M1A Regulator-Mediated Methylation Modification And Gene Signatures And Their Prognostic Value In Multiple Myeloma

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Abstract

Introduction: N1-methyladenosine (m1A), a methylation modification on RNA, is gaining attention for its role across diverse biological processes. However, the potential roles of m1A regulator-mediated methylation modification in multiple myeloma (MM) remain unclear.

Methods: We investigated the mRNA expression of m1A regulators in normal plasma (NP, n = 9) and MM (n = 174) bone marrow plasma cells and comprehensively evaluated the m1A modification patterns of 559 MM samples based on the expression of 10 m1A-related regulatory genes. A univariate Cox regression model, Kaplan–Meier survival curve, unsupervised clustering and gene enrichment analysis were used to explore the associations between m1A-related regulatory genes and MM patient survival and prognosis. Additionally, the m1Ascore was constructed to quantify m1A modification patterns of individual tumors using the principal component analysis algorithm.

Results: Compared with NP, the expression of five genes (TRMT61A, TRMT61B, YTHDF1, YTHDF2, YTHDF3) was upregulated in MM patients. The Kaplan–Meier survival curve showed the high expression of the above five genes was associated with shorter overall survival. Three distinct m1A modification patterns were determined, of which cluster B showed the worst outcome; the expression of ALKBH3, YTHDF2, TRMT10C, TRMT6, and TRMT61B was high and the expression of ALKBH1 and YTHDC1 was low in cluster B. In addition, the relationships between the m1Ascore and survival, m1Aclusters, m1A gene clusters, and PD-L1 expression all indicated that high m1Ascore was associated with clinical benefits and therapeutic advantages.

Conclusion: These findings indicate that m1A-related regulatory genes play a crucial role in regulating MM progression and that the m1Ascore could be used for diagnostic and prognostic purposes.

1. Introduction

Multiple myeloma (MM) is one of the most common malignant tumors in the blood system, with symptoms related to anemia, immunosuppression, bone destruction, and renal failure[1]. Myeloma biomarkers relevant to treatment can be both prognostic and predictive, and approaches to target these biomarkers are likely to have the greatest impact on outcomes[3], so it is critical to identify novel biomarkers for early MM detection and prognostication.

The dynamic and reversible chemical modification of RNA plays a vital role in posttranscriptional gene regulation[4]. Decades of research have revealed more than 100 different types of posttranscriptional modifications to RNA molecules, including 5-methylcytosine (m5C), N6-methyladenosine (m6A), and N1-methyladenosine (m1A). In the case of chemical conversion, m1A can undergo Dimroth rearrangement under alkaline conditions to become m6A[6]. In contrast to m6A, which is enriched around the stop codon and in the 3'UTR, m1A preferentially associates with the start codon region in mammalian mRNA[6]. The unique chemical properties of m1A, with both a positive charge and a

methyl group, potentially allow each modified site to have a stronger effect than m6A in terms of RNA structure or protein–RNA interactions[4,7]. Similar to the modification of DNA and protein, m1A modification is a kind of dynamic reversible process in mammalian cells that is regulated by methyltransferases, demethylases, and binding proteins, also known as “writers”, “erasers” and “readers”[8]. The formation process of m1A methylation is catalyzed by methyltransferases consisting of TRMT10C, TRMT61B, TRMT6, and TRMT61A[9,10], while the removal process is mediated by demethylases, including ALKBH1 and ALKBH3[11]. In addition, a group of specific RNA-binding proteins composed of YTHDF1/2/3 and YTHDC1 can recognize the m1A motif, thus affecting m1A functions[12]. An in-depth understanding of these regulators would help reveal the role and mechanism of m1A modification in posttranscriptional regulation.

In this study, using MM data from the Gene Expression Omnibus (GEO) database, we attempted to identify special m1A-related regulatory genes related to the progression of MM.

2. Results

2.1 Landscape of genetic variations of m1A regulators in MM

We first summarized the differential expression of m1A regulators between normal plasma (NP, n = 9) and MM (n = 174) bone marrow plasma cells. Compared with NP, the expression of the eraser gene ALKBH1 was significantly downregulated in MM patients (Fig. 1A). The expression of the writer genes TRMT61A and TRMT61B and the reader genes YTHDF1, YTHDF2 and YTHDF3 were upregulated in MM patients.

Another GEO dataset (n=559) with the most comprehensive clinical annotation was used to further investigate the prognostic values of m1A regulators. A univariate Cox regression model revealed the prognostic values of 10 m1A regulators in patients with MM. The Kaplan–Meier survival curve revealed seven regulators that were significantly correlated with survival. Among them, the high expression of six regulators (YTHDF1/2/3, TRMT6, TRMT10C, and TRMT61B), especially YTHDF2 and trmt61B, was associated with shorter overall survival (OS) (Fig. 1B-G). High expression of YTHDC1 was associated with longer OS (overall survival). The comprehensive landscape of m1A regulator interactions, regulator connections, and their prognostic significance in MM patients was depicted with the m1A regulator network (Fig 1H). From the network diagram, we found that ALKBH1 was significantly negatively correlated with YTHDF2 and TRMT61B.

The above analyses indicated that the expression imbalance of m1A regulators, especially the high expression of three readers (YTHDF2, TRMT61B, and ALKBH1), may play a crucial role in MM occurrence and progression.

2.2 M1A methylation modification patterns mediated by 10 regulators

Three fully distinct modification patterns were eventually identified using unsupervised clustering, including 248 patients in pattern A and 190 patients in pattern B and 121 patients in pattern C. We termed these patterns m1Aclusters A, B, and C. Prognostic analysis of the three main m1A modification subtypes revealed a particular survival disadvantage in the m1Acluster B modification pattern (Fig. 2A). Clusters A and C were better than cluster B in terms of survival and showed similar outcomes. There was a significant distinction in the m1A transcriptional profile between m1Aclusters A and C and m1Acluster B (Fig. 2B). As shown in Fig. 2C, cluster B was characterized by the increased expression of five regulators (ALKBH3, YTHDF2, TRMT10C, TRMT6, and TRMT61B) and presented variable decreases in other m1A regulators (Fig. 2C). In contrast, m1Acluster C, with better survival, exhibited significant decreases in the expression of the same regulators. This probably means that these regulators, especially ALKBH3, YTHDF2, and TMRT61B, imply a poor prognosis.

To explore the biological behaviors related to these distinct m1A modification patterns, we performed GSVA enrichment analysis. M1Aclusters A and C were markedly enriched in base excision repair and homologous recombination (Fig. 2D, E). M1Acluster B presented enrichment pathways associated with cell proliferation-related pathways, including nucleotide excision repair, DNA replication, aminoacyl tRNA biosynthesis, RNA polymerase, purine metabolism, and pyrimidine metabolism (Fig. 2D), suggesting that m1A modification may mediate the proliferation of MM cells.

2.3 Generation of m1A gene signatures and functional annotation

To further investigate the potential biological behavior of each m1A modification pattern, we identified 865 m1A phenotype-related DEGs using the limma package (Fig. 3A). The Metascape platform was used to perform GO enrichment analysis for the DEGs. Surprisingly, these genes showed enrichment of biological processes remarkably related to the regulation of RNA biogenesis and metabolic processes (Fig. 3B), which confirmed again that m1A regulators played a nonnegligible role in RNA modification.

To further validate this regulatory mechanism, we then performed unsupervised clustering analyses based on the 865 obtained m1A phenotype-related genes to classify the patients into different genomic subtypes. Consistent with the clustering grouping of m1A modification patterns, the unsupervised clustering algorithm also revealed three distinct m1A modification genomic phenotypes, and we named these three clusters m1A gene clusters A, B, and C (Fig. 3C). An intermediate prognosis was observed in gene cluster A, which included 273 patients. One hundred and twelve patients with MM were included in gene cluster C, which was proven to be related to a better prognosis. Patients in gene cluster B (174 patients) experienced poorer prognosis (Fig. 3D), with high expression of TRMT6, TRMT10C, TRMT61B, ALKBH3, and YTHDF1/2/3 and low expression of ALKBH1 and YTHDC1, especially TRMT61B, YTHDF2, ALKBH3 and ALKBH1 (Fig. 3E), as mentioned above. In the three m1A gene clusters, prominent differences in the expression of m1A regulators were observed, which was in accordance with the expected results of m1A methylation modification patterns.

To further identify the prognostic value of the m1Ascore, the patients were divided into low- and high-m1Ascore groups with a cutoff value of -2.3971 using the survminer package. The Kruskal–Wallis test revealed that patients with a low m1Ascore had inferior survival (Fig. 4A). Then, we specifically examined the ability of the m1Ascore to serve as a prognostic biomarker for patients with MM. Gene cluster B showed the lowest median score, and m1Acluster B also showed the lowest median score (Fig. 4B-C). Compared with patients with low m1Ascores, patients with high m1Ascores had a prominent survival probability (74% vs. 63%), which again proved that low m1Ascores could be closely linked to poor prognosis (Fig. 4D). In addition, patients with high m1Ascores showed obviously higher expression levels of PD-L1, which indicated a potential response to anti-PD-L1 immunotherapy (Fig. 4E).

3. Discussion

M1A modification is widely distributed in RNA and has been proven to play an important role in ensuring the correct folding of tRNAs and promoting the formation of rRNA subunits[17,18]. In mRNA, m1A was found to be highly enriched in the 5'UTR and tended to be located in highly structured regions [6]. In addition, some studies identified an m1A modification site in MALAT1[9], a lncRNA that could be employed in cancer diagnosis and prognosis[19]. Increasing evidence suggests that changes in m1A-related regulatory genes are closely associated with a variety of diseases, including gastrointestinal cancer and glioblastoma[20,21]. However, the role of m1A methylation in the occurrence and prognosis of MM remains unclear.

By exploring the GEO database, we analyzed a total of 183 samples (NP=9, MM=174) to understand alterations in m1A-related regulator genes in the MM samples. The expression of five m1A regulatory genes was upregulated in MM patients, including the writer TRMT61A/B and the readers YTHDF1/2/3. The expression of the eraser ALKBH1 was significantly downregulated. Then, the Kaplan–Meier curve showed that the expression of m1A-related regulatory genes could be used to assess MM patient risk, especially the high expression of TRMT6, TRMT61B, and YTHDF1/2/3 associated with shorter overall survival and distinct situations of YTHDC1. This result suggested that the expression of m1A-related regulatory genes could be a useful prognostic marker for MM. Cluster analysis revealed three distinct m1A modification patterns based on the expression of 10 m1A regulators. M1Acluster B was characterized by the increased expression of ALKBH3, YTHDF2, TRMT10C, TRMT6, and TRMT61B and presented apparent decreases in ALKBH1 and YTHDC1, with the worst outcome. M1Acluster C with better survival exhibited significant contrary changes in the expression of these regulators, which means that these regulators (especially ALKBH1/3, TRMT61B, and YTHDF2) have the potential to serve as important biomarkers for MM. In our study, the GSVA results revealed the biological functions of m1A-related regulatory genes involved in MM, which were related to the proliferation of cells, including nucleotide excision repair, DNA replication, aminoacyl tRNA biosynthesis, RNA polymerase, purine metabolism, and pyrimidine metabolism, which provide clues to their contribution to MM pathogenesis.

The next unsupervised cluster analysis showed three distinct m1A modification genomic phenotypes consistent with the m1A modification patterns. In accordance with the expected results of the m1A modification patterns, the expression of m1A regulators (high expression of TRMT6, TRMT61B, YTHDF2, and ALKBH3 and low expression of ALKBH1 and YTHDC1) was also observed in the m1A gene clusters. Patients in m1A gene cluster B experienced the worst prognosis, while those in gene cluster C showed the best prognosis, which confirmed that the ALKBH3, TRMT61B, YTHDF2, and ALKBH1 genes had good risk prediction value for MM survival, and elevated expression of the previous three regulators was associated with poor prognosis.

In addition to nuclear-encoded transcripts, M1A modification also exists in mitochondrial-coded mRNAs (mt-mRNAs)[22]. TRMT61B is a mitochondria-located methyltransferase that can modify not only mt-tRNA and mt-rRNA but also mt-mRNA[23]. YTHDF2 is overexpressed in a broad spectrum of human AMLs and contributes to the integrity of leukemia stem cell function. Therefore, YTHDF2 is considered a unique target for the treatment of AML[26].

From the 10 regulators network diagram, we found a strong positive correlation between YTHDF2 and TRMT61B, and they were significantly negatively correlated with ALKBH1. A previous study revealed the significant effects of ALKBH1 on subsequent translational initiation, which results in attenuated translation initiation and reduces the role of target tRNAs in protein synthesis[27]. The demethylases ALKBH1 and ALKBH3 have been characterized as m1A erasers[28]. However, we found that the expression of ALKBH1 and ALKBH3 had opposite effects on the prognosis of MM patients. Human ALKBH3 belongs to the AlkB family that utilizes nonheme iron (II) to catalyze biological oxidation and could participate in the demethylation of single-stranded RNA both in vitro and in vivo[27,29,30]. Previous research shows that hypermethylation of the ALKBH3 promoter CpG island is common in hematological malignancies, and ALKBH3 hypermethylation is associated with shorter OS in Hodgkin lymphoma[31]. This result is consistent with the upregulation of ALKBH3 in patients with poor prognosis found in our study.

The Kruskal–Wallis test revealed significant and similar differences in m1A scores in the m1A clusters and m1A gene clusters. Cluster B presented the lowest median score compared to the other clusters, and cluster C had the highest median score. Combining the above analysis with the m1Ascore survival curve, we found that patients with high m1Ascores had a prominent survival benefit. To test whether the m1Ascore could serve as an independent prognostic biomarker for MM, we next confirmed that patients with high m1Ascores had a higher survival rate. We also identified the predictive value of the m1Ascore for anti-PD-L1 immunotherapy, in which a high m1Ascore indicated higher expression of PD-L1.

4. Conclusions

Our study identified alterations in m1A-related regulatory genes in MM for the first time and found a clear relationship with clinicopathological features and prognosis. ALKBH3, TRMT61B, YTHDF2, and ALKBH1 effectively predicted MM patient survival and contributed to important biological processes. Similarly, the constructed m1Ascore was found to be a prognostic biomarker for patients with MM and could predict

the patients' clinical response to PD-L1 immunotherapy. To further clarify the molecular mechanism of m1A mRNA modification in MM development, future studies in vivo and in vitro are needed to confirm our findings and molecular understanding.

5. Methods

5.1 MM dataset source and preprocessing

Public gene expression data and full clinical annotation were searched in the GEO database. In total, 3 eligible MM cohorts (GSE13591, GSE47552, and GSE24080) were gathered in this study for further analysis, and the normalized matrix files were directly downloaded from platforms. The data were analyzed with R (version 4.1.2) and R Bioconductor packages.

5.2 Analysis of m1A-related regulatory gene expression

We first analyzed the mRNA expression of m1A regulators in normal plasma and MM bone marrow plasma cells based on samples from GSE13591 and GSE47552. Then, another dataset (GSE24080) with prolific clinical information was used to elucidate alterations of m1A-related regulatory genes in MM samples. The data were analyzed with the limma R package.

5.3 Unsupervised clustering for 10 m1A regulators

A total of 10 regulators were extracted from GSE24080 to identify different m1A modification patterns mediated by m1A regulators. These 10 m1A regulators included 4 writers (TRMT6, TRMT10C, TRMT61A, and TRMT61B), 2 erasers (ALKBH1 and ALKBH3) and 4 readers (YTHDC1, YTHDF1, YTHDF2, and YTHDF3). Unsupervised clustering analysis was applied to identify distinct m1A modification patterns based on the expression of 10 m1A regulators and classify patients for further analysis. The number of clusters and their stability were determined by the consensus clustering algorithm[13]. The ConsensusClusterPlus package was used to perform the above steps.

5.4 Gene set variation analysis (GSVA) and functional annotation

To investigate the difference in biological processes between m1A modification patterns, we performed GSVA enrichment analysis using the GSVA R package. GSVA is a nonparametric and unsupervised method and can easily adapt to the analysis of RNA-seq data[14]. The gene sets of "c2.cp.kegg.v6.2.-symbols" were downloaded from the MSigDB database for GSVA. Adjusted P values less than 0.05 were considered statistically significant. The clusterProfiler R package was used to perform functional annotation for m1A-related genes, with the cutoff values of | log fold change (FC) |> 0.1 and adjusted P value<0.05.

5.5 Identification of differentially expressed genes (DEGs) between distinct m1A phenotypes

To further analyze m1A-related regulators, we identified DEGs based on different modification patterns of m1A. The limma R package was applied to identify DEGs, and the significance criteria were set as adjusted P value < 0.001. Gene Ontology (GO) enrichment analysis of DEGs was performed by the Metascape platform.

5.6 Generation of an m1A gene signature

To preferably quantify the m1A modification pattern, we constructed the m1A gene signature to calculate a series of scores to assess individual MM patients, and it was termed the m1Ascore. The steps for establishing the m1A gene signature were as follows.

We first extracted overlapping genes from DEGs identified from different m1Aclusters and then performed unsupervised clustering analysis on the overlapping genes. The consensus clustering algorithm was used to determine the number and stability of gene clustering, and the patients were divided into several groups for further analysis. Next, we used univariate regression analysis to analyze the prognosis of each overlapping gene and extracted the genes with significant prognoses for further analysis.
Principal components 1 and 2 were selected as signature scores, and m1A-related gene signatures were constructed through principal component analysis (PCA).

We then defined the m1Ascore using a method similar to the gene expression grade index (GGI)[15,16]:

$$m1Ascore=\sum(PC1_i+PC2_i)$$

where i is the expression of m1A phenotype-related genes.

5.7 Statistical analysis

The survival curves for the prognostic analysis were generated via the Kaplan–Meier method, and log-rank tests were used to identify the significance of differences. We adopted a univariate Cox regression model to calculate the hazard ratios (HRs) for m1A regulators and m1A phenotype-related genes. The independent prognostic factors were ascertained through a multivariable Cox regression model. Patients with detailed clinical data were eligible for inclusion in the final multivariate prognostic analysis. All statistical P values were two-sided, with P < 0.05 considered statistically significant. All data processing was performed in R 4.1.2 software.

6. Abbreviations

M1A = N1-methyladenosine; MM = Multiple myeloma; NP = normal plasma; m5C = 5-methylcytosine; m6A = N6-methyladenosine; GEO = Gene Expression Omnibus; OS = overall survival; GSVA = Gene set variation analysis; FC = fold change; DEGs = differentially expressed genes; GO = Gene Ontology; PCA = principal component analysis; GGI = gene expression grade index; HRs = hazard ratios.

7. Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during the current study are available in the Gene Expression Omnibus (GEO) database.

Competing interests

The authors report no conflicts of interest related to this study.

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Authors' contributions

Fu Jiaqi and Cui Xing wrote the main manuscript text. Fu Jiaqi and Yu Manya prepared figures 1-4. Han Xingjun, Gao Wei and Cui Jinwei did the preparatory work. All authors reviewed the manuscript.

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Figures

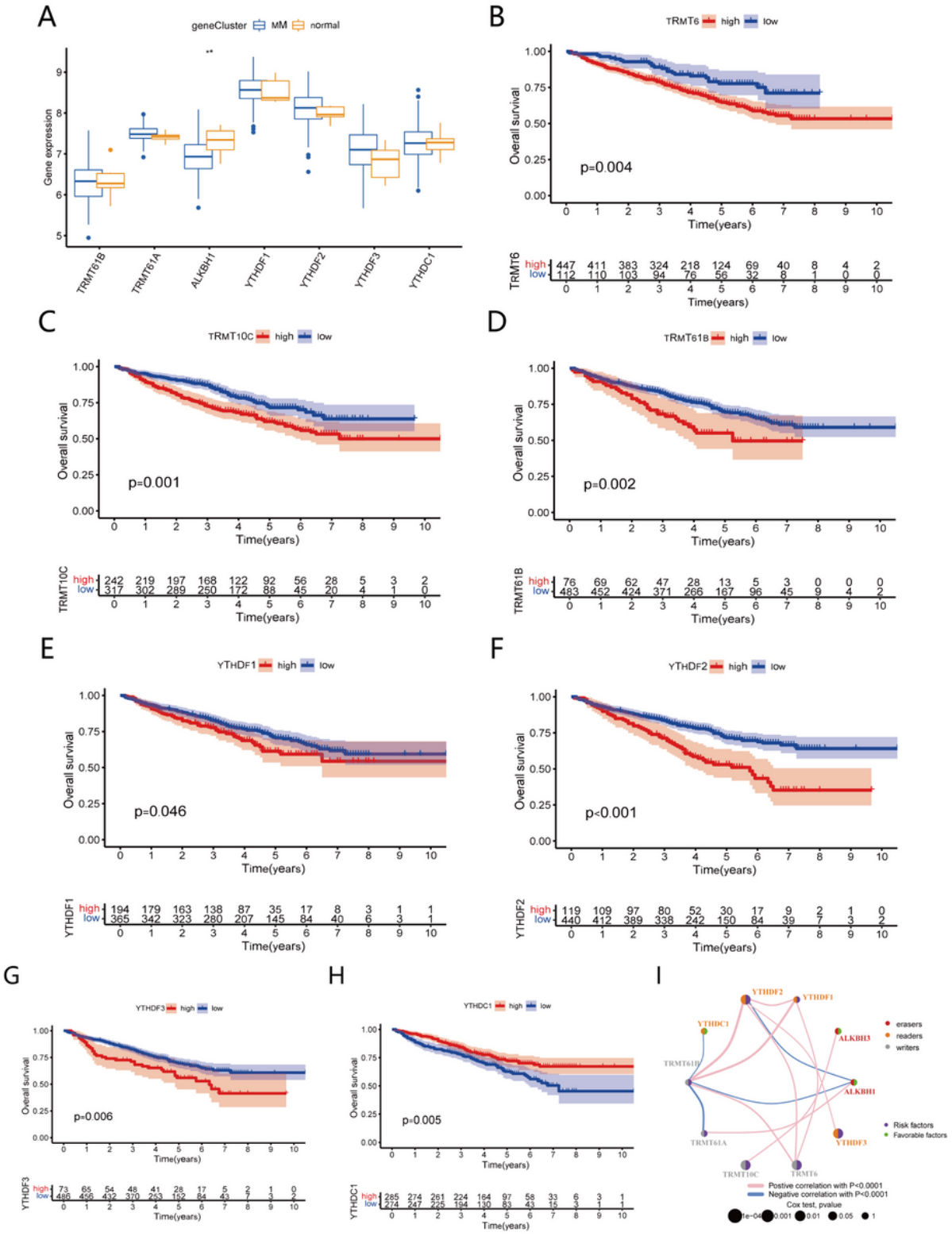

Figure 1

Landscape of genetic variations of m1A regulators in multiple myeloma.

(A) Alterations in m1A-related regulatory genes in MM patients. The upper and lower ends of the boxes represent an interquartile range of values. The lines in the boxes represent the median value, and black dots show outliers. The asterisks represent significant P values (P<0.1, **P< 0.01). (B-G) High expression

of (B) TRMT6, (C) TRMT10C, (D) TRMT61B, (E) YTHDF1, (F) YTHDF2 and (G) YTHDF3 is associated with poor prognosis in HCC patients. (H) Low expression of YTHDC1 is associated with a better prognosis. (I) The interaction between m1A regulators in MM. The circle size represents the effect of each regulator on the prognosis, and the range of values calculated by the log-rank test was P< 0.001, P< 0.01, P< 0.05 and P< 0.1. Purple dots in the circle indicate risk factors for prognosis; green dots in the circle indicate protective factors for prognosis. The lines linking regulators showed their interactions, and the thickness shows the correlation strength between regulators. Negative correlations are marked with blue, and positive correlations are marked with red. The regulator erasers, readers and writers are marked with red, orange and gray, respectively.

Figure 2

M1A methylation modification patterns mediated by 10 regulators.

(A) Survival analyses for the three m1A modification patterns based on 599 patients with gastric cancer from GSE24080. Kaplan–Meier curves with a log-rank P value of 0.002 showed a significant survival difference among the three m1A modification patterns. m1Acluster B showed worse overall survival than the other two m1Aclusters. (B) Principal component analysis of the transcriptome profiles of the three m1A modification patterns, showing a remarkable difference in the transcriptome between different modification patterns. (C) Unsupervised clustering of 10 m1A regulators. The m1Acluster, ISS staging system, sex, immunoglobulin type and age were used as patient annotations. Red represents high expression of regulators, and blue represents low expression. (D-E) GSVA enrichment analysis showed the activation states of biological pathways in distinct m1A modification patterns. A heatmap was used to visualize these biological processes; red represents activated pathways, and blue represents inhibited pathways. The MM cohort was used as sample annotations. (D) m1Acluster A vs. m1Acluster B; (E) m1Acluster B vs. m1Acluster C.

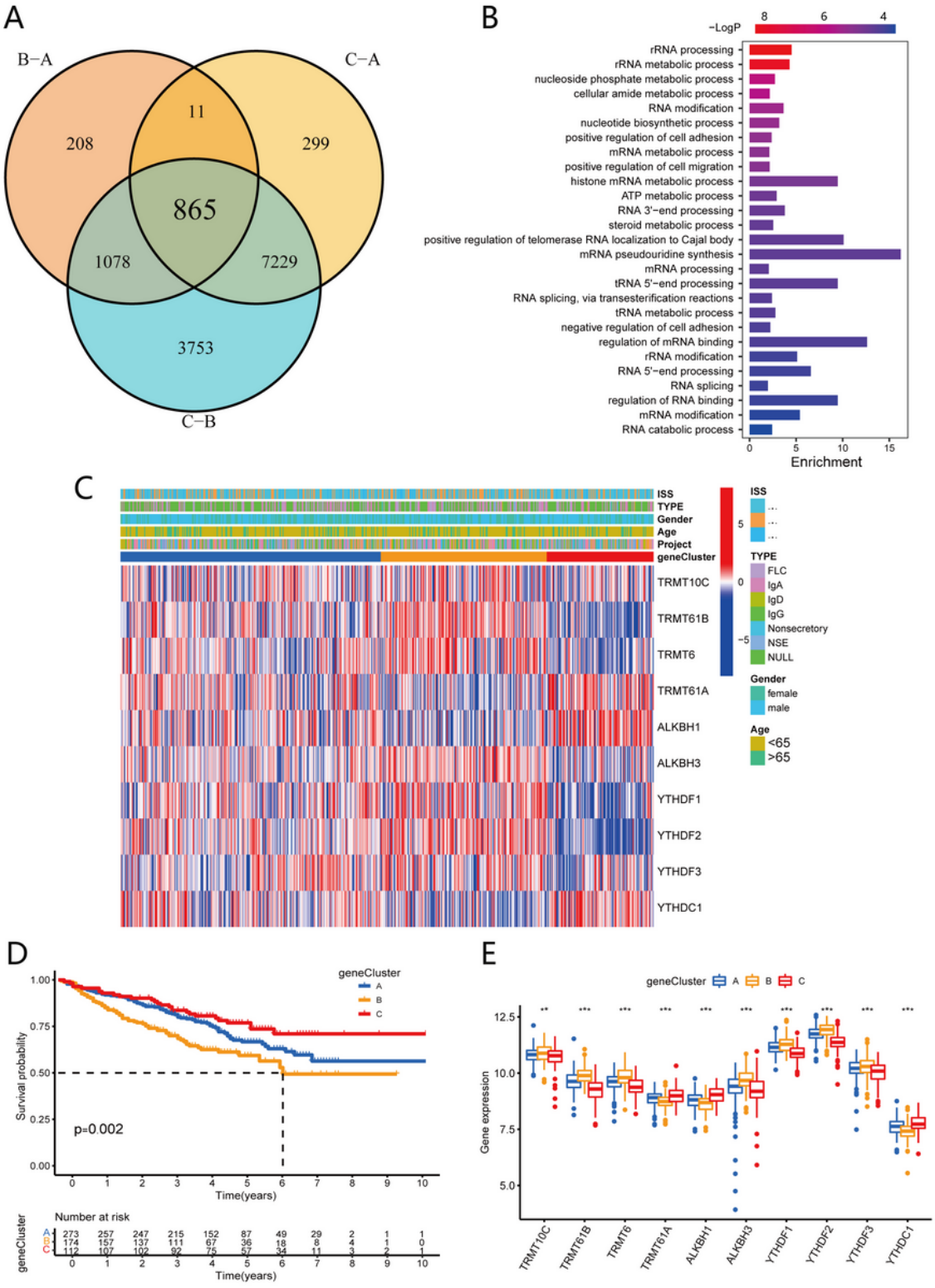

Figure 3

Generation of m1A gene signatures and functional annotation.

(A) Identification of differentially expressed genes (DEGs) between distinct m1A phenotypes. (B) Functional annotation for m1A-related genes using GO enrichment analysis. The color depth of the bar plots represents the number of enriched genes. (C) Unsupervised clustering of overlapping m1A

phenotype-related genes to classify patients into different genomic subtypes, termed m1A gene clusters A-C. m1Acluster, ISS staging system, sex, immunoglobulin type and age were used as patient annotations. (D) Kaplan–Meier curves indicated that m1A modification genomic phenotypes were markedly related to the overall survival of 559 patients, of which 273 patients were in gene cluster A, 174 patients were in gene cluster B, and 112 patients were in gene cluster C (P< 0.0001, log-rank test). (E) The expression of 10 m1A regulators in three gene clusters. The upper and lower ends of the boxes represent the interquartile range of values. The lines in the boxes represent the median value, and black dots show outliers. The asterisks represent significant P values (*P< 0.05; **P< 0.01; ***P< 0.001).

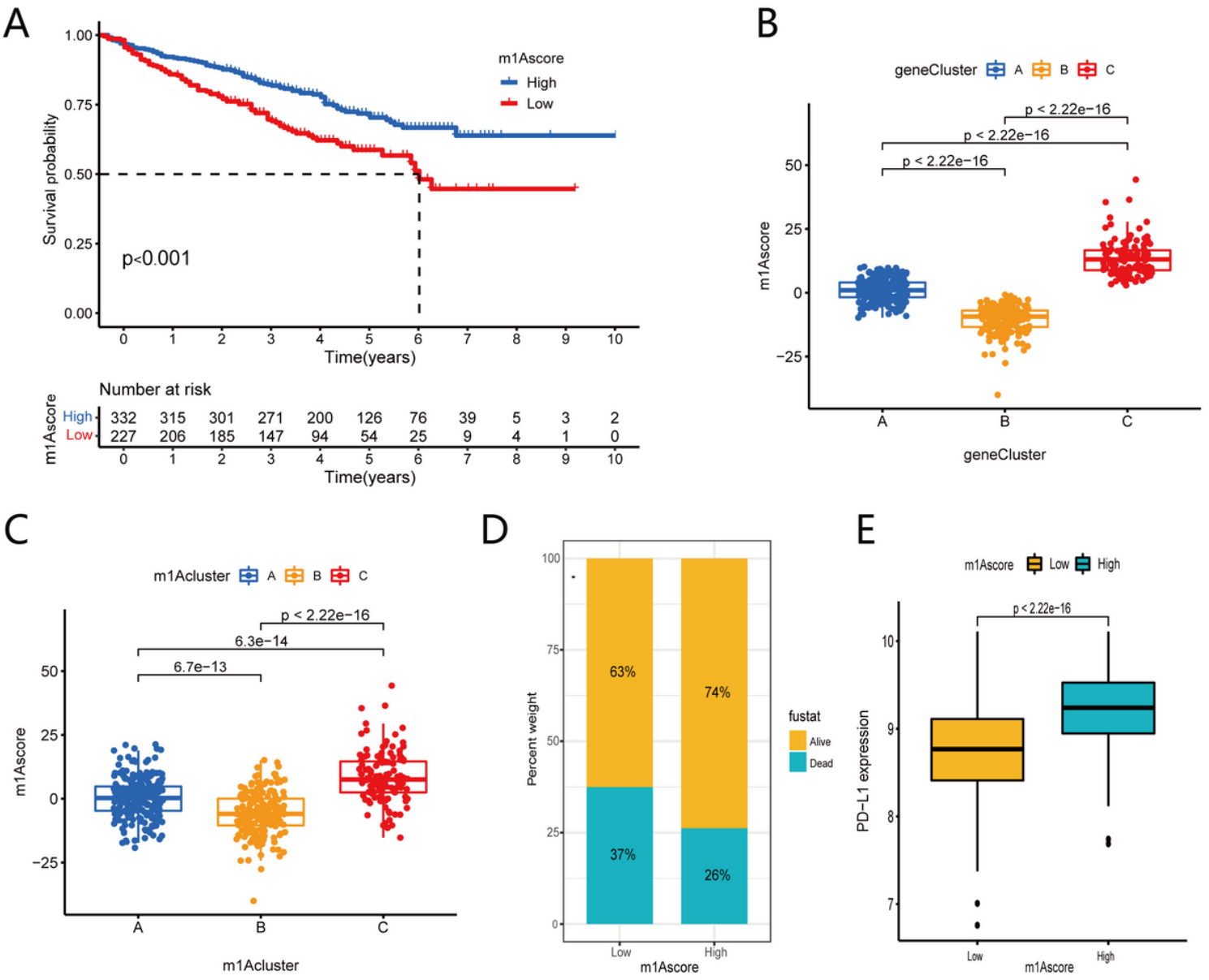

Figure 4

Prognostic values of m1Ascore in multiple myeloma.

(A) Survival analyses for the low-m1Ascore (227 patients) and high-m1Ascore (332 patients) patient groups (P< 0.001). (B) Differences in the m1Ascore among the three gene clusters. (C) Differences in the

m1Ascore among the three m1A clusters. (D) The proportion of patients who survived in the low- or high-m1Ascore groups. Alive/dead 63%/37% in the low-m1Ascore groups and 74%/26% in the high-m1Ascore groups. (E) Differences in PD-L1 expression between the low- and high-m1Ascore groups (P< 2.22e-16, Wilcoxon test).