Investigate the Function and Immune Correlation of the Methylation Modification of m6A Regulators in DLBCL

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Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is the major subtype of non-Hodgkin lymphoma, whose treatment still has a major challenge. The dysregulation of N6-methyladenosine (m6A) modification is a common type of messenger ribonucleic acid (mRNA) modification. However, there is a lack of research into m6A RNA methylation regulators in DLBCL. Methods: We down loaded the data of 48 patients from The Cancer Genome Atlas (TCGA) database. The relationship between the expression level of m6A RNA methyla tion regulators and clinicopathological variables in DLBCL was analyzed by R language. Results: The results showed that the expression of m6A gene was abnormal in DLBCL tissues compared with normal tissues (P<0.05). GO functional analysis showed that m6A gene was involved in different biological functions during the development of DLBCL. The alteration of m6A gene is correlated with tumor stage and immune function. Next, according to ssGSEA algorithm, IGF2BP2 and YTHDF2 are correlated with a number of signaling pathways, which can provide a theoretical basis for further research on the occurrence and development mechanism of DLBCL. Conclusions: The m6A RNA methylation regulators are involved in DLBCL cancer progression.

Keywords
m6A, DLBCL, IGF2BP2, YTHDF2

1. Introduction

DLBCL is the major subtype of non-Hodgkin lymphoma, with clinical and biological heterogeneity [1]. Although the standard first-line chemotherapy regimen R-CHOP (rituximab + cyclophosphamide + doxorubicin + vincristine + prednisone) enables disease-free survival in more than 50% of DLBCL patients, approximately one-third of cases are not cured by standard immunochemotherapy, which remains a challenging clinical problem [2]. In recent years, with the progress of science and technology, more and more genes are involved in the occurrence and development of DLBCL. To further determine how these genes play a regulatory role in the occurrence and development of DLBCL is also one of the research hotspots.

M6A has been identified as the most common and abundant internal RNA modification in eukaryotes, playing different roles in various biological processes [3]. Accumulating evidence confirms that the most important role of m6A is to regulate eukaryotic transcriptome mRNA splicing, export, localization, translation and stability, thereby altering a series of malignant biological behaviors, such as cell proliferation, invasion and transfer [4]. In addition, several studies have shown that m6A RNA methylation plays an important role in tumorigenesis and development, and m6A methylation may serve as a new diagnostic biomarker and therapeutic target for cancer [5].

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2. Methods

2.1 Data collection

The RNA-seq transcriptome data of 48 DLBCL patients and their corresponding clinical and prognosis information were acquired from TCGA database (https://www.aclbi.com/static/index.html#/tcga).

2.2 m6A RNA methylation modulator selection

A total of 19 genes are recognized as vital m6A methylation modulators, including METTL3, METTL14, WTAP, RBM15, RBM15B, ZC3H13, YTHDC1, YTHDC2, YTHDF3, YTHDF1, YTHDF2, HNRNPC, IGF2BP1, IGF2BP2, IGF2BP3, RBMX, HNRNPA2B1, FTO and ALKBH5.

2.3 Bioinformatics analysis

The expressions of 19 regulatory factors in 48 tumor tissues and 928 normal tissues were visualized and analyzed by heatmap and barchart used the TCGA database. Next, the Consensus Cluster Plus package was used to divide tumor samples into two groups. A Gene Ontology (GO) functional enrichment analysis was performed to verify the function of the 18 m6A methylation modulators.

2.4 Immunity correlation

TCGA dataset immune correlation analysis dataset was used to analyze the correlation between m6A methylation modulators and immune cells, and the multi-gene correlation map was displayed by R software package P heatMap.

2.5 Gene and pathway correlation

According to ssGSEA algorithm, the correlation between IGF2BP2, YTHDF2 and pathways was calculated to obtain the relationship between genes and pathways.

2.6 Statistical analysis

The Wilcoxon’s test was used to compare the expression level of the m6A RNA methylation regulators between the tumor and normal tissues. \( P \) values of less than 0.05 were considered statistically significant.

3. Results

3.1 The expression of m6A RNA methylation regulators in DLBCL

We compared the expression of m6A RNA methylation in tumor and normal tissues based on RNA data extracted from TCGA database, and identified 19 differentially expressed regulators. The heatmap and the barchart both showed that 18 regulatory were significantly upregulated in cancer tissues. However IGF2BP2 expression was significantly lower in cancer tissues (Figure 1A, B).

As shown in Figure 1 (C, D), Pearson showed correlation expression among 19 regulatory. The highest correlation was observed for YTHDC1 and YTHDC2 with a correlation coefficient of 0.93. The correlation coefficient between YTHDC1 and METTL14 is 0.91, respectively.

3.2 Cluster classification based on m6A RNA methylation

We used R's Consensus Cluster Plus package to identify different subgroups of DLBCL samples. As shown in Figure 2 (A, B), All patients were successfully divided into two subgroups based on the most stable k value. As shown in Figure 2 (C, D), Principal component analysis shows that subgroup 1 and subgroup 2 can be merged.

3.3 Confirmation of the function of m6A RNA methylation regulators

We conducted GO functional analysis to verify the function of the m6A RNA methylation regulators. Our results showed that the biological processes involved in these genes are regulation of mRNA metabolic process, regulation of mRNA stability, and regulation of mRNA catabolic process (Figure 3A).

Among the genes related to m6A methylation, multiple genes participate in regulation of mRNA metabolic process. However, METTL3, METTL14, YTHDF3, YTHDF1, YTHDF2, HNRNPC, IGF2BP1, IGF2BP2,
IGF2BP3, FTO and ALKBH5 involved in regulation of mRNA stability, regulation of RNA stability and regulation of mRNA catabolic process (Figure 3B).

The most significant biological processes in which m6A RNA methylation regulators are involved are regulation of mRNA metabolic process, regulation of mRNA stability, methyltransferase complex, nuclear speck (Figure 3C, D).

Figure 1. Expression pattern of m6A RNA methylation regulators in DLBCL. (A) Comparison of expression levels of m6A RNA methylation regulators between normal tissues and tumor tissues. (B) Barchart visualizing the differentially expressed regulators in DLBCL. The normal tissues were marked blue, and the cancer tissues were marked red. (C) Spearman correlation analysis of m6A RNA methylation regulators in DLBCL. (D) Circles represent the m6A-related mRNA.

Figure 2. (A) Consensus clustering matrix for k=2. (B) Relative area change under the cumulative distribution function curve for k=2 to 6. (C) PCA of the total RNA expression profile of two clusters in TCGA database. (D) Kaplan-Meier OS curves of DLBCL patients.
3.4 Relationship between the m6A and the clinicopathological features

To examine the relationship between the m6A RNA methylation regulators and the clinicopathological features of DLBCL patients, we analyzed the clinical significance of these regulators. The results showed that the expression of IGF2BP1 was significantly correlated with tumor (T) stage (Figure 4A).

3.5 Relationship between m6A and immunity

As shown in Figure 4B, YTHDF2 was positively correlated with Endothelial cell, and the correlation coefficients $r$ were 0.40, IGF2BP2 was positively correlated with Endothelial cell, Macrophage and NK cell. RBM15, YTHDC1, YTHDF1, HNRNPC and HNRNPA2B1 was positively correlated with Endothelial cell. IGF2BP2 was negatively correlated with B cell.

3.6 Correlation between m6A and pathways

We collect related pathways include collection of genes, according to the ssGSEA algorithm, gene expression and pathways score by calculation correlation, Passage relations of m6A RNA methylation regulators IGF2BP2 and YTHDF2 were obtained. As shown in Figure 5 A, IGF2BP2 was associated with Tumor Inflammation Signature, Cellular response to Hypoxia, EMT Markers, ECM Related genes, Angiogenesis, Apoptosis, Inflammatory response, PI3K-Akt-mTOR pathway, P53 pathway, TGFB, IL-10 Anti Inflammatory Signaling Pathway, Genes up-regulated by reactive oxygen species (ROS). As shown in Figure 5 B, YTHDF2 was associated with Cellular response to Hypoxia, Tumor proliferation signature, G2M checkpoint, PI3K-Akt-mTOR pathway, MYC targets, TGFB. The above findings can further provide a basis for studying the mechanism of the occurrence and development of DLBCL.
Figure 4. (A) Correlation between m6A RNA methylation regulators and the clinicopathological features of DLBCL patients. (B) Relationship between m6A RNA methylation regulators and immunity.

Figure 5. (A) IGF2BP2 is associated with various pathways. (B) YTHDF2 is associated with various pathways.
4. Discussion

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma with heterogeneity in many aspects [6]. The latest cancer statistics report shows that the incidence and mortality of non-Hodgkin's lymphoma have increased [7]. Clinically, approximately 50–70% of patients with DLBCL can be controlled with the R-CHOP regimen, while some patients relapse and are refractory [8]. Therefore, it is urgent to find a new and effective treatment.

m6A, as the richest post-transcriptional modification in RNA, has been reported to be associated with different metabolic processes in various RNA [9]. Studies have shown that m6A modifications are involved in the occurrence and development of various cancers [10]. In this study, we analyzed the expression of m6A modification-related genes in DLBCL, and found that DLBCL tissues were abnormally expressed compared with normal tissues (Figures 1A, B).

Our study shown that m6A regulators are aberrantly expressed in DLBCL and that their interactions are complex. The same m6A regulator can be positively correlated with another regulator, and at the same time, negatively correlated with another regulator. More interestingly, our study also found that DLBCL patients could be divided into two groups based on differentially expressed m6A regulators. Compared with specific genes, multiple dysregulated genes derived from patient tumor tissue can provide better accuracy in cancer prognosis and potentially provide more effective and personalized treatment regimens [11]. However, our results indicated that there was no significant difference in survival between the two groups.

Another highlight achieved in this study is that we constructed a functional enrichment map based on m6A regulators. Our results showed that the main biological processes involved in these m6A regulators include regulation of mRNA metabolism, regulation of mRNA stability and regulation of mRNA catabolism. The above results show that m6A regulators in DLBCL are involved in different biological processes, thereby promoting the occurrence and development of the disease. Further experiments can be carried out to verify the above results to provide a basis for the occurrence, development and treatment of DLBCL. Due to the abnormal expression of multiple m6A in DLBCL and the complex relationship between some regulatory factors, bioinformatics analysis has become an effective way to explore the core genes of DLBCL and provide potential targets for tumor therapy. In our study, we used the TCGA dataset to extract the clinical data of patients. Bioinformatics analysis revealed that the regulator IGF2BP2 was associated with clinical stage of DLBCL patients, thus providing an important candidate gene for further investigation.

IGF2BP2 is a member of the insulin-like growth factor mRNA-binding protein family. IGF2BP2 is localized in the cytoplasm and can also enter the nucleus. Several studies have reported that IGF2BP2 is associated with the development of various tumors [12] and metabolic diseases such as liver cancer [13] and colorectal cancer [14], and can participate in cell proliferation by m6A modification of Mrna [15] to activate MAPK and PI3K/Akt [16] signaling pathways. Numerous studies have revealed the close relationship between m6A and the immune microenvironment [17]. This study found that IGF2BP2 was associated with clinical stage of DLBCL, and consistent with other studies, there is a significant positive correlation between IGF2BP2 and PI3K-Akt-mTOR signaling pathway. In addition, the study further found that IGF2BP2 was also positively correlated with apoptosis, tumor inflammatory characteristics, P53 signaling pathway. The above results indicated that IGF2BP2 may be involved in the development of DLBCL, and there is a certain relationship with DLBCL cell apoptosis. These findings demonstrate that IGF2BP2 may be used as a tumor biomarker in DLBCL in the future.

YTHDF2 is one of the main readers of m6A. Studies have shown that YTHDF2 is involved in the occurrence and development of various malignant tumors. Zhang found that the abnormal expression of YTHDF2 was negatively correlated with the survival of patients with liver malignancies, and promoted the expression of tumor hepatocytes and the metastasis of tumors. In addition, studies have found that YTHDF2 is involved in the occurrence and development of gastric cancer, acute myeloid leukemia, multiple myeloma, pancreatic cancer and prostate cancer, and is related to disease prognosis. Our study found that YTHDF2 was abnormally expressed in DLBCL tissues and was positively correlated with the immune microenvironment. At the same time, we found that the abnormal expression of YTHDF2 in DLBCL tissues was significantly positively correlated with signaling pathways such as PI3K-Akt-mTOR. The above results indicate that YTHDF2 plays an important role in the progression of malignant tumors, and the development of specific YTHDF2 inhibitors can provide new targeted therapy options for different tumors and improve the overall survival rate of patients.

In conclusion, this study investigated the expression and functional enrichment of multiple m6A regulators in DLBCL tissues. More importantly, we have pioneered the relationship between m6A regulators and immune cell...
infiltration in DLBCL. In addition, studies have found that IGF2BP2 and YTHDF2 regulated the occurrence and development of DLBCL through various mechanisms in DLBCL, and are expected to become new biological markers for the diagnosis and treatment of DLBCL in the future.

References


