Expression and Prognostic Significance of m6A-Related Genes in TP53-Mutant Non-Small-Cell Lung Cancer

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Abstract

Background: TP53 is an important tumor suppressor gene on human chromosome 17. TP53 mutations have been confirmed in more than 60% of tumor patients' somatic cells. N6-methyladenosine is an enzyme that plays an important role in mRNA splicing, translation, and stabilization. It is a common internal modification of nucleotides, that is regulated by methyltransferases ("writer"), binding proteins ("reader"), and demethylases ("eraser"). However, its role in TP53 mutant non-small-cell lung cancer remains unknown. In this study, we investigated 17 common N-6-Methyladenosine regulators' contributions and prognostic values.

Results: Wilcox test showed that 9 of 17 m6A regulators were expressed differently between TP53 mutant and wild type NSCLC ($p<0.05$). A new way of tumor typing is attempted by consensus clustering. Using univariate cox regression analysis, ALKBH5, HNRNPA2B1 were associated with the prognostic of TP53 mutant patients. Then we built signatures of N-6-Methyladenosine regulators with prognostic ability based on the multivariate cox regression in TP53 mutant cohorts. Moreover, ROC curve showed that this signature could predict the prognostic in mutant cohort well and the riskscore for patients was calculated. With this riskscore, patients with TP53 mutations can be divided into high and low risk groups, and there was a significant difference between two groups. Finally, we found 338 DEGs between high and low risk groups, then GO enrichment analysis, PPI network and GSEA enrichment analysis were used to analysis different signaling pathways between two groups. In conclusion, our study showed m6A regulators can be used as predictive prognostic tools in TP53 mutant patients.

Conclusion: Signature consisting of the “erasers” ALKBH5 and “readers” HNRNPA2B1 have potential diagnostic value and prognostic significance in patients with TP53 mutations in non-small cell lung cancer.

Introduction

Lung cancer remains the deadliest malignancy in the world, and non-small cell lung cancer accounts for 85% of all types of lung cancer individuals. Non-small cell lung cancer can be mainly classified to three histological subtypes: lung squamous-cell carcinoma (LUSC), lung adenocarcinoma (LUAD) and large-cell lung cancer, and the vast majority of diagnosed NSCLC patients consists of LUAD and LUSC[1]. Although great progress has been made in the detection and the treatment in NSCLC in recent years, the 5-year survival remains for NSCLC remains low, only 16.6%[2].

TP53, also known as p53, is the most frequently mutated gene in NSCLC patients, up to 80% in squamous-cell carcinoma[3]. TP53 gene is one of the widely studied tumor suppressor genes, names from the fact that it encodes protein with the molecular weight of 53 kDa. p53 protein is an important regulator of cell growth, proliferation and damage repair. p53 protein can inhibit cell’s resting in G1/S phase and repair the damages. DNA damage and oncogene activation can stimulate the acetylation and activate p53, to realize the function of TP53 transcription factor as a cellular stress sensor[4]. TP53 is the most frequently mutated gene in human malignancies, its mutation is mainly missense mutations of DNA. The tumor suppressive function of p53 protein in mutant was converted to oncogenic function compared with TP53-wild type. In vivo experiments confirmed that Trp53 knockout mice had a higher risk of developing cancer[5].

Scientists have discovered a reversible modification of RNA methylation that occurs at the 6th position of the RNA molecule adenine nitrogen atom (N-6 methylation, m6A). m6A methylation is a common post-transcriptional modification in eukaryotes and plays an important role in mRNA metabolism and translation, as well as cell differentiation and embryonic development[6], that often found enriched in 3'-UTR and near the termination codons of mRNA[7]. Enzymes involved in the modification of mRNA methylation can be classified into three types according to their functions, called "writers", "erasers" and "readers". The function of the "writers" is add methyl to nucleotides. Such enzymes include METTL3/14/16, RBM15/15B, WTAP, KIAA1429 and ZC3H13. METTL3, as a catalytic subunit, combines with METTL14 to form a hetero complex[8]. However, METTL3 was also found to play the role as a "reader" and locate in the cytoplasm[9]. WTAP can bind to the hetero complex and plays an important role in the recruitment of the hetero complex[10]. Formed METTL3/14-WTAP complex can be induced into the nucleus by ZC3H13, and form ZC3H13-KIAA1429-HAKAI complex in the nucleus to regulate the m6A process[11]. RBM15/15B can also promotes the methylation for certain RNAs. mRNA methylation caused by "writers" can be demethylated by "erasers" (including FTO and ALKBH5), which makes the process reversible[12]. "readers" can be divided according to different domains, domains such as YTH domains (YTHDF1-3, YTHDC1/2), HNRNPs domains (HNRNPC and HNRNPB2A1) and some same RNA binding domains, respectively[13–15]. These regulators can bind specifically to the methyl on mRNA in unknown ways, which may be associated with RNA-binding proteins[12]. These genes build a network of interactions that worked by acting on m6A-modified mRNAs.

So far, many studies have revealed the roles of m6A methylation modification in various tumors, particularly hepatocellular carcinoma, breast cancer, gastric cancer and lung cancer[16–19]. However, the expression and prognostic significance in TP53 mutant non-small cell lung cancer is still unknown.

To further investigate the role of m6A modification in TP53 mutant lung cancer patients, we conducted an in-depth analysis of 17 m6A gene expression profiles in 469 lung cancer patients. Of 469 patients, 233 have mutated TP53 and 236 had wild-type TP53. All the data comes from The Cancer Genome Atlas (TCGA database). Then, bioinformatics and statistical analysis were performed to investigate the potential value of m6A modified factors in TP53 mutant NSCLC patients.

Material And Methods

Dataset

All data were downloaded from TCGA database (https://portal.gdc.cancer.gov/), including 1026 NSCLC patients' RNA-seq transcriptome profiling and 561 NSCLC patients' single nucleotide variation with corresponding clinical information. Taking the intersection of the two and get a matrix including 236 TP53 wild-type and 233 TP53 mutation.

Selection Of M6a Methylation Regulators

17 m6A regulators were finally identified in our analysis based on our search[20, 21] which including 8 "writers" (METTL3/14/16, WTAP, RBM15/15B, KIAA1429, ZC3H13), 7 "readers" (YTHDF1/2/3, YTHDC1/2, HNRNPA2B1,

HNRNPC), 2 "erasers" (FTO, ALKBH5). We obtained a matrix of 17 genes for 469 patients and performed subsequent bioinformatics analysis.

Bioinformatics Analysis

R 4.0.2 (R studio) was applied to all analysis. First we divided all samples into TP53 mutant cohort and TP53 wild-type cohort according to TP53 mutation. We compared expression levels between TP53 mutant cohort and TP53 wild-type cohort by using limma package (Wilcox-Text). All the samples in each cohort are then divided into two clusters that are separate and related to each other within the group, using the package named “ConsensusClusterPlus”. Univariate cox analysis was applied to independent prognostic analysis for 17 m6A related genes, HR value (Hazard Ratio) was used to determining protective gene or risk gene in two cohorts. Multivariate cox regression was used to construct an independent prognostic model by regularizing and screening 17 genes, and the risk score of screened genes was calculated. The calculation formula of risk score is

$$Risk\ \ score = \sum_{j=1}^{n} Coefj * ij.$$

R package “Survival” and “glnmet” were applied to univariate cox and multivariate regression cox in R version 4.0.2 respectively.

We then investigated the different pathways between high risk group and low risk group in TP53 mutant cohort by GSEA, and the GSEA test run 1000 times. Differentially expression genes (DEGs) were acquired between high risk group and low risk group in TP53 mutant cohort. ggplot2 package was applied to GO pathway enrichment analysis of these DEGs. A protein-protein interaction graph of these DEGs was constructed in STRING (http://string-db.org/). MCODE plugin of Cytoscape software is applied to PPI visualization, and Bingo plugin is used to build GO pathways diagrams. Finally, relationship between expression of m6A regulators and prognostic of patients with TP53 mutations was demonstrated.

Statistics

Wilcox test was performed to compare m6A expression difference between mutant group and wild-type group. Chi-square was used to compare the difference of clinical features between different subgroup. Kaplan-Meier method was used for the analysis of overall survival in different subgroups. Univariate and multivariate cox regression was applied to assess the prognostic power of risk score obtained from lasso regression analysis. The missing data was deleted from the analysis. All the statistical analysis was performed in R version 4.0.2, and $p < 0.05$ was considered statistically significance.

Results

Differential expression of m6A related genes in Lung cancer

By analyzing the expression of 17 m6A related genes in 233 TP53 mutant samples and 236 TP53 wild-type samples, we found that the expression of 9 genes were significantly different between two cohorts (Fig. 1A-B).

The coexpression analysis showed that WTAP was significantly negatively correlated with YTHDC1, ZC3H13, METTL3 and METTL16 in the wild-type, while the negative correlated genes in mutant cohort are FTO and METTL3, YTHDC2 and HNRNPC. Most of the other genes are positive correlated in two cohorts (Fig. 1C-D).

Clustering And Grouping Prognoses For Mutant And Wild-type Cohorts

The wild-type cohort and the mutant cohort were divided into two clusters by consensus clustering respectively, based on the expression profiles of all genes in TCGA (Fig. 2A-B). In the process of consensus clustering, we analyzed the possibility of clustering count (k value) from 2 to 9 (Fig. 2C-H). Finally, k = 2 was applied to the mutant cohort and the wild-type cohort. The clinicopathological characteristics of the patients in each cluster are listed in Table 1.

Table 1
The clinicopathological grouping of all non-small-cell lung cancer patients.

Wild-type cluster 1			**Wild-type cluster 2**			**Mutant type cluster1**			**Mutant type cluster 2**		
Age	≤ 65	25	Age	≤ 65	64	Age	≤ 65	88	Age	≤ 65	37
	> 65	41		> 65	89		> 65	72		> 65	23
Gender	Male	30	Gender	Male	65	Gender	Male	69	Gender	Male	35
	Female	36		Female	88		Female	91		Female	25
Stage	Stage I-II	46	Stage	Stage I-II	126	Stage	Stage I-II	124	Stage	Stage I-II	47
	Stage III-IV	20		Stage III-IV	27		Stage III-IV	36		Stage III-IV	13
T	T1-2	54	T	T1-2	138	T	T1-2	137	T	T1-2	53
	T3-4	12		T3-4	15		T3-4	22		T3-4	6
N	N0-1	51	N	N0-1	130	N	N0-1	131	N	N0-1	53
	N2-3	12		N2-3	23		N2-3	17		N2-3	6
M	M0	46	M	M0	109	M	M0	107	M	M0	35
	M1	5		M1	6		M1	5		M1	8
	Mx	15		Mx	38		Mx	48		Mx	17
Total	66		Total	153		Total	160		Total	60	

We then analyzed the clinicopathology and survival of two subgroups of each cohort. We found that two subgroups of wild-type have a significant difference in T stage, while a more significant difference in M status consists in mutant cohort. There was a significant difference in stage exists in both cohorts (Fig. 3A-B).

However, survival analysis, according to the results of both in the wild-type cohort ($p = 0.121$) and mutant cohort ($p = 0.089$), survival situation between the subgroups were no significant differences ($p < 0.05$) (Fig. 3C-D).

Independent prognostic signatures building and comparison in wild-type and mutant cohorts

We then constructed a prognostic model by using univariate cox regression and multivariate cox analysis. Univariate cox regression was applied to m6A related regulators to screen prognostic-associated genes. HNRNPA2B1 gene has a good correlation with prognostics in cases without TP53 mutant, and is positively correlated with prognostic risk. This “risk gene” in cases with TP53 mutant are HNRNPA2B1 and ALKBH5, with which $P < 0.05$ (Fig. 4A-B). When we expanded the range to $P < 0.1$, 5 of 17 genes in the wild cohort came into view (FTO, METTL14, HNRNPC, HNRNPA2B1 and YTHDC2), while the number in the mutant cohort is 10 (FTO, RBM15, METTL3/14/16, HNPNPC, HNPNPA2B1, YTHDC1, ALKBH5 and ZC3H13). Interestingly, high expression of FTO or METTL14 means different prognostic risk in cases with and without TP53 mutant. Forest map shows that high expression of FTO and METTL14 was considered low risk in the wild-type patients, which was exactly the opposite in the mutant patients (Fig. 4A-B).

We constructed prognostic signatures of 17 genes in mutant cohorts by using multivariate cox regression analysis to predict patients’ prognostic risk score with the coef value of each gene calculated. By this method, we established prognostic signatures containing 2 genes in the mutant cohorts. Then based on the calculated risk score, the patients were divided into the high-risk group and the low-risk group. Signature of 2 genes (HNRNPA2B1 and ALKBH5) also did well in separating mutant patients into two groups according to risk score and was statistically significant in overall survival (Fig. 4C). The results of ROC curve also showed that it was feasible to evaluate the overall survival rate of lung cancer patients using risk score obtained by multivariate cox analysis in the TP53 gene mutant patients. (Fig. 4D). Significant gender differences were revealed when exploring the relationship between demographics and clinicopathology (Fig. 5C). To validate the signature`s performance in predicting the patients outcome, univariate and multivariate cox regression were applied to evaluate the accuracy of demograghy, clinicalpathlogy and risk score. And the result showed that whether in univariate or multivariate cox regression analysis, risk score performed well in predicting the prognosis of TP53 mutant patients (Fig. 5A-B). This result indicates that the independent prognostic model constructed by ALKBH5 and HNRNPA2B1 has better predictive value than TNM staging in TP53 mutant patients. In the framework of the relationship of the risk score and patient survival we constructed, we can perceive that in the Fig. 5D, cases were arranged in order of risk score from low to high. In the scatter plot, we observed that green plots become sparse as the risk score increases, and the red plots are the opposite (Fig. 5D-E). It can also be concluded that with the increment of HNRNPA2B1 and ALKBH5 expression, an increasing trend occurred in the risk score (Fig. 5F). These finds confirmed the accuracy of the independent prognostic model.

The following we explored the distinction of m6A regulators between high-risk and low-risk group. The results showed that the expression of ALKBH5 in high-risk group was higher than low-risk which difference was statistical significance, as similarly found in the result of HNRNPA2B1 transcript (Fig. 5G). Among them, more than half of m6A regulators expression differential between two groups (Fig. 5H).

M6a-regulated Signaling Pathways And Functional Enrichment

In order to explore the elements for the different prognostics between high and low risk groups, GSEA analysis was conducted to excavate the different signaling pathways and cell functional enrichment. As is shown in Fig. 7, biological functions related to cell proliferation and DNA synthesis were highly enriched in high-risk group which including cell cycle, spliceosome, folate involved one carbon metabolism, aminoacyl-tRNA synthesis, RNA degradation, DNA replication, purine and pyrimidine metabolism, mismatch repair (MMR) and nucleotide excision repair (NER), etc. IgA producing intestinal immune networks was observed to be silent in high-risk group and enriched in low-risk group (Fig. 6). FDR < 0.05 of signaling pathways and cell functions was used as inclusion criteria in GSEA analysis.

Then we searched for the differentially expression genes (DEGs) between high and low-risk groups. Using the R version tool, DEseq package, 338 DEGs get into our field of view. Heatmap and volcano plot were shown in figure S1. The 338 DEGs are analyzed in BINGO plugin of cytoscape, and the functional enrichments of three modules of GO analysis (BP, CC, MF) were output. In the cellular component analysis, we observed that most of the biological functions of enrichment on extracellular region and plasma membrane. Molecular functions dominated by these DEGs are more enrichment in receptor binding, organic acid transmembrane transporter activity, IgE binding, symporter activity, etc. Biological processes associated are mainly the regulation of response to external stimulus, cytolysis, humoral immune response, response of fibrinolysis, etc. (Fig. 7A). And these molecular functions and biological process can also be verified in the heatmap that output by R version (Fig. 7B).

We then put all the DEGs to STRING web tool (http://string-db.org/) to analyze the protein-protein interaction network to further determine the molecular mechanism of the DEGS (Fig. 7C). And the PPI network obtained from the String database is then imported in cytoscape for visualization. Green nodes represent the down-regulated genes and red nodes represent the up-regulated genes (in high-risk groups) (Fig. 7).

Relationship between m6A regulators and prognostic in TP53 mutant individuals

Finally, we assessed the relevance between m6A regulators expression and overall survival in TP53 mutant patient cohort. All the cases were categorized into two groups by median expression value of each gene, and Kaplan-Meier survival curves were plotted between high-expression and low-expression groups. According to the feedback information from the survival curves, we got that 4 regulators including ALKBH5, METTL3, HNRNPA2B1 and YTHDC1 which expression levels were significantly correlated with survival (figure S2).

Discussion

Lung cancer is the most common malignancy tumor among men and the second leading cause of death of malignancy in women that ranks only second to the breast cancer. In general, most patients are diagnosed with lung cancer with local or distant metastasis, hence the average five-year survival rate for diagnosed lung cancer patients is as low as 15%[22]. Therefore, systematic palliative is the most common clinical treatment for lung cancer, including radiotherapy, chemotherapy and immunotherapy[23]. As the most common mutated gene in human malignant tumors, TP53 gene plays a role in tumor formation, development and treatment. About 50% patients have TP53 mutations, which are often missense mutations[24]. Compared with wild-type patients, TP53 mutant individuals have poor prognostics and strong tolerance to chemoradiotherapy, hence it can be used as a good prognostic biomarker for patients with NSCLC[25]. p53 protein is the expression product of

TP53 gene, which is often not detected in normal individuals. But the abnormal activation and accumation of p53 in cell can be caused while TP53 gene is in a mutated state, and the mutant protein can be endowed with functional acquisition. It is in this way that the TP53 gene participate in many stages with tumor development and mediates the treatment tolerance of tumors[24].

Chemical modification of RNA is considered to have important biological functions. So far, more than 160 RNA chemical modification have been found in eukaryotes of which N-6 methylation that always as known as m6A is the most common. m6A was found in RNA transcript, RNA splice and RNA degradation in mammals. Babieri I showed that METTL3 and FTO could regulate the transcription of CEBP through its interaction with CEBP protein family and thus promotes the procession of AML [26]. However, with the discovery of the first “eraser”, FTO, the m6A-mediated methylation of the 6th nitrogen atom of RNA adenine has been recognized as a dynamic reversible regulation [27].

According to current studies, m6A plays a role in variety of human tumors, and can also be an important marker to predict prognostic in some tumors. As the core gene of the “writers”, METTL3 plays a vital part in many tumors. In LUAD, METTL3 can augment EGFR expression and promote the cyclization of mRNA through eIF3 [28]. In addition, high expression of METTL3 can be considered as a signal of poor prognosis in lung adenocarcinoma, hepatocellular carcinoma and gastric cancer[29]. In lung squamous cell carcinoma, tumors with high expression of demethylase FTO show greater aggressitivity and proliferation, and have greater apoptotic resistance (usually by acting on M2F1). Hence, FTO is clinically recognized as a prognostic factor for lung squamous cell carcinoma [30]. The two mentioned m6A regulators in our study are ALKBH5 and HNRNPA2B1. ALKBH5 is a member of Alk protein family, and its dysregulation has been found in many tumors. ALKBH5 contains a DSBH domain, which can bind to the ATP domain of DDX3 gene, thus affecting cell cycle, apoptosis, RNA degradation and other cellular processes[31]. In the present study, ALKBH5 expressions was varied in different malignant tumors. ALKBH5 expression is often low in colon cancer[32] and pancreatic cancers[33], while high in breast cancers[34] and lung cancers[35] is always a sign of poor prognosis. We focus on expounding the role of ALKBH5 in NSCLC. Evidence suggest that, reduced levels of FOXM1 N-6 methylation can inhibit proliferation and invasion phenotypes of lung cancer cells. This reduction can occurred in ALKBH5 knock-down individuals[36]. HNRNPA2B1 is closely related to a function called m6A-swicth. It has a HNRNPs domain which can specific structures thus to regulate RNA-protein interactions[12]. In addition, it can regulate the splicing of transcriptional exons and the formation of pre-miRNA, which is similar to METTL3—m6A “readers”[13].

In our study, we are devoting to constructed a signature that contains m6A regulators that were expected to predict the prognostic in TP53 mutant non-small cell lung cancer patients. We first analyzed the differences of m6A in TP53 mutant and wild-type NSCLC patients, and found that more than a half of m6A regulators expression was significantly different in two groups. We used the method of the consensus clustering to classify the TP53 mutant and wild-type cohorts into any subgroups respectively, in an attempt to find a better prediction method for tumor prognosis. Both two cohorts were classified into two groups under consensus clustering, and clinicalpathological heatmap told that clustering was associated with stage and N state in wild-type patients. Stage and M state were significantly related to clustering in patients with a TP53 mutated status. Unfortunately, our clustering was not a perfect predictor of patient outcome, regardless of whether an individual had a TP53 mutant. The following univariate cox regression analysis reveals that m6A regulators predicted

different prognostic risk with different status of TP53 gene. For example, high expression of ALKBH5 in TP53 mutant patients predicted a poor outcome, but has no significant effect in TP53 mutant individuals. We then established the prognostic signature in patients with TP53 mutant. Multivariate cox regression showed that a signature consisting ALKBH5 and HNRNPA2B1 could categorize the patients into high- and low- risk groups. Kaplan-Meier survival curve indicates there are significant differences between two groups obtained by the signature. The validity of the prediction was verified by univariate independent prognostic model and multivariate independent prognostic model. The forest map shows that the calculated risk score is a better predictor of patient prognostic risk than all stage, T stage, N stage, and M stage, which indicates the success of our signature. The risk curve also confirms the conclusion. It is worth mentioning that the majority of m6A regulators in high-risk group has a significantly higher expression. In order to analysis possible mechanism, GSEA enrichment analysis was carried out between high and low risk group next. It was found that the enriched pathways in high risk group were more related to cell proliferation and gene expression. The conclusion predicts the possible impact of m6A in patients with TP53 mutations. And the DEGs between groups were identified for further functional exploration. These DEGs were put in GO enrichment analysis and visualized. The results show that these DEGs were significantly enriched in functions as receptor binding, cytolysis and so on, which mostly occurred in extracellular region and plasma membrane.

In our study, we systematically analyzed the potential value and possible mechanism of m6A regulators in TP53 mutant NSCLC patients, and also obtained some consequential findings. However, there are still some limitations in this paper. First, all the data are from the TCGA database and should be validated in lung cancer patients; second, all the cases are from the United States, which could lead to regional and racial biases in the results; third, the exact mechanism is still unclear.

In short, these findings are exciting as they may provide new insights and explore new target for the diagnosis and treatment of patients with TP53 mutations in NSCLC patients.

Declarations

Ethics approval and consent to participate

All data are sourced from open accessed repository that require no ethical approval and consent to participate applicability.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

All the data in this study are download from public databases as described in the passage. Data could be acquire from https://portal.gdc.cancer.gov/.

Conflict of interests

The authors declare that there is no relative conflict of interests.

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Authors' contributes

Zhuochen Zhao, Junhu Wan and Manman Guo jointly responsible for the data acquisition and analysis; Yangxia Wang, Zhengwu Yang and Zhuofang Li involved in the production of the figures and tables; Zhuochen Zhao and Junhu Wan conceived and wrote the manuscript; Liang Ming approved the final manuscript.

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Figures

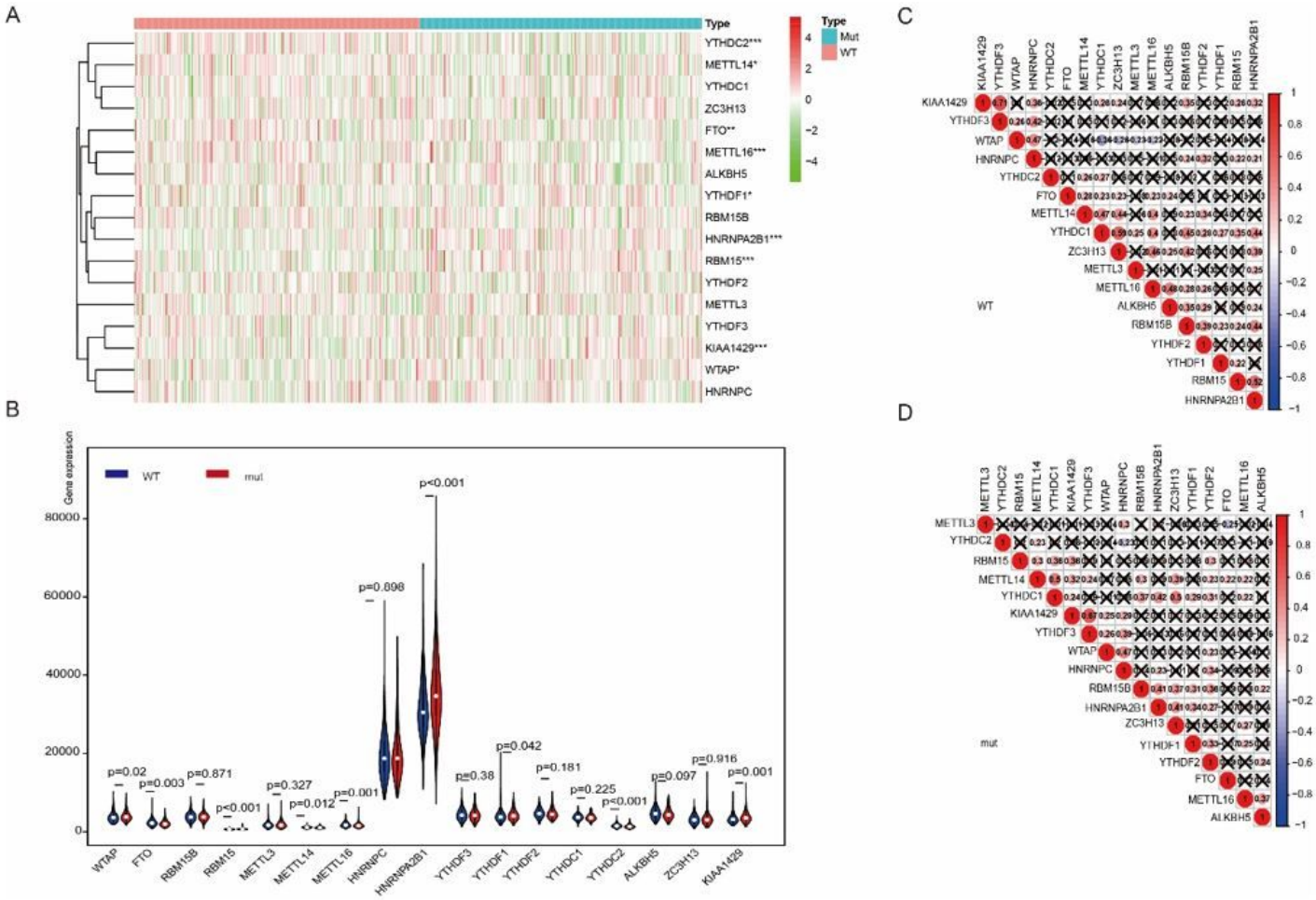

Figure 1

Differential expression of m6A regulators in TP53 wild-type and mutant type and correlation in respective cohorts. (A). The heatamp of m6A regulators expression in every individual. (B). Violin plot of m6A regulators expression differential in wild-type and mutant type. (C-D). Coexpression in m6A regulators based on the Pearson correlation in wild and mutant cohorts. *p<0.05, **p<0.01, ***p<0.005

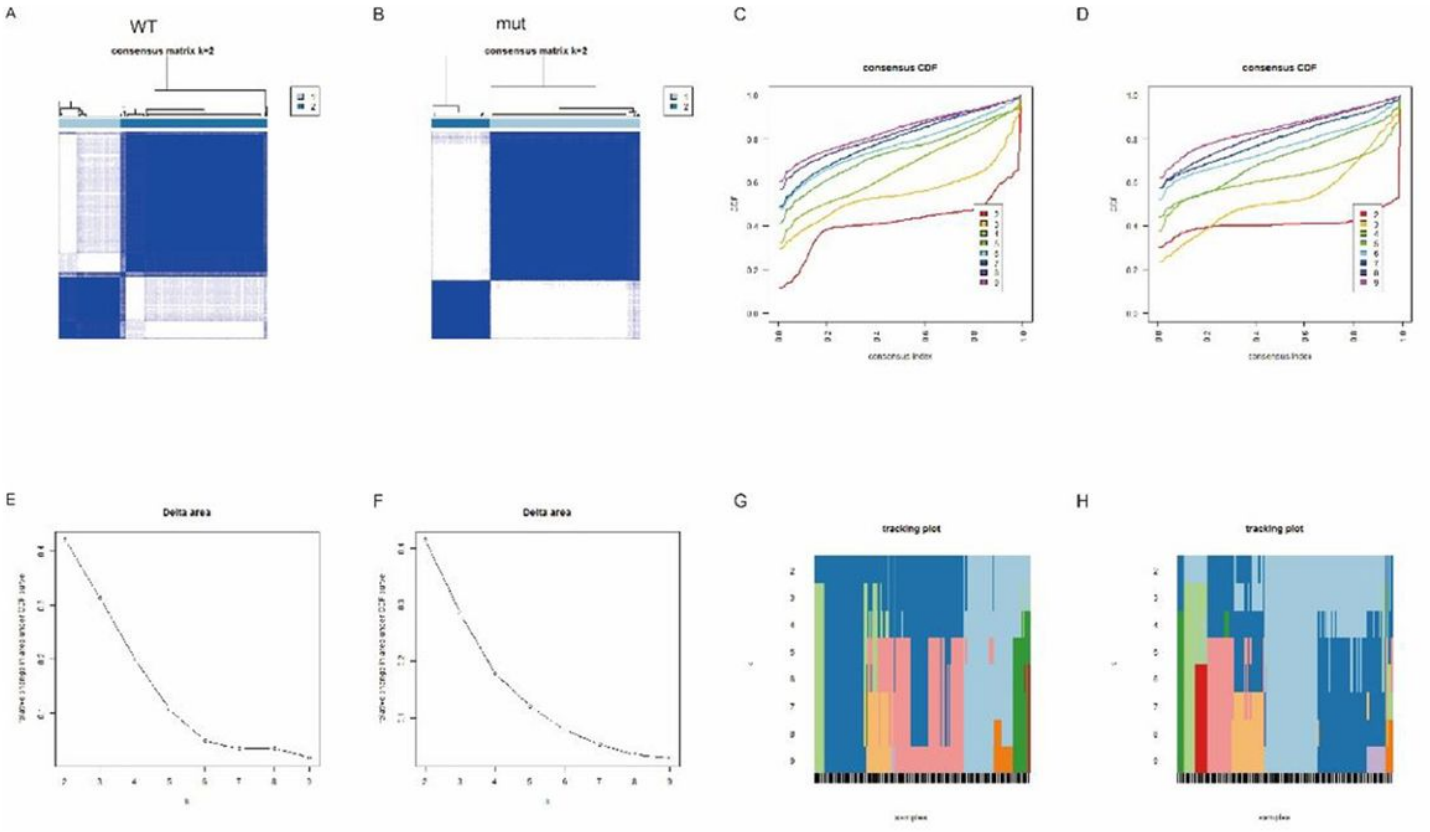

Figure 2

Consenscluster of respective cohorts.

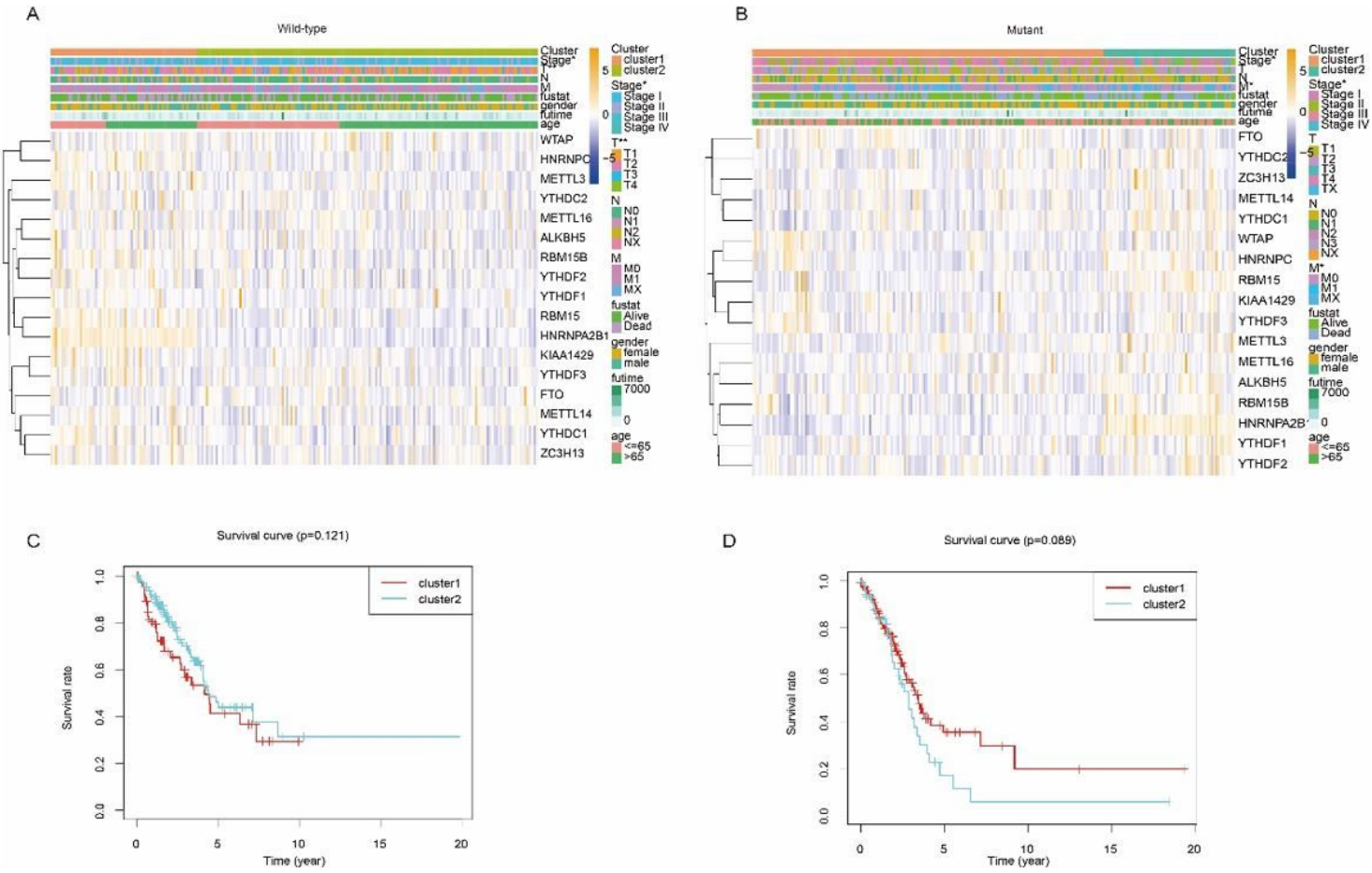

Figure 3

Survival and clinicalpathology characteristics between two subgroups in TP53 wild-type and mutant-type correspondingly. (A-B), relationship between m6A regulator and clinicalpathology; (C-D), Kaplan-Meier overall survival curves, elucidates the suvival of the two subgroups. * differences are statistically sinificant.

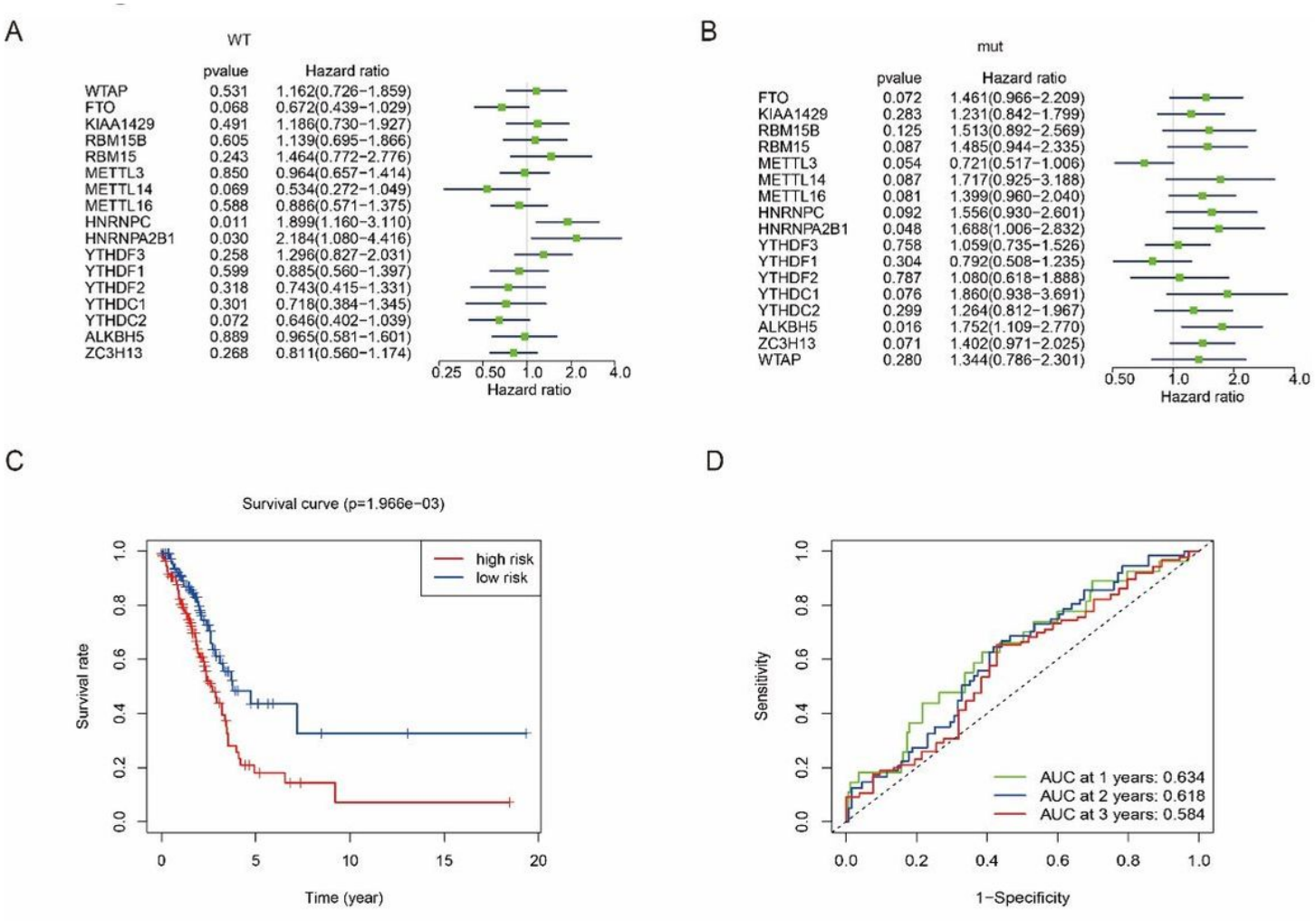

Figure 4

(A-B) Univariate cox for m6A regulators in patients with and without TP53 mutant. (C) Mutant cases were divided into two groups by gene signature, and the Kaplan-Meier curves between two groups, with pval=1.966e-03. (D) ROC curve of gene signature to predict the prognostic in TP53 mutant patients, AUC represents the sensitivity of prediction.

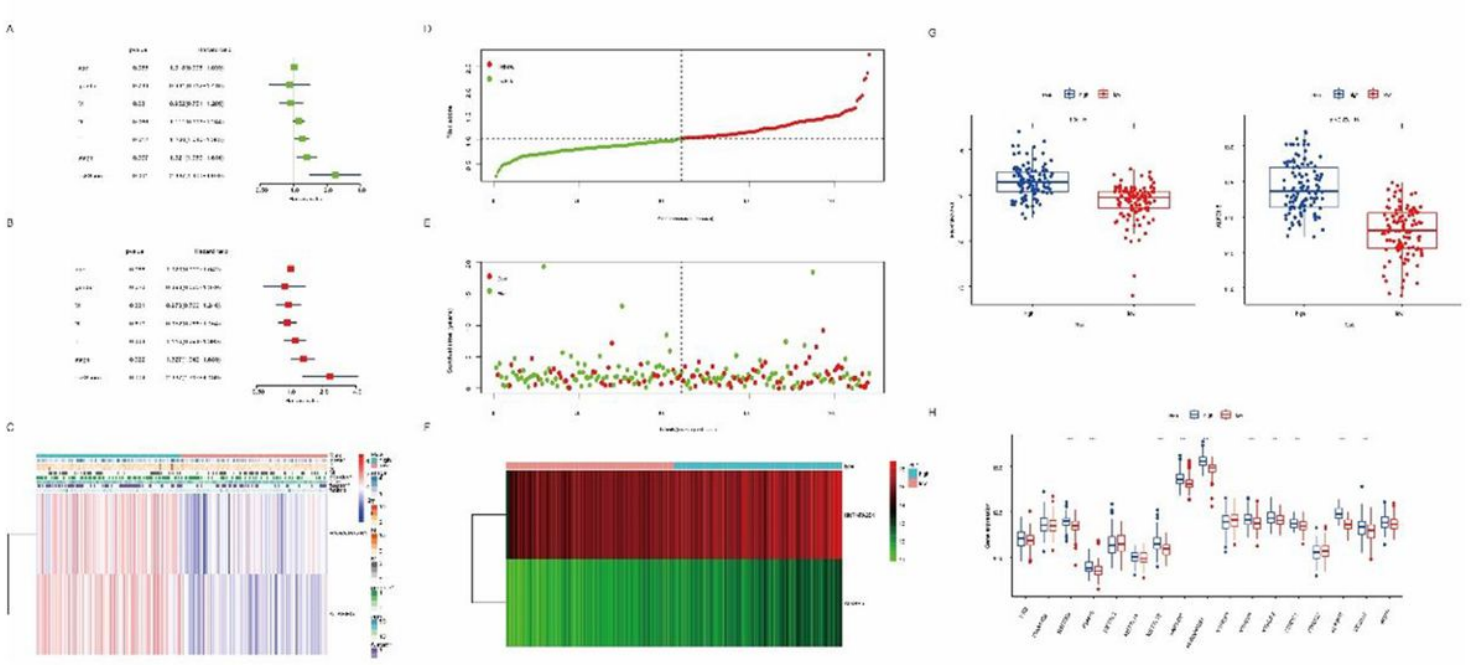

Figure 5

(A-B) Verification of gene signatures by univiriate cox regression and multiviriate cox regression. (C) Associated between clinicopathologic features and m6A regulators. (D-E) Risk score, survival time and survival status of patients. (F) The relationship between expression of m6A regulators and prognostic risk. (G) Expression of HNRNPA2B1 and ALKBH5 in two groups. (H) Expression of all m6A regulators. HR, harzard ratio.

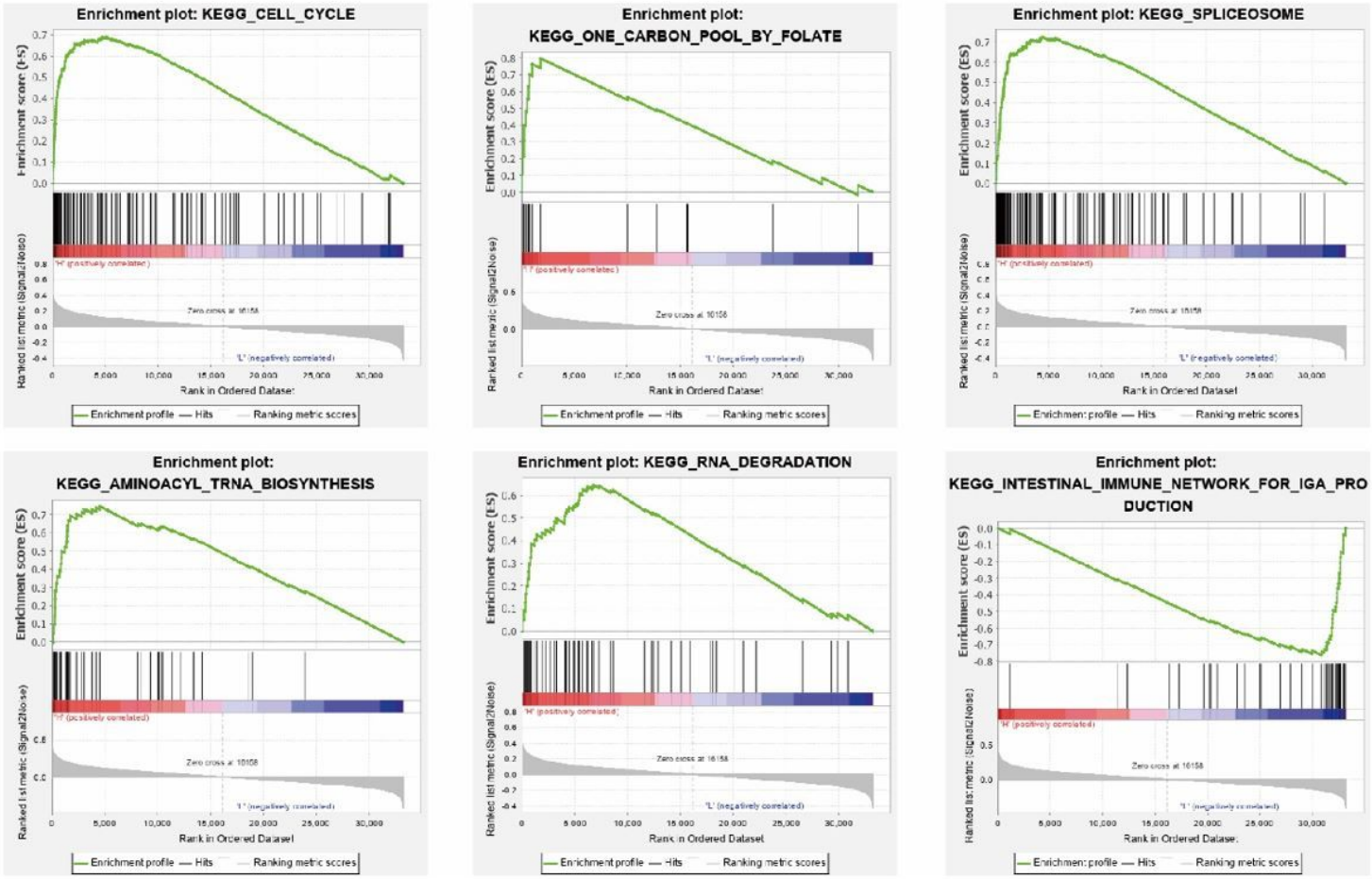

Figure 6

Performed GSEA analysis between two groups to seek different signaling pathways and cellular functions. It was revealed that cell functions in high-risk patients is more associated with spliceosome, cell cycle, folate and one carbon pool metabolism, aminoacyl tRNA synthesis, RNA degradation, DNA replication, purine/pyrimidine metabolism, MMR and NER, etc. The IgA secreting intestinal immune network was found in low-risk group, and that only this cellular function was enriched in low-risk group and silenced in high-risk group.

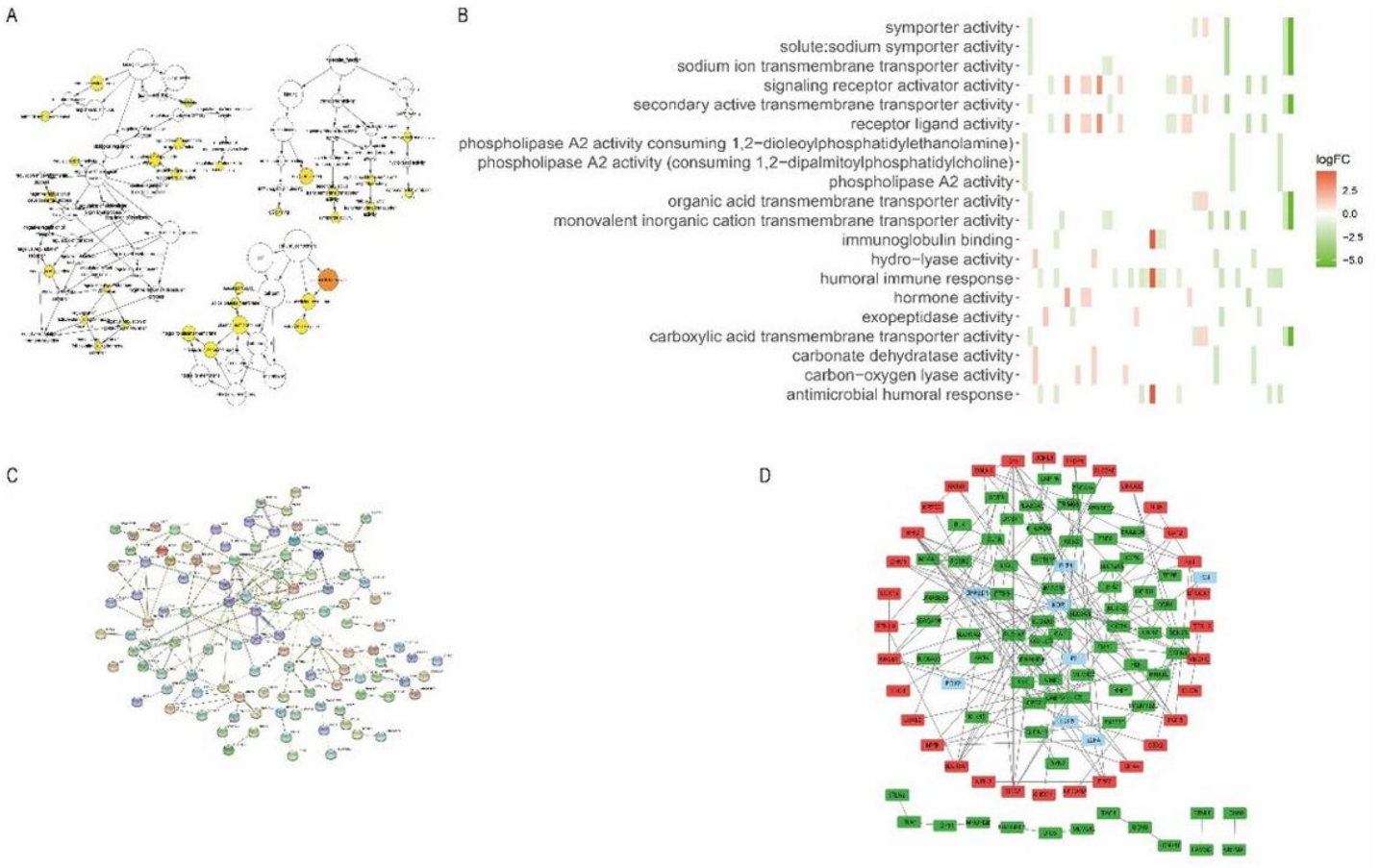

Figure 7

(A) Tree modules (CC, BP, MF) of GO analysis in cytoscape plugin BINGO. (B) Heatmap of GO functional enrichment analysis. (C) Protein-protein interaction natwork which accomplished in STRING. (D) Visualization of PPI netwaorks in cytoscape. CC, cellular component; BP, biological process; MF, molecular functions; GO, Gene Ontology.

Supplementary Files

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

- AdditionalFile1.docx