAP	0.007	1.178(1.047–1.326)
HNRNPA2B1	<0.001	1.023(1.011–1.035)
METTL3	0.866	0.981(0.789–1.220)
FTO	0.872	0.976(0.729–1.307)
YTHDF2	0.978	1.001(0.933–1.074)
HNRNPC	0.110	1.029(0.993–1.067)
YTHDF1	0.674	1.008(0.971–1.046)
METTL16	0.087	1.345(0.958–1.887)
RBM15	0.116	1.150(0.966–1.369)
ALKBH5	0.063	0.952(0.903–1.003)
YTHDF3	0.288	1.028(0.977–1.081)
YTHDC1	0.273	1.090(0.935–1.271)

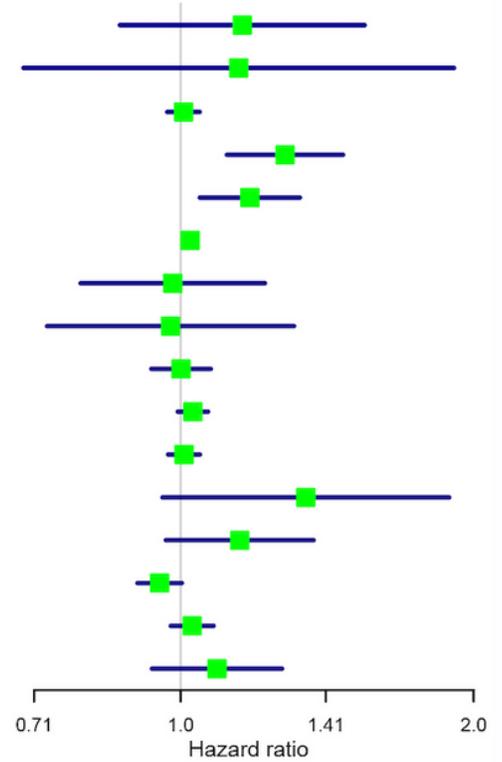


Figure 4

Univariate Cox analysis of the 16 m6A RNA methylation regulators.

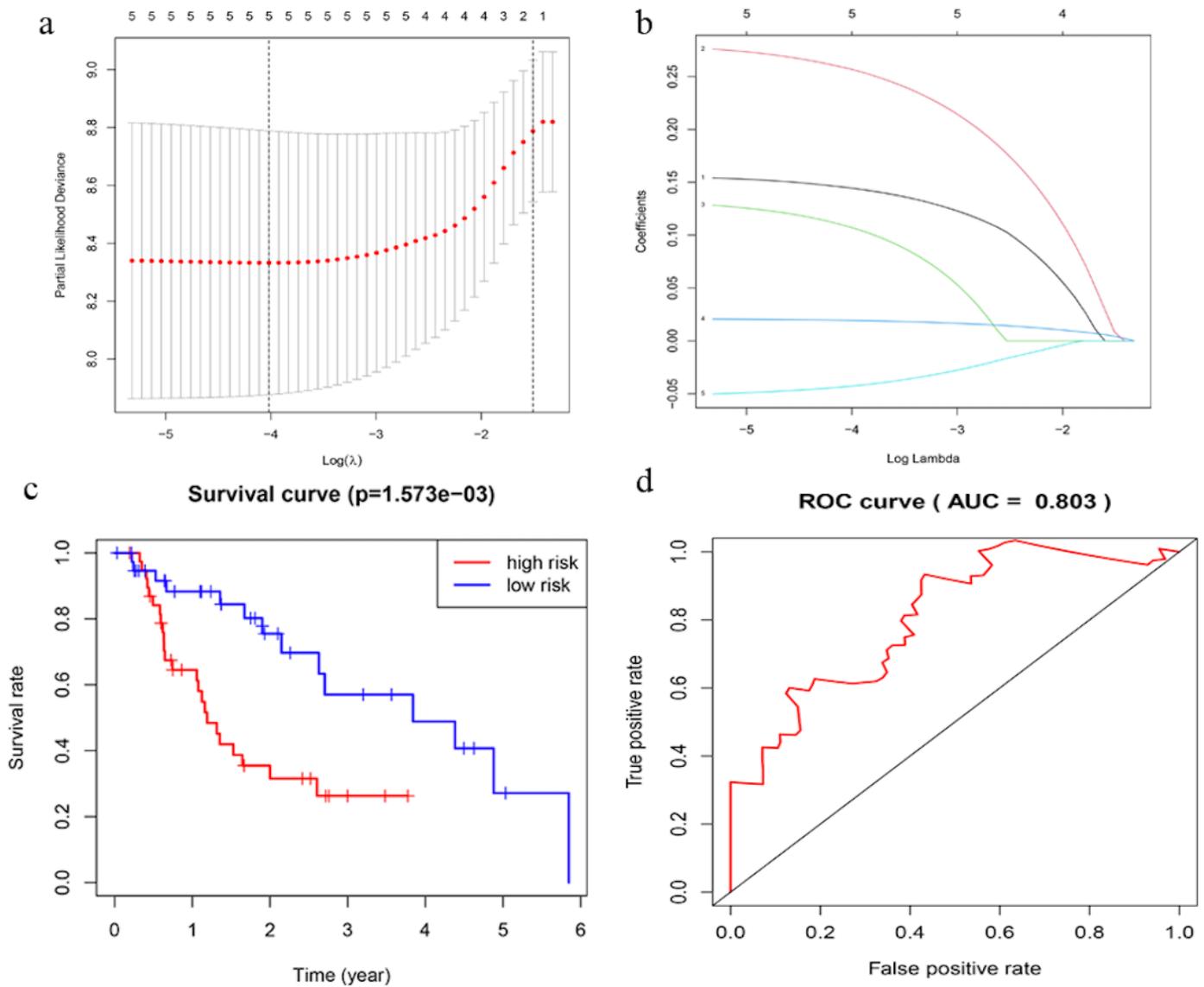


Figure 5

Construction of prognostic risk signature with five m6A RNA methylation regulators. a,b LASSO Cox analysis of the 5 m6A RNA methylation regulators. c The survival analysis of the two subgroups stratified based on the median of risk scores. d The ROC curve for evaluating the prediction efficiency of the prognostic signature.