The prognostic values of m6A RNA methylation regulators in Uveal melanoma

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Abstract

Purpose The aim of this study were to identify gene signatures and prognostic values of m6A methylation regulators in uveal melanoma (UM). Methods The RNA sequencing dataset and corresponding clinical information were downloaded from TCGA and GEO database. Based on the expression of m6A RNA methylation regulators, the patients were further clustered into different groups by applying the “ClassDiscovery” algorithm. Survival analysis was performed using log-rank tests and LASSO regression model. The association between mutations and m6A regulators was assessed by student t tests and clinical characteristics was examined by using chi-square test. Result Totally, we identified two molecular subtypes of UM (C1/2) by applying consensus clustering to m6A RNA methylation regulators. Contrasted to the C1 subtype, the C2 subtype associates with a better prognosis, longer survival time and lower percentage of Monosomy 3. The malignant hallmarks of mTORC1 signaling, oxidative phosphorylation, interferon-a response and apoptosis signaling are also significantly enriched in the C1 subtype. Moreover, a 2-m6A regulators signature was screened out by LASSO method, which can robustly predict UM patients survival. Conclusions m6A RNA methylation regulators take a crucial role in the potential malignant progression and prognostic value of UM and might be regarded as a new promising biomarker for UM prognosis and treatment strategy development.

Introduction

Uveal melanoma is the most common type of malignant tumor of the adult eye, with 50% UM patients will eventually die of their disease.[1, 2] The prognosis for patients with UM remains poor, though there are certain advances in diagnosis and treatment of UM.[3, 4] Therefore, is important to explore the molecular mechanism underlying the the survival events of UM and identify new prognostic factors and therapeutic targets.

It is well known that both DNA and histone proteins control gene expression by dynamic and reversible chemical modifications. RNA modification, like DNA and protein modification, is dynamically regulated by methyl-transferases.[5] The most prevalent RNA methylation is N6-methyladenosine (m6A), which exists in about 25% of transcripts at the genome-wide level and firstly discovered in the 1970s. m6A RNA methylation regulators modifies translocation, stability, RNA splicing and translation.[6] m6A is dynamically regulated by the ‘writers’ (RNA methyltransferases) , such as METTL14, METTL3 and WTAP, is removed by ‘erasers’ (the demethylases), such as ALKBH5 and FTO, and ‘readers’ (the binding proteins), such as YTHDF2 and YTHDF1.[7] RNA methyltransferases, the demethylases, and the binding proteins are often upregulated in a variety of human cancer types, increasing the expression of Oncogenes and Oncoproteins, increasing the proliferation, progression, and metastasis of cancer cells.[8]

m6A modification not only plays a vital role in the pathogenesis of a variety of human disease including obesity, neuronal disorders and immunological disease, but also has been shown to take apart in tumor initiation and promote progression of cancer and recurrence.[9] In addition, growing evidence suggests that gene mutation and abnormal expression of m6A regulators are intimately associated with

the malignant progress of various cancers.[10] Although it is recognized that RNA methylation plays a critical role in different types of cancers, little is known about the relationship between m6A-related genes and UM.

Hence, in this study, we systematically evaluated the expression of m6A regulators in 80 UM samples from The Cancer Genome Atlas (TCGA) dataset as well as the association between the genetic alterations and clinical characteristics and validation in 28 UM samples from Gene Expression Omnibus (GEO) dataset. We found that the expression of m6A regulators plays critical roles in the malignant process of UM, and provide a potential biomarker with two identified m6A regulators as prognostic signatures.

Methods

2.1 Data processing

The RNA sequencing dataset and corresponding clinical information of 80 uveal melanoma patients were downloaded from TCGA (http://cancergenome.nih.gov/) and the dataset of 28 uveal melanoma patients obtained from GEO (https://www.ncbi.nlm.nih.gov/geo). To investigate the relationship between these m6A regulatorys and mutation, CNV, mutation expression data of 80 uveal melanoma patients were retrieved from TCGA.

2.2 Bioinformatic analysis of m6A regulators

We first selected thirteen m6A RNA methylation regulators from previously published articles, and then we restricted the RNA expression data of thirteen regulators in the TCGA and GEO datasets. Based on the expression of m6A regulators, we clustered the uveal melanoma patients into different subtypes by applying the "ClassDiscovery" algorithm in R package. Principal component analysis was performed to determine whether these m6A regulators could definitely divided into different uveal melanoma subtypes. To evaluate the interactive relationships among m6A regulators, correlation analyses of m6A regulators was applied and we also mapped the m6A regulators to STRING database (http://stringdb.org). GO and Hallmark gene set enrichment analysis were conducted by using the "clusterProfiler" package to assesses the functions associated with different subtypes .

To determine the prognostic value of m6A regulators, LASSO was used to construct prognostic markers with m6A regulators. According to the expression level of each sample, LASSO determines the qualified m6A regulators for the risk system and generates corresponding coefficients for each of m6A regulators. Base on the risk model, the risk score of each sample was evaluated. The patients were divided into high-risk group and low-risk group by using the cutoff risk score. The survival curves of Kaplan-Meier were drawn and the differences among groups were compared by log-rank tests.

2.3 Statistical analysis

All statistical analyses were conducted using R(v.3.5.2). In order to compare with two groups, the statistical significance of normal distribution variables was estimated by Student t tests. The correlation coefficient was calculated by Spearman and distance correlation analyses. The association between m6A regulatory genes and clinicopathological characteristics were analyzed with chi-square test. The Kaplan–Meier survival analysis was applied to compare the overall survival of the patients in the different groups or in the low- and high-risk groups. $P<0.05$ was regarded as statistically significant in all statistical tests and estimated values were regarded as significant at a CI of 95%.

Results

3.1 Subgroup analysis of m6A regulators

As a result, a total of thirteen m6A RNA methylation regulators changed mRNAs expression values and clinicopathological characteristics of \UM were obtained from TCGA and GEO. Based on “ClassDiscovery” algorithm, 80 UM patients from TCGA and 28 UM patients from GEO can be identified two clusters of groups, respectively. (Figure 1A and B). Then, we contrasted the clinical features of these two subgroups, namely, C1 and C2. The subgroups analysis of clinical characteristics showed that only time and Chromosome.3.status have a signifcant difference (Table 1). The others clinical characteristics like stage, gender and age have no statistical significance. To find out the potential correlation of overall survival with C1 and C2. Kaplan-Meier survival analysis was performed and the curves showed that overall survival of samples in C2 is longer than the samples in the C1 group (Figure 1C ,D). Then, expression levels of thirteen m6A RNA methylation regulators in UM patients with different C1/2 groups were shown in Figure 1E ,F.

3.2 Gene mutation and m6A regulators

Then, we assessed the relationship between gene mutation and m6A regulatorys. we firstly evaluated the gene mutation in 80 UM samples at TCGA database and found 20 highly variant mutated genes. (Figure 2A).The heatmap of m6A regulatorys expression and 20 highly variant mutated genes indicated that SF3B1, CYSLTR2 and ADAMTSL1 were the most significantly regulated the expression of m6A regulatorys. (Figure 2B). Kaplan-Meier analysis of these 3 mutant genes showed that only SF3B1 have a significant difference with overall survival. And then studied the relationship between SF3B1, CYSLTR2 and ADAMTSL1 mutation status and expression levels of each m6A RNA methylation regulator in TCGA database, respectively. The results showed that there are significant differences between with mutant-SF3B1 and wildtype-SF3B1 for the expression levels of ALKBH5, FTO, WTAP, YTHDF1, YTHDF2, YTHDC2 and KIAA1429 respectively(Figure 2C).Compared with mutant-CYSLTR2 and wildtype-CYSLTR2, the

expression levels of ALKBH5, FTO, METTL14, WTAP, YTHDF2, YTHDC2, ZC3H13, KIAA1429 and RBM15 are significantly different (Figure 2D). The subgroup analysis of mutant-ADAMTSL1 and wildtype-ADAMTSL1 also showed that m6A regulators of ALKBH5, METTL14, WTAP, YTHDF2, YTHDC1, YTHDC2, KIAA1429 and RBM15 are also significantly different (Figure 2E).

3.4 Clustered molecular subtype of uveal melanoma

The above results revealed that the clustered molecular subtype was intimately related to the prognosis of uveal melanoma. For better understanding of the interrelations among the thirteen m6A regulators, we also analyzed the interrelation (Figure. 3A) and correlation (Figure. 3C) among these regulators. ALKBH5 seems to be the hub gene of the 'Eraser', and correlated or co-expressed with METTL3, WTAP, YTHDF2, METTL14, YTHDF1, YTHDC1, YTHDC2, RBM15, KIAA1429. The correlation analysis of these regulators showed that ALKBH5 was also significantly negatively correlated with METTL3, RBM15, KIAA1429, YTHDC1, YTHDC2 and HNRNPC. Principal components analysis showed that C1 samples and C2 samples in TCGA datasets could be well differentiated based on the expression of m6A regulators. (Figure. 3B). To investigate biologic pathways shared by the different C1/2 subtype, we performed GSEA analysis. According to the following criteria: p value<0.05 and | NES | ≥1. 49 BP terms were differentially enriched in C2 expression phenotype. The top 5 BP terms indicated that pathways are commonly enriched T cell mediated pathways , including positive regulation of T cell mediated cytotoxicity, antigen processing and presentation of endogenous antigen, regulation of T cell mediated cytotoxicity, positive regulation of T cell mediated immunity and regulation of T cell mediated immunity. (Figure 3D). What's more, The GSEA analysis of malignant hallmarks of tumors showed that 9 terms including mTORC1 signaling, oxidative phosphorylation, interferon-a response and apoptosis signaling were significantly associated with the C1 subgroup expression phenotype. (Figure 3E).

3.5 Identification and confirmation of m6A regulators signature

For better predict the clinical and pathologic outcomes of UM with m6A regulators. Then we used LASSO modelling to evaluate associations between generally changed thirteen m6A regulators and overall survival in TCGA dataset. Totally, a 2-m6A regulators signature was screened out of thirteen m6A regulators to build the risk signature based on the minimum criteria.(Figure 4A, B)The risk score formula for OS was calculated as follows: risk score = 0.02 × (expression value of ALKBH5) + -0.01 × (expression value of YTHDC2). The risk system reckons a risk score for each patient. Applying the cut-off value (0.664) of the risk scores. 80 UM patients were divided into high-risk and low-risk groups (Figure 4C). The life status and 2 m6A regulators expression value of each patient are showed in Figure 4C as well. Kaplan-Meier curve indicated that there is a significant difference between high-risk and low-risk group with log-rank test of p=0.0052 (Figure 4D). To verify the predictive ability of the 2 m6A regulators, validation analysis was performed in GEO dataset. The curve of Kaplan-Meier revealed that the low-risk

group have a significantly better survival than the patients in high-risk group with log-rank p=0.047. The subgroups analysis of clinical characteristics between low- and high- risk groups showed that only time in TCGA and GEO have a significant difference (Table 2).Combination group analysis of 2 m6A regulators (ALKBH5 and YTHDC2) signature showed that patients with high expression of ALKBH5 and Low expression of YTHDC2 markers have the worse overall survival (p<0.0429) of all four groups. (Figure 5)

Discussion

The growing genome-wide studies demonstrated that most of the human genome is transcribed, which exists a complex network of large and small RNA molecules in human cells. However, only 1 to 2 % of the transcripts have the capacity for protein translation.[11-13] In fact, post-transcriptional regulation at the RNA level through cis-and trans-mechanisms is essential to control the gene expression procedures that determine cellular function and cell fate.[14] To date, more than 150 chemical modifications have been described for RNA. Among them, m6A is the most prevalent posttranscriptional modification of eukaryotic mRNAs and long noncoding RNAs. Recent studies have indicated that m6A regulators have been shown to play important regulatory roles in diverse biological processes in human cancer.[15] However, despite the increasing evidence for their implication in cancers, little is known about the potential role of m6A regulators in UM prognosis.

In this study, we demonstrated that the expression of m6A regulators is also intimately related to the prognosis and malignancy of UM. Based on the expression of m6A regulators, we identified two UM subgroups, namely C1/2 molecular subtype, by applying consensus clustered method. The C1/2 molecular subtype not only affected the clinical and prognosis features but also closely associated with biological signals and malignant hallmarks of UM. Survival analysis showed that C1 subtype have worse overall survival than C2 subtype. GSEA showed that C2 expression phenotype is mostly enriched in positive regulation of T cell mediated pathways and C1 expression phenotype is mostly enriched in malignant hallmarks of mTORC1 signaling, oxidative phosphorylation, interferon-a response and apoptosis signaling. In fact, T cells like active CD 4 + and CD 8 + cells have antitumor immunity and therapy functions.[16] As to C1 molecular subtype, lots of malignant hallmark of pathways were enriched. Thus, it is reasonable to believe that clustered molecular subtypes C1/2 are closely correlated to the malignancy and prognosis of UM. Moreover, extensive researches also suggests uveal melanoma with monosomy 3 is associated with a dramatically poor prognosis.[17-19]The same result also be found in our study. The subgroups analysis of Chromosome.3.status showed that the percentage of Monosomy 3 in C1 molecular subtype is 76.9% and is much higher than 26.7% in C2 molecular subtype (P=0.023). (Table 1).The different analysis of m6A regulators between C1/2 molecular subtype showed that “eraser” like ALKBH5, “writer” like METTL3, METTL14, and WTAP and “readers”, like YTHDF1 and YTHDF2 have a significant difference. (Figure 1E ,F). The different expression of these m6A regulators may eventually lead to the various of survival outcomes.[20] Among the m6A regulators, previous studies indicated that the eraser ALKBH5 can induce breast cancer stem cell and glioblastoma stem-like cell proliferation and tumor initiation;[21] the writers METTL3 and METTL14 were reported to enhance

glioblastoma growth and suppress Liver cancer metastasis;[22-24] the reader YTHDF1 and YTHDF2 induce cancer cell proliferation in Colon cancer and lung oncogenic effects;[25, 26] These findings manifested that high or low expression of specific m6A regulators are related to misregulated RNAs in tumors, and the same m6A regulators may have different functions in various tumors.[27, 28]

By analyzing the mutation annotation files of the TCGA-UVM cohort, we identified 20 highly variant mutated genes and 3 of them (SF3B1, CYSLTR2 and ADAMTSL1) are the most significantly influence the expression of m6A regulatorys. SF3B1 (splicing factor 3subunit B1) mutations can be generally found in 10% to 21% of cases of UM. Previous researches have shown that mutations in SF3B1 have been associated with favorable prognostic features in UM patients.[29] Survival analysis also indicated that SF3B1-mutated UM had a better survival than the SF3B1 wild-type. In our research, the result showed that the mutation of SF3B1 will generally significantly down-regulated the expression of m6A regulatorys, including “eraser” such as ALKBH5 and FTO; “writer” such as WTAP and KIAA1429; “reader” such as YTHDF1, YTHDF2 and YTHDC2. (Figure2 C) As regards the mutation of CYSLTR2 and ADAMTSL1, the results revealed that “writer” and “reader” will generally down-regulated, However, the “eraser” such as ALKBH5 and FTO are up-regulated. Although There are no significant results for the survival analysis of CYSLTR2 and ADAMTSL1, the mutated CYSLTR2 and ADAMTSL1 have a poor tendency of overall survival. Therefore, it easily refer to think that the mutant of SF3B1 may lead to down-regulate the expression of “eraser” such as ALKBH5 and FTO and finally result in a better survival in UM. Then the mutant of CYSLTR2 and ADAMTSL1 can cause a poor survival in UM by up-regulating the expression of “eraser”. Massive studies also reported that either CYSLTR2 or ADAMTSL1 mutations occur early in cancer progression and are important initiating events in UM.[30]

What’s more, we also distinguished a prognostic risk signature with two identified m6A regulators (ALKBH5 and YTHDC2), which divided the overall survival of UM into high- and low-risk subgroups. Kaplan-Meier analyses indicated that high-risk subgroups with a poor survival. Stratified analysis of clinical characteristics between low- and high- risk groups also revealed that lots of risk factors like Stage, TNM and Chromosome.3.status are take higher percentage in high- risk groups.(Table 2) Furthermore, combination group analysis of ALKBH5 and YTHDC2 revealed that low expression of ALKBH5 combined with high expression of YTHDC2 have a better overall survival among four groups. (Figure 5) Corelation analysis also indicated that ALKBH5 was significantly negative correlated with YTHDC2.(Figure 3C) In sum , ALKBH5 and YTHDC2 might be regarded as a new promising biomarkers for UM and down-regulated the expression of ALKBH5 will be a new therapic target and can get a better prognosis. For example, in human breast cancer cells, knockdown ALKBH5 contributed to significantly decrease the number of cancer stem cells and the opportunity of tumorigenesis. In addition, the high expression of ALKBH5 in glioblastoma can lead to stem-like cell proliferation and tumorigenesis.[31]

In summary, we firstly comprehensively evaluated the expression, potential function, and prognostic value of m6A regulatory genes in UM from TCGA dataset and have validated in GEO dataset, which should be helpful for UM early diagnosis and might be regarded as a new promising biomarker for UM prognosis and treatment.

Abbreviations

TCGA—The Cancer Genome Atlas database

GEO —– Gene Expression Omniniub

UM— Uveal melanoma

KEGG—Kyoto encyclopedia of genes and genomes pathway

GO—gene ontology

LASSO— the least absolute shrinkage and selection operator

OS —overall survival

Declarations

Declarations

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Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request

Authors' contributions

JT designed the study. QW and JQL wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Conflict of interests

All authors declare that they have no competing interests.

References

1. Stang A, Parkin DM, Ferlay J, Jockel KH: **International uveal melanoma incidence trends in view of a decreasing proportion of morphological verification**. *International journal of cancer* 2005, **114**(1):114-123.
2. Singh AD, Turell ME, Topham AK: **Uveal melanoma: trends in incidence, treatment, and survival**. *Ophthalmology* 2011, **118**(9):1881-1885.
3. Rietschel P, Panageas KS, Hanlon C, Patel A, Abramson DH, Chapman PB: **Variates of survival in metastatic uveal melanoma**. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005, **23**(31):8076-8080.
4. Bol KF, Mensink HW, Aarntzen EH, Schreibelt G, Keunen JE, Coulie PG, de Klein A, Punt CJ, Paridaens D, Figdor CG *et al*: **Long overall survival after dendritic cell vaccination in metastatic uveal melanoma patients**. *American journal of ophthalmology* 2014, **158**(5):939-947.
5. Yu J, Chen M, Huang H, Zhu J, Song H, Zhu J, Park J, Ji S-J: **Dynamic m⁶A modification regulates local translation of mRNA in axons(Article)**. *Nucleic Acids Research* 2018(No.3):1412-1423.
6. A EE-B, O. B: **m6A modification and implications for microRNAs**. *Microrna* 2017(No.2):97-101.
7. Vu LP, Cheng Y, Kharas MG: **The Biology of m6A RNA Methylation in Normal and Malignant Hematopoiesis**. *Cancer discovery* 2019(No.1):25-33.
8. Chai RC, Wu F, Wang QX, Zhang S, Zhang KN, Liu YQ, Zhao Z, Jiang T, Wang YZ, Kang CS: **m(6)A RNA methylation regulators contribute to malignant progression and have clinical prognostic impact in gliomas**. *Aging* 2019, **11**(4):1204-1225.
9. Lan Q, Liu PY, Haase J, Bell JL, Huttelmaier S, Liu T: **The Critical Role of RNA m(6)A Methylation in Cancer**. *Cancer research* 2019, **79**(7):1285-1292.
10. Sun T, Wu R, Ming L: **The role of m6A RNA methylation in cancer**. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2019, **112**:108613.
11. Joshi P, Kooshki M, Aldrich W, Varghai D, Zborowski M, Singh AD, Triozzi PL: **Expression of natural killer cell regulatory microRNA by uveal melanoma cancer stem cells**. *Clinical & experimental metastasis* 2016, **33**(8):829-838.
12. Larsen AC, Holst L, Kaczkowski B, Andersen MT, Manfe V, Siersma VD, Kolko M, Kiilgaard JF, Winther O, Prause JU *et al*: **MicroRNA expression analysis and Multiplex ligation-dependent probe amplification in metastatic and non-metastatic uveal melanoma**. *Acta ophthalmologica* 2014, **92**(6):541-549.
13. Starkey MP, Compston-Garnett L, Malho P, Dunn K, Dubielzig R: **Metastasis-associated microRNA expression in canine uveal melanoma**. *Veterinary and comparative oncology* 2018, **16**(1):81-89.

14. Kwok CT, Marshall AD, Rasko JE, Wong JJ: **Genetic alterations of m(6)A regulators predict poorer survival in acute myeloid leukemia**. *Journal of hematology & oncology* 2017, **10**(1):39.
15. Yang Y, Hsu PJ, Chen YS, Yang YG: **Dynamic transcriptomic m(6)A decoration: writers, erasers, readers and functions in RNA metabolism**. *Cell research* 2018, **28**(6):616-624.
16. M1 K, B2. S-T: **Immunotherapy of Uveal Melanoma: Vaccination Against Cancer**. *Methods Mol Biol* 2017:273-278.
17. Aziz H, Salvi S, Singh N, Dar S, Hayden B, Singh A: **Uveal Melanoma Regression After Brachytherapy: Relationship with Monosomy Chromosome 3**. *INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE* 2015(No.7).
18. M.D.1 FH, M.D.2 CAM, M.D.2 ADS, M.D.1 JPR, Charles V. Biscotti M.D.1: **Uveal melanoma: An analysis of cellular features and comparison to monosomy 3 status**. *DIAGNOSTIC CYTOPATHOLOGY* 2016(No.5):377-383.
19. Tura A, Merz H, Reinsberg M, Lüke M, Jager MJ, Grisanti S, Lüke J: **Analysis of monosomy-3 in immunomagnetically-isolated circulating melanoma cells in uveal melanoma patients**. *PIGMENT CELL & MELANOMA RESEARCH* 2016(No.5):583-589.
20. Zhou J, Wang J, Hong B, Ma K, Xie H, Li L, Zhang K, Zhou B, Cai L, Gong K: **Gene signatures and prognostic values of m6A regulators in clear cell renal cell carcinoma - a retrospective study using TCGA database**. *Aging* 2019.
21. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, Chen Y, Sulman EP, Xie K, Bogler O *et al*: **m(6)A Demethylase ALKBH5 Maintains Tumorigenicity of Glioblastoma Stem-like Cells by Sustaining FOXM1 Expression and Cell Proliferation Program**. *Cancer Cell* 2017, **31**(4):591-606 e596.
22. Liu X, Si W, Liu X, He L, Ren J, Yang Z, Yang J, Li W, Liu S, Pei F *et al*: **JMJD6 promotes melanoma carcinogenesis through regulation of the alternative splicing of PAK1, a key MAPK signaling component**. *Molecular cancer* 2017, **16**(1):175.
23. Lin S, Choe J, Du P, Triboulet R, Gregory RI: **The m⁶A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells(Article)**. *Molecular Cell* 2016(No.3):335-345.
24. Lin S, 2, Choe J, 2, Du P, 2, Triboulet R, 2, Gregory RI, 2,3,4: **The m6A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells**. *MOLECULAR CELL* 2016(No.3):335-345.
25. Chen M, Wei L, Law CT, Tsang FHC, Shen J, Cheng CLH, Tsang LH, Ho DWH, Chiu DKC, Lee JMF *et al*: **RNA N6-methyladenosine methyltransferase-like 3 promotes liver cancer progression through YTHDF2-dependent posttranscriptional silencing of SOCS2**. *Hepatology* 2018(No.6):2254-2270.
26. Tanabe A, Tanikawa K, Tsunetomi M, Takai K, Ikeda H, Konno J, Torigoe T, Maeda H, Kutomi G, Okita K *et al*: **RNA helicase YTHDC2 promotes cancer metastasis via the enhancement of the efficiency by which HIF-1α mRNA is translated(Article)**. *Cancer Letters* 2016(No.1):34-42.
27. Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, Huang H, Nachtergaele S, Dong L, Hu C *et al*: **FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N⁶-Methyladenosine RNA Demethylase(Article)**. *Cancer Cell* 2017(No.1):127-141.

28. Nishizawa Y, Konno M, Asai A, Koseki J, Kawamoto K, Miyoshi N, Takahashi H, Nishida N, Haraguchi N, Sakai D *et al*: **Oncogene c-Myc promotes epitranscriptome m⁶A reader YTHDF1 expression in colorectal cancer(Article)**. *Oncotarget* 2018(No.7):7476-7486.
29. Yavuzyigitoglu S, MD,Koopmans, Anna E., MD, PhD,Verdijk, Robert M., MD, PhD,Vaarwater, Jolanda, BSc,Eussen, Bert, BSc,van Bodegom, Alice, BSc,Paridaens, Dion, MD, PhD,Kili, Emine, MD, PhD,de Klein, Annelies, PhD: **Uveal Melanomas with SF3B1 Mutations**. *Ophthalmology* 2016(No.5):1118-1128.
30. Slater K, Hoo PS, Buckley AM: **Evaluation of oncogenic cysteinyl leukotriene receptor 2 as a therapeutic target for uveal melanoma**. *CANCER AND METASTASIS REVIEWS* 2018(No.2-3):335-345.
31. Zhang C, Zhi WI, Lu H, Samanta D, Chen I, Gabrielson E, Semenza GL: **Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells**. *Oncotarget* 2016, **7**(40):64527-64542.

Tables

Table 1:Clinicopathological characteristics of C1/2 molecular subtypes.

TCGA group	C1	C2	p
n	61	19	
vital_status = DEAD (%)	20(32.8)	3 (15.8)	0.255
race = white (%)	44 (100.0)	11 (100.0)	NA
age (mean (SD))	64.08 (13.46)	62.82 (17.28)	0.739
gender = MALE (%)	36 (59.0)	9 (47.4)	0.529
stage (%)			0.238
	1 (1.6)	0 (0.0)	
Stage II	26 (42.6)	13 (68.4)	
Stage III	31 (50.8)	5 (26.3)	
Stage IV	3 (4.9)	1 (5.3)	
m (%)			0.702
m0	39 (66.1)	12 (63.2)	
m1	1 (1.7)	1 (5.3)	
m1b	2 (3.4)	0 (0.0)	
mx	17 (28.8)	6 (31.6)	
n = nx (%)	21 (34.4)	6 (33.3)	1
t (%)			0.747
t2a	7 (11.5)	5 (26.3)	
t2b	2 (3.3)	0 (0.0)	
t3	1 (1.6)	0 (0.0)	
t3a	17 (27.9)	8 (42.1)	
t3b	4 (6.6)	1 (5.3)	
t3c	1 (1.6)	0 (0.0)	
t4a	17 (27.9)	3 (15.8)	
t4b	7 (11.5)	2 (10.5)	
t4c	2 (3.3)	0 (0.0)	
t4d	2 (3.3)	0 (0.0)	
t4e	1 (1.6)	0 (0.0)	
histological_type (%)			0.888
Epithelioid Cell	10 (16.4)	3 (15.8)	
Spindle Cell	22 (36.1)	8 (42.1)	
Spindle Cell|Epithelioid Cell	29 (47.5)	8 (42.1)	
age_group = younger (%)	31 (50.8)	9 (47.4)	1
time (mean (SD))	13.11 (10.41)	21.92 (22.29)	0.02
AGE = >60 (%)	37 (60.7)	10 (52.6)	0.724
GEO group	C1	C2	
n	13	15	
time (mean (SD))	57.31 (46.75)	89.67 (40.50)	0.05
age = Age>=60 (%)	7 (53.8)	10 (66.7)	0.761
age_group = younger (%)	6 (46.2)	5 (33.3)	0.761
vital_status = DEAD (%)	11 (84.6)	5 (33.3)	0.019
Chromosome.3.status = Monosomy 3 (%)	10(76.9)	4 (26.7)	0.023

Table 2: The subgroups analysis of clinical characteristics between low- and high- risk groups.

TCGA group	High-risk	Low-risk	p
n	52	28	
vital_status = DEAD (%)	10 (19.2)	3 (10.7)	0.505
race = white (%)	37 (100.0)	18 (100.0)	NA
age (mean (SD))	63.64 (13.30)	64.05 (16.37)	0.903
gender = MALE (%)	29 (55.8)	16 (57.1)	1
stage (%)			0.34
	1 (1.9)	0 (0.0)	
Stage II	23 (44.2)	16 (57.1)	
Stage III	24 (46.2)	12 (42.9)	
Stage IV	4 (7.7)	0 (0.0)	
m (%)			0.517
m0	32 (62.7)	19 (70.4)	
m1	2 (3.9)	0 (0.0)	
m1b	2 (3.9)	0 (0.0)	
mx	15 (29.4)	8 (29.6)	
n = nx (%)	18 (35.3)	9 (32.1)	0.972
t (%)			0.661
t2a	6 (11.5)	6 (21.4)	
t2b	2 (3.8)	0 (0.0)	
t3	1 (1.9)	0 (0.0)	
t3a	15 (28.8)	10 (35.7)	
t3b	2 (3.8)	3 (10.7)	
t3c	1 (1.9)	0 (0.0)	
t4a	14 (26.9)	6 (21.4)	
t4b	7 (13.5)	2 (7.1)	
t4c	2 (3.8)	0 (0.0)	
t4d	1 (1.9)	1 (3.6)	
t4e	1 (1.9)	0 (0.0)	
histological_type (%)			0.085
Epithelioid Cell	9 (17.3)	4 (14.3)	
Spindle Cell	15 (28.8)	15 (53.6)	
Spindle Cell|Epithelioid Cell	28 (53.8)	9 (32.1)	
age_group = younger (%)	25 (48.1)	15 (53.6)	0.815
time (mean (SD))	12.86 (10.66)	19.55 (19.21)	0.048
AGE = >60 (%)	32 (61.5)	15 (53.6)	0.651
GEO group	High-risk	Low-risk	p
n	18	10	
time (mean (SD))	68.67 (43.48)	85.40 (50.04)	0.033
age = Age>=60 (%)	10 (55.6)	7 (70.0)	0.729
age_group = younger (%)	8 (44.4)	3 (30.0)	0.729
vital_status = DEAD (%)	10 (55.6)	6 (60.0)	1
Chromosome.3.status = Monosomy 3 (%)	10 (55.6)	4 (40.0)	0.693

Figures

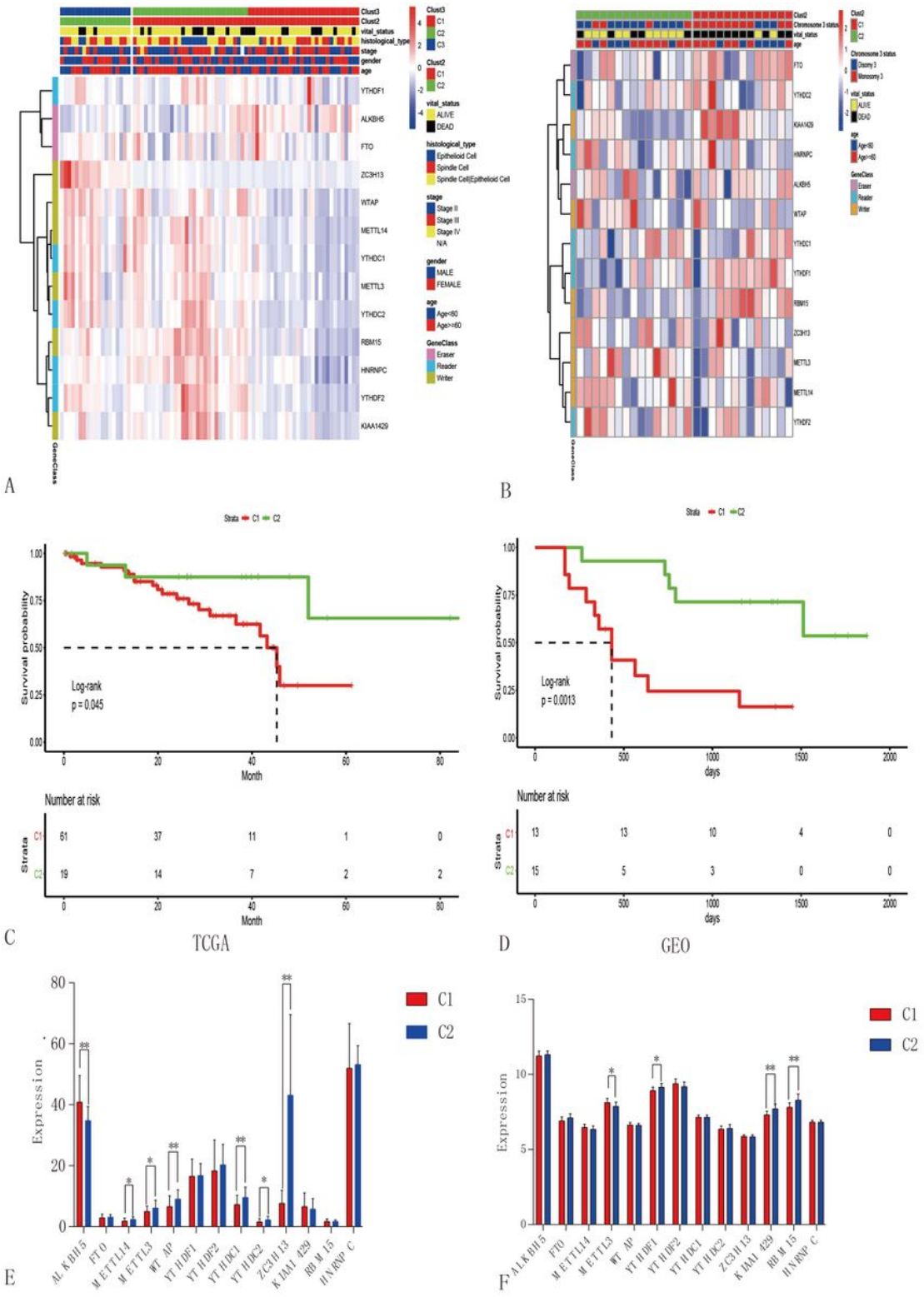

Figure 1

Expression of m6A RNA methylation regulators in uveal melanoma from the different database. (A-B) Heatmap and clinicopathologic features of the two clusters (C1/2) defined by the m6A RNA methylation regulators consensus expression downloaded from TCGA and GEO database. (C-D) Differential overall survival of uveal melanoma in the C1/2 subtypes (E-F) The expression levels of m6A RNA methylation regulators in uveal melanoma with different C1/2 subtypes. * P < 0.05, ** P < 0.01.

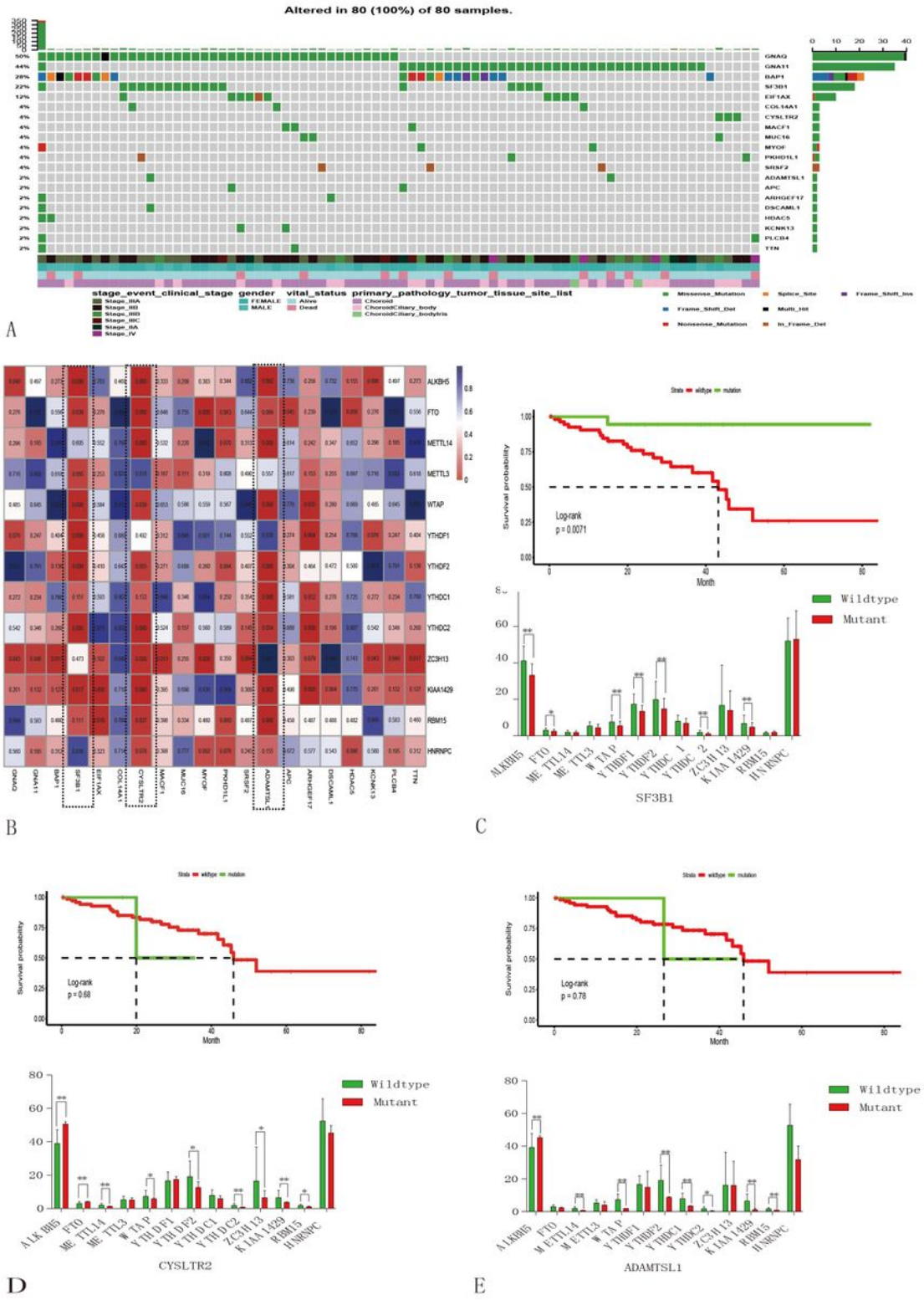

Figure 2

Correlation between different mutated genes and mRNA expression levels of thirteen m6A regulatory genes respectively.(A) Distribution of highly variant mutated genes in 80 UM samples at TCGA database. (B) The heatmap of m6A regulatorys expression and 20 highly variant mutated genes.(C-E) Kaplan-Meier survival analysis for SF3B1, CYSLTR2 and ADAMTSL1 mutated genes and the expression levels of m6A RNA methylation regulators in uveal melanoma with different mutant subtypes. * P < 0.05, ** P < 0.01.

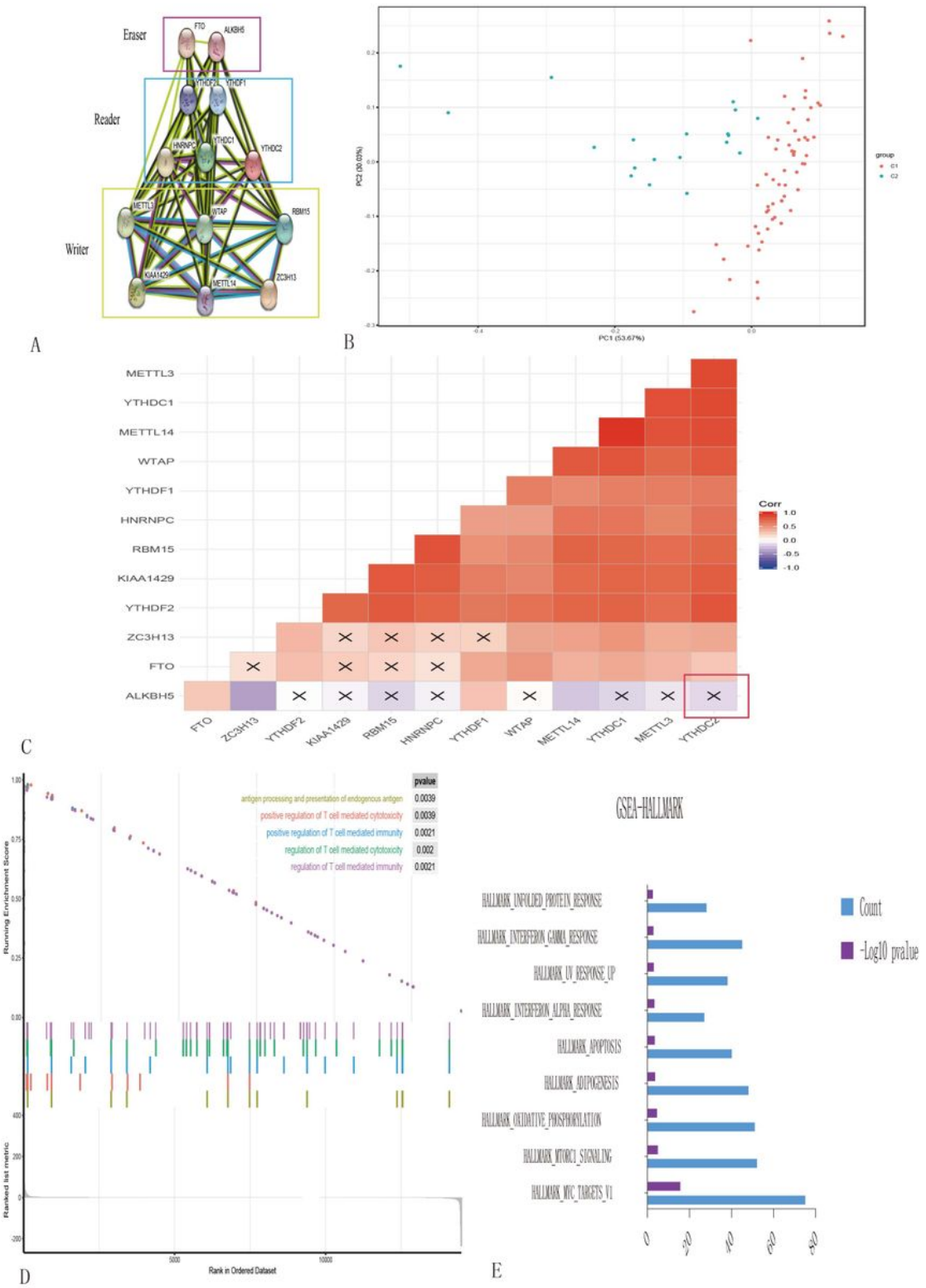

Figure 3

Interaction among m6A RNA methylation regulators and functional annotation of uveal melanoma in C1/2 subtypes. (A) The m6A modification-related interactions among the 13 m6A RNA methylation regulators. (B) Principal component analysis of the total RNA expression profile in the TCGA dataset. (C) Spearman correlation analysis of the 13 m6A modification regulators. X means P < 0.05 (D) The top 5 BP

terms were differentially enriched in C2 expression phenotype. (F) GSEA revealed that 9 malignant hallmarks pathways were significantly associated with the C1 subgroup expression phenotype .

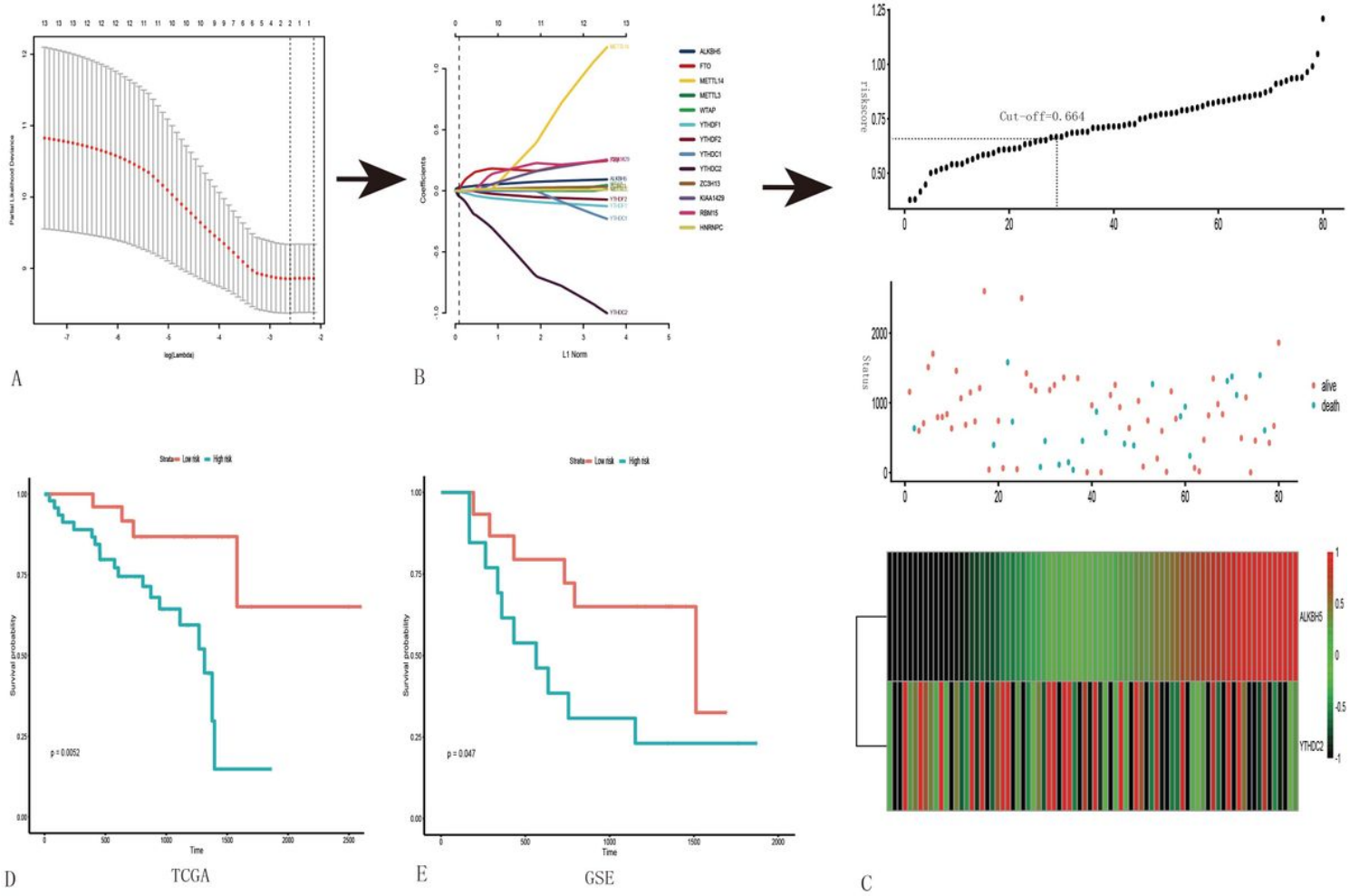

Figure 4

Risk signature with two m6A RNA methylation regulators. (A-C) The process of building the signature containing two m6A RNA methylation regulators and the coefficients calculated by LASSO method. (D–E) Kaplan–Meier overall survival (OS) curves for patients in the TCGA(B) and GEO(E) datasets assigned to high- and low-risk groups based on the risk score.

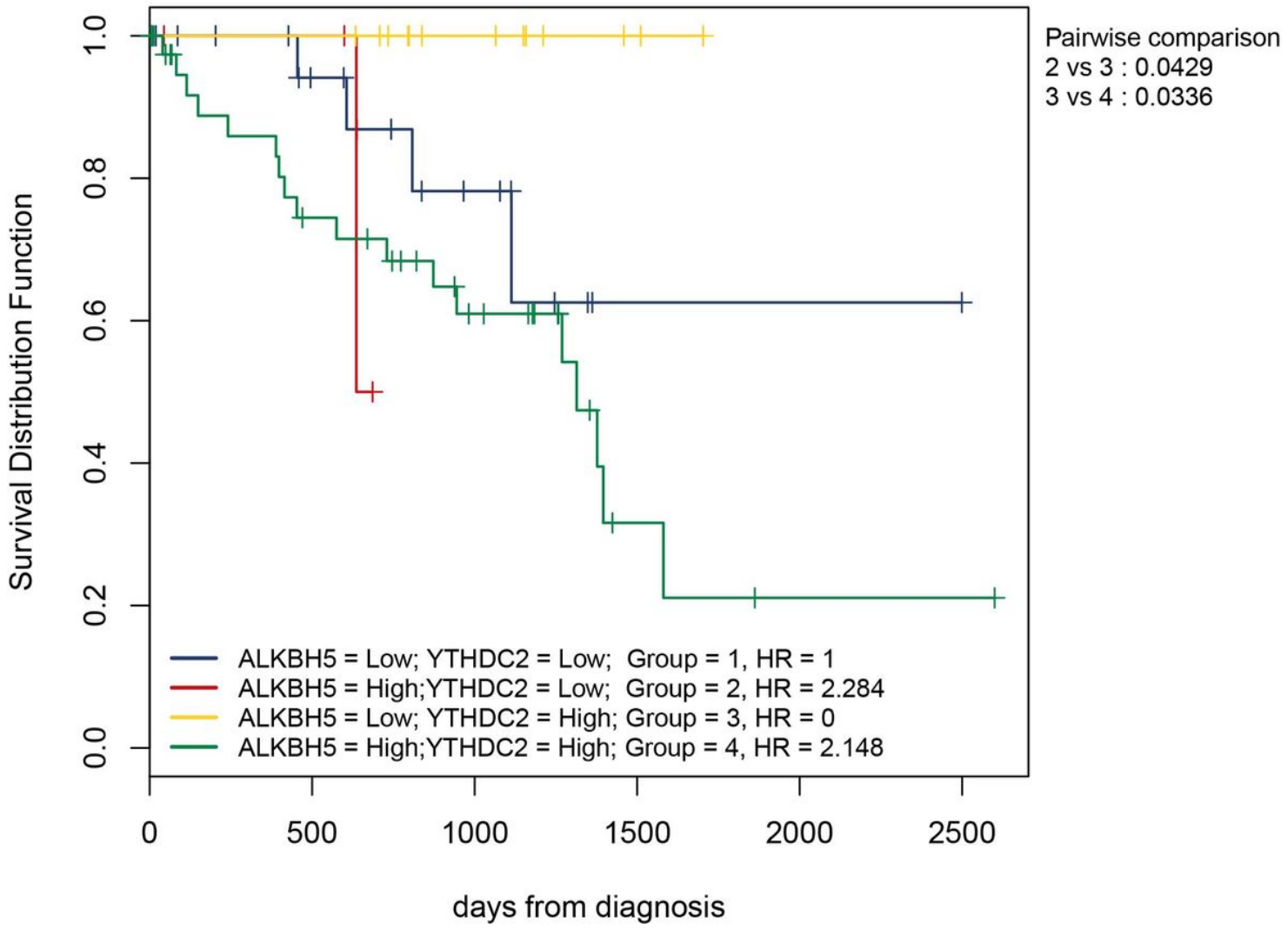

Figure 5

Combination group analysis of two m6A methylation regulators (ALKBH5 and YTHDC2) signature. Kaplan-Meier survival analysis showed that low expression of ALKBH5 combined with high expression of YTHDC2 have a better overall survival among four groups.