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Research Article

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Expression and Prognosis of m6A Methylation Factor in HNSCC

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Abstract

In this study, We comprehensively analyzed 20 genes from The Cancer Genome Atlas(TCGA) HNSCC database (YTHDF1, FTO, YTHDF2, YTHDF3, HNRNPA2B1, METTL14, METTL3, IGF2BP1, HNRNPC, IGF2BP2, RBMX, IGF2BP3, YTHDC1, YTHDC2, RBM15, RBM15B, VIRMA,ZC3H13,WTAP,ALKBH5) to predict whether there is an impact on the prognosis of patients with HNSCC. The importance of 20 m6A regulators such as VIRMA in the pathogenesis of HNSCC was emphasized. These findings will provide a therapeutic approach and new research direction for the expression of m6A in patients with HNSCC and its prognosis.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is a development of the mucosal epithelium of the mouth, pharynx and larynx, and is one of the most common types of head and neck cancer, generally associated with smoking, or exposure to some tobacco-derived carcinogens, excessive alcohol consumption and other undesirable lifestyle habits[1-3]. Although more efforts have been made to prevent and treat HNSCC, the number of people suffering from HNSCC has been increasing in recent years and the results remain unsatisfactory, with a 5-year adaptation amount of beneath than 50% [4]. Due to the molecular heterogeneity and variety of etiology of head and neck tumors, it is important to identify novel molecular biomarkers to improve the prognosis of patients with HNSCC. This study aimed to conduct a comprehensive study of the correlation between m6A methylation regulators and m6A-related genes and normal genes in HNSCC. In this study, the expression of 20 m6A methylation regulators in HNSCC and the factors associated with the prognosis of HNSCC were identified. At last,we found that m6A methylation regulation plays an important role in the development and prognosis of HNSCC and can be used to predict the prognosis of patients with HNSCC.

2. Methods and Materials

2.1 Data Collection

We retrieved mRNA expression and clinical data from the Cancer Genome Atlas (TCGA) database via cBioPortal. All data is publicly available and open. We only use anonymous statistical gene expression. To collect mRNA expression data for 502 tumors and 44 normal tissues, as well as clinically relevant information, including age, gender, clinical stage, smoking status, and HPV infection, the researchers used the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) to obtain transcriptome data and matching clinical data for HNSCC.

2.2 RNA Sequence Analysis and m6A-Related Genes

We selected 20 m6A methylation regulators considered important from the TCGA dataset,includingYTHDF1,FTO,YTHDF2,YTHDF3,HNRNPA2B1,METTL14,METTL3,IGF2BP1,HNRNPC,IGF2BP2,RBMX,IGF2BP3,YTHDC1,YTHDC2,RBM15,RBM15B,VIRMA,ZC3H13,WTAP,ALKBH5,we mainly studied the relationship between these genes, as well as the clinicopathological characteristics and overall survival of patients with HNSCC.

2.3 Bioinformatic Statistics

We used raw counts of RNA-sequencing data (level 3) and corresponding clinical information from 20 were obtained from The Cancer Genome Atlas (TCGA) dataset (https://portal.gdc.cancer.gov/) , in which the method of acquisition and application complied with the guidelines and policies. The m6A-related genes are derived from Juan Xu's research on the molecular characterization and clinical significance of m6A modulators across 33 cancer types [5]. All the above analysis methods and R package were implemented by R foundation for statistical computing (2020) version 4.0.3 and software packages ggplot2 and pheatmap. We conducted a Kaplan–Meier (KM) survival analysis on the UALCAN website (http://ualcan.path.uab.edu/index.html). to assess the effect of m6A methylation regulator on survival, and in the test, p-value less than 0.05 was considered a significant threshold Next, tumor samples were divided into two groups using the Consensus Cluster Plus.and PCA analysis

was performed to validate the packet results and finally, in order to understand the prognosis of m6A methylation modulators in patients with HNSCC,we used univariate Cox regression analysis to determine the prognosis of m6A methylation modulators [6,7]. It has been shown that through this study, three genes have a significant correlation with survival rate (P < 0.05) [8]. Using the LASSO cox regression technique, these genes were chosen for further investigation and development of possible risk profiles. Three genes were discovered to be significant prognostic variables. The following formula was used to compute each patient's risk score:

$$Risk\ score = \Sigma_{i=1}^{n} coef_i \times x_i$$

4. Statistical Analysis

The expression levels of these 20 m6A methylation regulators present in tumor tissue and normal tissues were compared using Wilcoxon's test, and The Wilcoxon rank sum test was used to calculate the relationship between the expression of 20 gene in HNSCC patients with age, sex, tumor location, smoking status, and HPV status. and the link between m6A regulators in the clinicopathological aspects of patients with HNSCC was analyzed using one-way analysis of variance. Patients were divided into two groups based on the median level of risk score, operating system differences between the two groups were further analyzed, and the prognosis of the predictive model was compared using the Kaplan-Meier survival curve. Statistical significance is defined as a P value of less than 0.05.

5. Results

5.1 In HNSCC, m6A RNA Methylation Regulators are Expressed

The goal of this research was to see if the m6A methylation gene was linked to HNSCC prognosis. The heatmap showed that FTO (P<0.01) · IGF2BP2 (P<0.001) · IGF2BP1 (P<0.001) · IGF2BP3 (P<0.001) · VIRMA (P<0.001)) · YTHDF3 (P<0.001) · YTHDF2 (P<0.001) · METTL14 (P<0.001) · WTAP (P<0.001) · RBM15 (P<0.001) · METTL3 ((P<0.001) · HNRNPC (P<0.001)) · RBMX (P<0.001),HNRNPA2B1 (P<0.001) · YTHDF1 (P<0.001) and RBM15 (P<0.001) were significantly upregulated in cancer tissues (see Figure 1A). In addition, ZC3H13□YTHDC2、RBM15 expression in cancer tissues was considerably lower than in normal tissues (P□0.01); see Figure 1A. The highest associations among these m6A-related genes were found between VIRMA and YTHDF3, HNRNPA2B1 and RBMX, and METTL14 and YTHDC1. (see Figure1B). We performed subgroup analyses,In the figure 2, we can see that in the factor of less than or equal to 60 years old, METTL14, RBM15, YTHDC1, YTHDF2, HNRNPA2B1 These genes are highly expressed(see Figure2A) and for sex, HNRNPA2B1, ALKBH5 is higher in male than female in gene expression, which is meaningful for HNSCC gene expressionsee(Figure2B). For the location of tumors, the expression of IGF2BP1 in the T3+T4 stage is much higher than that in the T1+T2 stage, which is statistically significant,(Figure2C-D) while HPV infection and smoking are not statistically significant in the expression of genes(Figure2E-F).

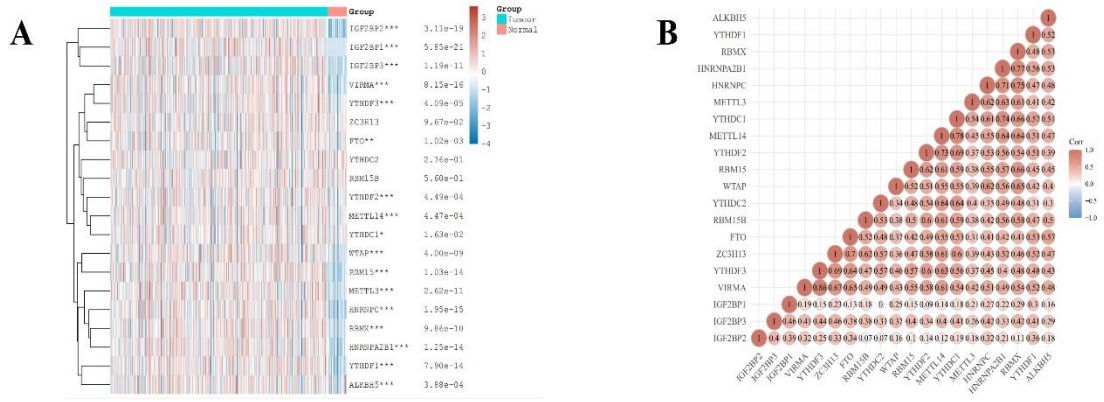

Figure 1: Expression pattern of m6A RNA methylation regulators in HNSCC. (A) Comparison of expression levels of 20 m6A RNA methylation regulators between normal tissues and tumor tissues. ** represents for P<0.01; *** represents for P<0.001. m6A, N6-methyladenosine; HNSCC, head and neck squamous cell carcinoma. (B) Spearman correlation analysis of 20 m6A RNA methylation regulators in HNSCC.

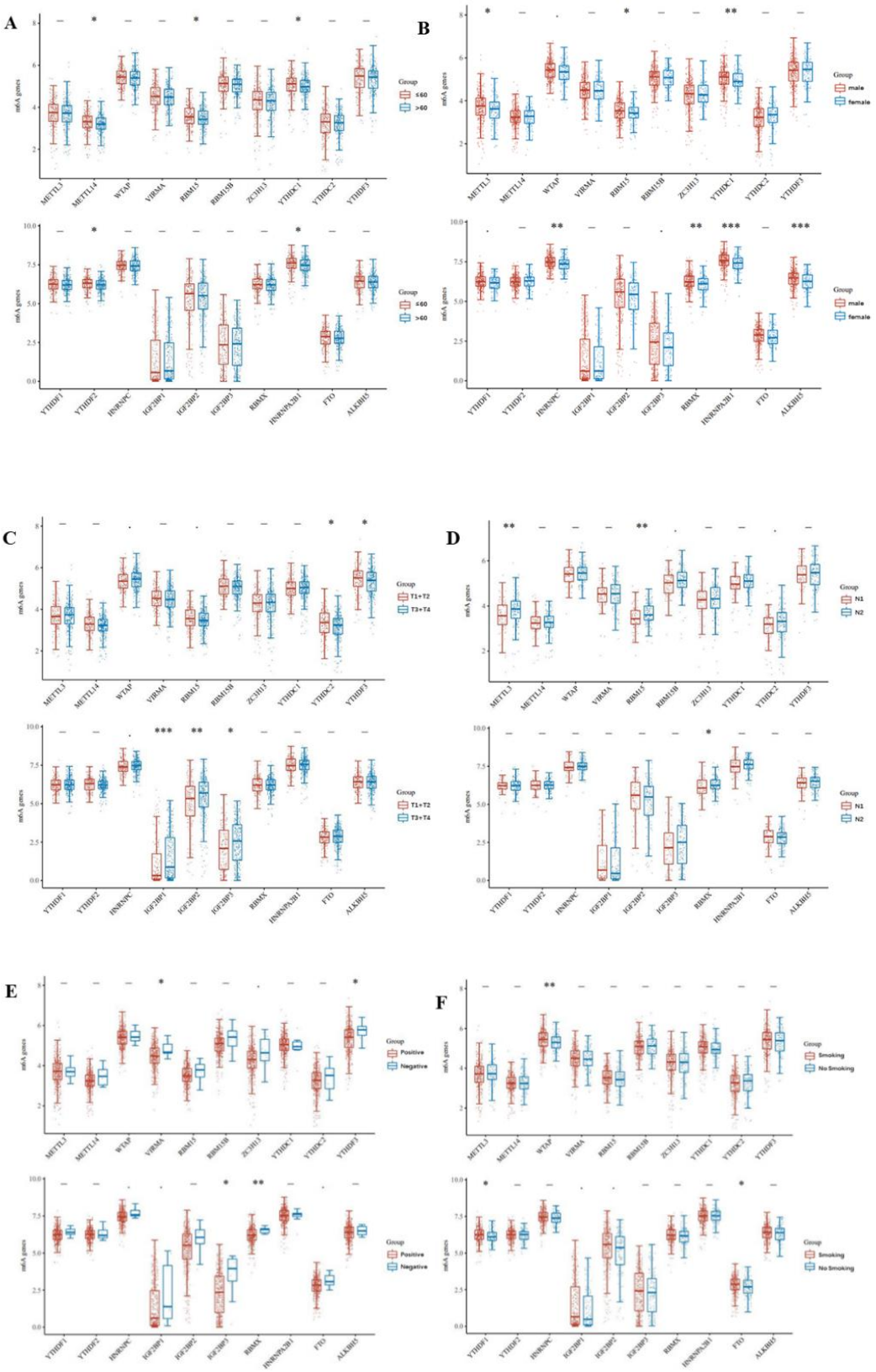

Figure 2: Methylation subgroup analysis in patients with HNSCC.(A) Expression of m6A gene related to age in HNSCC.(B) Expression of m6A gene related to sex in HNSCC.(C,D)Expression of m6A gene related to tumor location in HNSCC.(E) Expression of m6A gene related to HPV status in HNSCC.(F) Expression of m6A gene related to smoking status in HNSCC.

5.2 Survival Analysis of m6A Methylation-Related Genes

The survival information for 20 genes was found using the web program UALCAN (http://ualcan.path.uab.edu/index.html). Figure 3(A-B) demonstrates that patients' survival rates were substantially lower ($P < 0.05$) when two genes, HNRNPC and IGF2BP2, had high levels of expression.

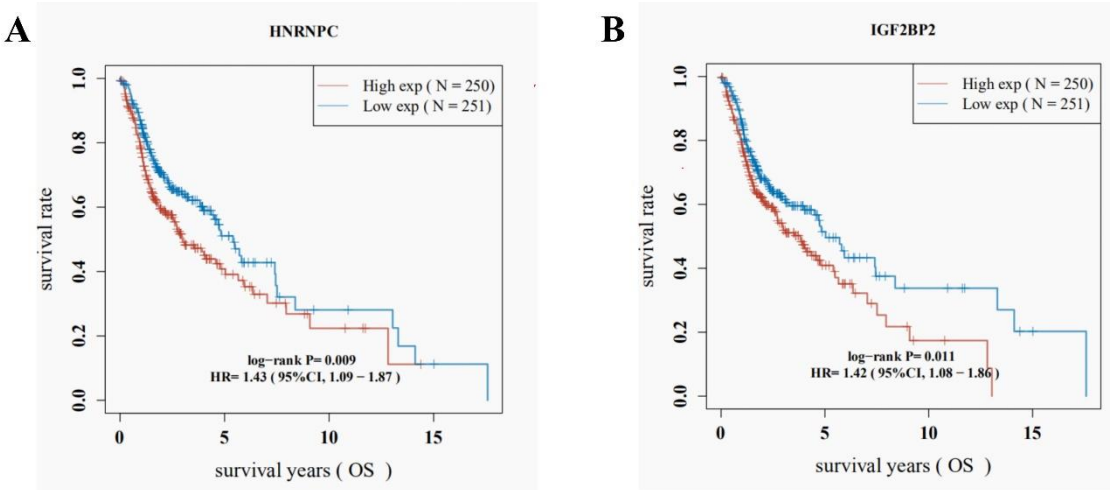

Figure 3: (A–B) Prognostic information for two of 20 genes, which had a significantly worse survival rate ($P < 0.05$).

5.3 The Expression of m6A RNA Methylation was Used to Classify the Clusters

The 502 HNSCC cancer tissues were grouped using the Consensus Cluster Plus package. We tried to divide these samples into two or three groups based on the value of the cumulative distribution function. We found that splitting the samples into two groups made sure that they were distinctly different from one another. (see Figure 4A,4B). PCA was then used to corroborate the categorisation. According to the findings, the Cluster 2 subgroup had a greater OS than the Cluster 1 subgroup (See Figure 4C for further information).

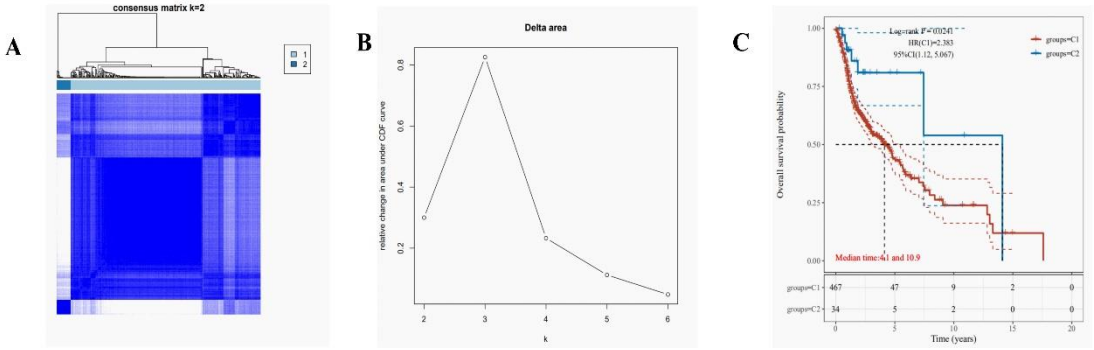

Figure 4: Consensus cluster classification by m6A RNA methylation regulators. (A) Consensus clustering matrix for k=2. (B) Relative area change under the cumulative distribution function curve for k=1 to 6. (C) Kaplan-Meier OS curves of HNSCC patients. Clusters 1 and 2 were marked red and blue, respectively.

5.4 Prognostic Role of m6A RNA Methylation Regulators in HNSCC

To investigate the predictive relevance of m6A RNA methylation regulators in HNSCC, we used a Cox univariate analysis (see Figure 5A). In particular, increased IGF2BP2 and HNRNPC expression was associated with a lower chance of survival in HNSCC patients [IGF2BP2 hazard ratio (HR) =1.4172, 95% confidence interval (CI) =1.0817–1.8568]. [HNRNPC hazard ratio (HR) =1.4313, 95% confidence interval (CI) =[1.0943–1.872]. To develop prognostic risk characteristics for HNSCC, the Kaplan-Meier method was used to calculate operating system differences between different clusters. Significantly better OS was found in patients in specific cluster 2 compared to other cluster patients. (See Figure 5B), next, we performed a LASSO regression analysis of these 20 m6A factors based on the TCGA database, and then we obtained three genes, RBM15, YTHDC1, and HNRNPC, which were used to calculate the risk scores of patients with HNSCC. (See Figures 5C,5D). We compared the prognostic effects of risk factors through ROC, and the results showed that the area under the curve (AUC) of 1-year survival was 63.7%, which can be used as a prognostic biomarker for HNSCC (See Figures 5E) .

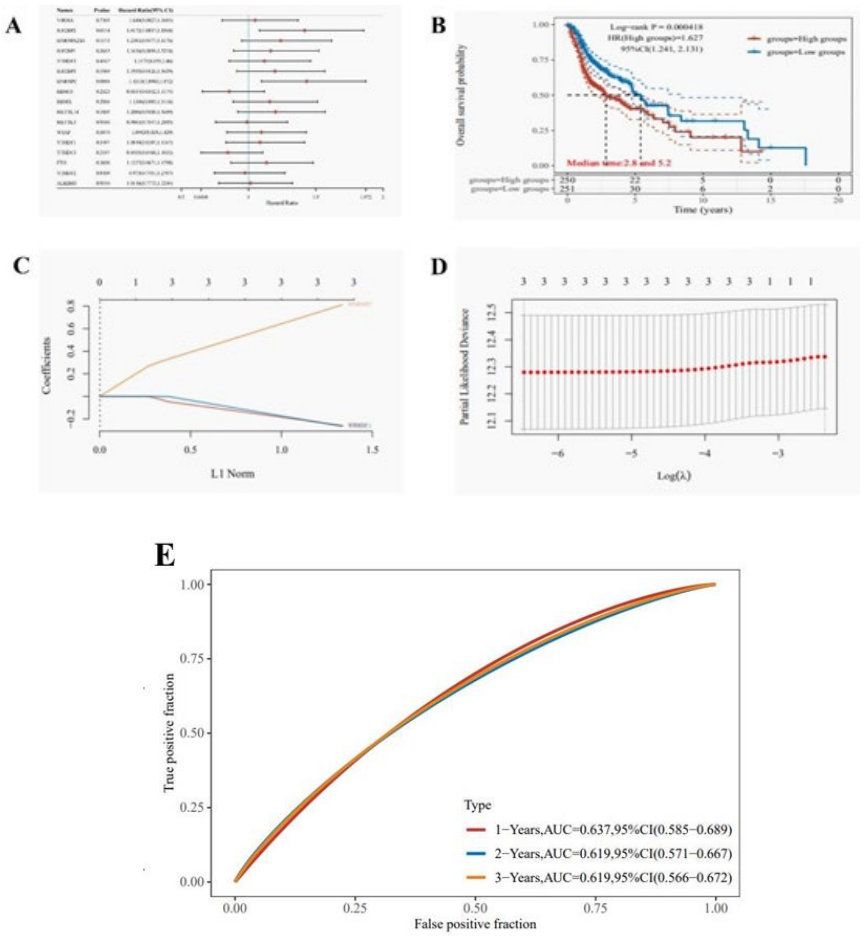

Figure 5: The risk signature comprising three m6A RNA methylation regulators. (A) Univariate Cox analysis of 12 m6A RNA methylation regulators in HNSCC patients. HRs and 95% CIs were calculated. (B) Kaplan-Meier OS curves of HNSCC patients assigned to the high- and low-risk groups. (C-D) Coefficients of three m6A RNA methylation regulators calculated by LASSO Cox regression algorithm. (E) ROC curves of the survival model in HNSCC (AUC = 63.7%).

6. Discussion

According to epidemiological statistical studies, HNSCC is the sixth most common cancer in the world, and the incidence of HNSCC continues to rise every year. Although the five-year survival rate has increased, it is also less than ideal, and the overall survival rate remains at 40%-50%, and the prognosis for patients with HNSCC is still very poor [9-11].

In recent studies, m6A methylation modification has played an important role in both tumor occurrence and metastasis, but we know very little about the effects and internal roles of m6A methylation in regulation, development and prognosis in HNSCC, so it is necessary to conduct in-depth research on the effect of m6A methylation on HNSCC.

We analyzed the relationship between different expression patterns and regulators between tumor tissue and normal tissue, and the results showed that sixteen of the 20 regulators(IGF2BP2,IGF2BP1,IGF2BP3,VIRMA,YTHDF3,FTO,YTHDF2,METTL14,WTAP,RMB15,METTL3,HNRNPC,RBMX-,HNRNPA2B1,YTHDF1,ALKBH5) expressed significantly differently in tumor tissue compared with normal tissue.

In recent years, the concept of precision medicine has promoted subcomponent typing for individual research subjects, the most classic example of which is the molecular typing of breast cancer, and different subgroups have different pathogenic mechanisms and clinical prognostic characteristics. Similarly, a single sample is grouped using different functional genomes to further select subgroups of interest for differential analysis. Based on gene expression levels, the data were divided into two subgroups using R's Consensus Cluster Plus package. Principal component analysis shows that there is a separation between subgroup 1 and subgroup 2. Total survival analysis showed a significantly improved survival in subgroup 2.

The Lasso (Least absolute shrinkage and selection operator) method is a compression estimate. It constructs a penalty function to get a more refined model, so that it compresses some coefficients and sets some coefficients to zero. Therefore, it retains the advantages of subset shrinkage, which is a kind of processing biased estimation with complex collinearity data, and the selection of variables can be realized while parameter estimation, and the multicollinearity problem in regression analysis is better solved, when the lambda minimum model reaches the optimal. In general the LASSO algorithm analyzes all independent variables and selects the most influential variables .

Much more accurate than traditional regression methods. According to Lasso analysis, we obtained three meaningful genes(RBM15,YTHDC1,HNRNPC) from 20 genes, and the predictive ability of m6A methylation-related genes for HNSCC prognosis was assessed by ROC curve. The predictive ability of m6A methylation-related genes for HNSCC prognosis was assessed by ROC curve. The results showed that m6A methylation-related genes affected HNSCC survival. Finally, we concluded that m6A methylated genes play a role in the survival of HNSCC.

The heterogeneous nuclear ribonucleoproteins subfamily includes the RNA-binding protein known as HNRNPC (hnRNPs) [12,13]. HNRNPC interacts with pre-mRNAs in the nucleus and functions in translation, nuclear retention and export, alternative splicing, stability, and translation [14-16]. HNRNPC has been shown to be significantly expressed in a variety of malignancies, including hepatocellular carcinoma[17], breast cancer and glioblastoma [18,19]. The significance of HNRNPC in is, however, still poorly understood. Our research served as a springboard for further investigation.

IGF2BP belong to an evolutionarily conserved family of RNA binding membranes, including IGF2BP1, IGF2BP2, and IGF2BP319 in the human genome[20]. The similarity of the canonical structures of these three IGF2BP proteins leads to similar biochemical functions [21]. According to research reports, IGF2BP is expressed poorly in adult tissues, and highly expressed in embryonic tissues, which increases synthesis in malignant tumors, among which the prognosis of high level expression is poor, its overexpression promotes tumor growth and metastasis, and the function and mechanism of IGF2BP involved in tumor development are also slowly being confirmed [22].

Overall, In terms of cancer prognosis, the usage of m6A methylation-related genes offers a lot of promise. and our preliminary findings suggests that the expression levels of m6A methylation-related genes play a significant role in the progression of HNSCC and may serve as a role in the progression of HNSCC. However, this study also has certain limitations, and more research is needed in the future to further clarify these findings.

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