RNA-Binding Protein IGF2BP1 Associated With Prognosis and Immunotherapy Response in Lung Adenocarcinoma

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N6-methyladenosine (m6A) is the most common modification in eukaryotic RNAs and plays a vital role in the tumorigenesis and metastasis of various cancers. However, a comprehensive study of m6A methylation regulators in lung adenocarcinoma (LUAD) is still lacking. The present study aimed to systematically explore the role of m6A methylation regulators in LUAD. RNA sequencing data of 20 m6A methylation regulators and clinical data of LUAD patients were downloaded from The Cancer Genome Atlas (TCGA) database. The prognosis value of m6A methylation regulators in LUAD was evaluated using the Gene Expression Profiling Interactive Analysis (GEPIA) and PrognoScan database. The correlation between IGF2BP1 and immune infiltrates in LUAD was investigated via CIBERSORT and Tumor Immune Estimation Resource (TIMER). A total of 15 m6A modification regulators were significantly abnormally expressed in LUAD tissues. Survival analysis revealed that four genes (HNRNPC, HNRNPA2B1, IGF2BP1, and IGF2BP3) were significantly associated with poor prognosis in LUAD. Multivariate Cox regression analysis showed that only IGF2BP1 was an independent predictor of LUAD after adjusting common clinical parameters. The mutation rates of m6A modification regulators in LUAD were less than 10%. Further analysis revealed that IGF2BP1 expression was significantly correlated with immune infiltration, the expression of immune checkpoints, and tumor mutational burden (TMB) in LUAD. Our findings suggest that IGF2BP1 is an independent predictor and related to immunotherapy response in LUAD, which maybe a potential novel biomarker for LUAD prognosis and the status of tumor immunity.

Keywords: lung adenocarcinoma, TCGA, m6A modification regulators, prognosis, immunotherapy response

INTRODUCTION

Lung cancer is one of the most common malignancies worldwide. Lung adenocarcinoma (LUAD) is the main subtype of lung cancer. The development of lung cancer is the result of the combined effect of genetic and environmental factors. Despite the advancement of surgery, radiotherapy, chemotherapy, and targeted therapy, it remains a high incidence and low overall 5-year survival (Bray et al., 2018). Therefore, early diagnosis and prognostic evaluation are urgently needed to be performed in LUAD.

N6-methyladenosine (m6A) is the most prevalent and abundant transcriptional modification in eukaryotic RNAs and plays a key role in the process of cell self-renewal and differentiation...
The m^6^A modification is highly conservative, which is commonly found in 3’ untranslated region (UTR), protein coding sequences (CDS), and transcription starting site (TSS). It regulates the posttranscriptional level of mRNA without changing the base sequence (Niu et al., 2013). The m^6^A modification is dynamically and reversibly regulated by different regulators, including m^6^A methyltransferase (“writers”), m^6^A demethylase (“erasers”), and m^6^A-binding protein (“readers”). The m^6^A-modified mRNA can be specifically recognized and bound by the m^6^A-binding protein, thereby regulating the RNA maturation, splicing, transport, degradation, and translation (Maity and Das, 2016). The abnormality of m^6^A modification can lead to the occurrence of many human diseases, such as tumors, metabolic diseases, and neurological diseases (He et al., 2019; Zhang et al., 2021).

Previous studies have shown the disorders of the m^6^A component, and the abnormal modification process can lead to the overexpression or inactivation of downstream oncogenes or tumor suppressor genes in various tumors (Zhou et al., 2020). A recent study showed that METTL3 could reduce the stability of SOC52 mRNA through the m^6^A-YTHDF2-dependent pathway. Knockdown of METTL3 could suppress cell proliferation in gastric cancer cells (Jiang et al., 2020). Additionally, downregulation of FTO could inhibit the proliferation and differentiation capacity through reducing the abundance of m^6^A in acute myeloid leukemia (AML). The inhibitors and regulators of m^6^A modification regulators have been explored as therapeutic approaches for treating cancer, such as FTO inhibitors (including rhein, R-2HG, IOX3, and FB23) and METTL3/METTL14 inhibitors (3-deazaadenosine) (Zhou et al., 2020). However, a systematic analysis of the impact of m^6^A modification regulators on LUAD is still lacking.

Our study aims to systematically analyze the expressions of m^6^A modification regulators in LUAD and explore the prognostic value and the relationships with tumor immune, which might be novel targets for the diagnosis and treatment of LUAD.

**METHODS**

**Datasets**

The RNA-seq transcriptome data (format: HTSeq-FPKM) and corresponding clinical information of 513 LUAD samples and 59 normal samples were downloaded from The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov). The impact of m^6^A modification regulators on LUAD was evaluated using Gene Expression Profiling Interactive Analysis (GEPIA), PrognoScan database, cBioPorta, CIBERSORT, Tumor Immune Estimation Resource (TIMER), and gene set enrichment analysis (GSEA) databases.

**Differential Expression Analysis of m^6^A Modification Regulators**

We totally selected 20 m^6^A modification regulators to analyze, including two “erasers” (ALKBH5 and FTO), eleven “readers” (HNRNPA2B1, HNRNPC, IGF2BP1, IGF2BP2, IGF2BP3, RBMX, YTHDC1, YTHDC2, YTHDF1, YTHDF2, and YTHDF3), and seven “writers” (METTL14, METTL3, RBM15, RBMWTAP, VIRMA, WTAP, and Z3H13). The expressions of m^6^A modification regulators in LUAD and normal lung tissues were assessed using the Wilcoxon test. The heatmap and scatter plot were used to display the different expressions of the 20 m^6^A methylation regulators in LUAD and normal lung tissues by R software (version: 4.0.3). p < 0.05 was considered statistically significant.

**Immunohistochemistry**

We also analyzed the protein level of IGF2BP1 and CD20 in LUAD tissues by immunohistochemistry (IHC). A total of 30 specimens were obtained from the First Affiliated Hospital of Nanjing Medical University in China between December 2020 and January 2020, including 24 LUAD tissues and 6 normal lung tissues. The histological evaluation was performed on hematoxylin and eosin–stained sections. The LUAD tissue sections were immunostained with the primary antibody against IGF2BP1 (Proteintech, Ca#22803-1-AP, 1:100) and CD20 (Proteintech, Ca# 60271-1-Ig, 1:1,000) at 37°C. The degree of immunostaining was based on staining intensity and percentage of cells stained. The study was approved by the hospital’s Institutional Review Board. Written informed consent was obtained from all participants or their guardians before the study.

**Prognostic Value of m^6^A Modification Regulators**

The Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) was used to evaluate the prognosis value of m^6^A modification regulators in LUAD patients. The GEPIA database is an interactive web that includes
9,736 tumors and 8,587 normal samples from TCGA and the GTEx projects. GEPIA was used to generate survival curves, based on gene expression with the log-rank test and the Mantel–Cox test in 33 different types of cancers. The correlation between m6A modification regulators and survival in LUAD was further analyzed by the PrognoScan database (http://www.abren.net/PrognoScan/) based on the GEO database (GSE31210). PrognoScan searches for relationships between gene expression and patient prognosis (such as overall survival), across a large collection of publicly available cancer microarray datasets. Adjusting the prognostic variables (age, gender, smoking history, pT staging, and pN staging of the TNM classification), multivariate Cox regression analysis was used to analyze the correlation between m6A modification regulators and the prognosis of LUAD as well. A nomogram was used to predict the overall survival (1, 3, and 5 years) of LUAD patients. \( p < 0.05 \) is considered statistically significant.

**Genetic Alteration Analysis**

The cBioPortal for Cancer Genomics (http://www.cbioportal.org/) is a comprehensive gene database, including different datasets such as DNA mutation, gene amplification, and methylation. Four studies from the cBioPortal database were enrolled: LUAD (Broad, Cell 2012), LUAD (OncoSG, Nat Genet 2020), LUAD (TCGA, Firehose Legacy), and Non-Small Cell Cancer (MSKCC, Cancer Discov 2017). A total of 1989 LUAD samples were used to analyze the genetic variation of m6A modification regulators in LUAD. Gene mutations included the following types: inframe mutation, missense mutation, splice mutation, truncating mutation, amplification, and deep deletion.

**Correlation Between IGF2BP1 Gene and Immune Cell Infiltration**

The CIBERSORT algorithm (https://cibersort.stanford.edu/) was employed for estimating the fractions of 22 phenotypes of...
immune cells based on gene expression profiles. In this study, the CIBERSORT database was used to explore the correlation between IGF2BP1 and immune cell infiltration. Patients were divided into high-expression group and low-expression group according to the median value of IGF2BP1 expression. The difference of immune cell infiltration between the two groups was evaluated by the Wilcoxon test. \( p < 0.05 \) is considered statistically significant.

TIMER is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types (https://cistrome.shinyapps.io/timer/). TIMER applies a deconvolution previously published statistical method to infer the abundance of tumor-infiltrating immune cells from gene expression profiles. The TIMER database includes 10,897 samples across 32 cancer types from TCGA database. We analyzed the correlations between IGF2BP1 expression and gene markers of tumor-infiltrating immune cells in LUAD via correlation modules. The gene markers of tumor-infiltrating immune cells included markers of CD4+ T cells, B cells, monocytes, M2 macrophages, and dendritic cells. The gene expression level was displayed with log2 RSEM.

**Correlation Between IGF2BP1 Gene and TMB, MSI, and Immune Checkpoints**

In our study, patients were divided into high-expression group and low-expression group according to the median value of IGF2BP1 expression. Then, the differences of tumor mutational burden (TMB), microsatellite instability (MSI), and immune checkpoints between the two groups were evaluated by the Wilcoxon test. \( p < 0.05 \) is considered statistically significant.

**GSEA and Functional Enrichment of the IGF2BP1 Gene**

Gene set enrichment analysis (GSEA) by LinkedOmics (http://www.linkedomics.org/login.php) was applied to study the function of IGF2BP1 and related signal pathways in LUAD. In addition, Gene Ontology (GO) enrichment analysis was also used to regard the possible function of the IGF2BP1 gene in LUAD based on IGF2BP1-related genes.

**RESULTS**

**Differential Expression Analysis of m^6^A Modification Regulators**

To explore the role of m^6^A modification regulators in LUAD tumorigenesis, we systematically analyzed the expression patterns of 20 m^6^A modification regulators in LUAD tumor and normal lung tissues based on TCGA database. The heatmap for the expressions of m^6^A methylation regulators in normal and LUAD tissues showed significant differences in 15 m^6^A modification regulators (ALKBH5, FTO, HNRNPA2B1, HNRNPC, IGF2BP1, IGF2BP3, RBMX, YTHDF1, YTHDF2, [FIGURE 3](#)Survival analysis of m^6^A modification regulators in LUAD patients by the GEPIA database. Five genes that have significant association with poor prognosis of LUAD were presented \( p < 0.05 \).
METL14, METL3, RBM15, VIRMA, WTAP, and ZC3H13) (Figure 1). Compared with the normal tissues, the expressions of HNRNPA2B1, HNRNPC, IGF2BP1, IGF2BP3, RBMX, YTHDF1, YTHDF2, METL3, RBM15, and VIRMA were upregulated in LUAD tissues, while the expressions of ALKBH5, FTO, METL14, WTAP, and ZC3H13 were downregulated in LUAD tissues (Figure 2). In addition, we verified the protein expression level of IGF2BP1 in LUAD tissues by IHC. The result showed that the IGF2BP1 protein expression level was significantly increased in LUAD tissues compared to that in normal lung tissues (Supplementary Figure S1), which was consistent with the mRNA level of IGF2BP1. These results suggested that the m^6^A methylation regulators played a vital role in LUAD.

**Prognostic Value of m^6^A Modification Regulators**

We used the GEPIA database to evaluate the prognosis value of m^6^A modification regulators in LUAD patients. The overall survival analysis revealed that the expressions of five genes were significantly associated with the poor prognosis of LUAD, including HNRNPA2B1 (HR = 1.6, p = 0.0032), HNRNPC (HR = 1.8, p = 0.00011), RBM15 (HR = 1.4, p = 0.038), IGF2BP1 (HR = 1.4, p = 0.016), and IGF2BP3 (HR = 1.6, p = 0.0017) (Figure 3). Furthermore, the PrognoScan database was also used to evaluate the prognostic value of m^6^A modification regulators in LUAD. The result showed that HNRNPA2B1 (HR = 12.25, p = 0.020373), HNRNPC (HR = 5.77, p = 0.004359), IGF2BP1 (HR = 1.59, p = 0.037049), and IGF2BP3 (HR = 1.50, p = 0.002818) were significantly associated with the poor prognosis of LUAD (Supplementary Figure S2). These results confirmed the prognostic value of m^6^A modification regulators in LUAD.

Cox regression analysis showed that HNRNPA2B1, IGF2BP1, IGF2BP3, HNRNPC, and pT/pN staging were significantly associated with the prognosis of LUAD (Figure 4A). Multivariate Cox regression analysis revealed that IGF2BP1 (adjust HR = 1.19, 95%CI = 1.07–1.32, p = 0.001), pT staging (adjust HR = 1.42, 95%CI = 1.15–1.74, p < 0.001), and pN staging (adjust HR = 1.56, 95%CI = 1.29–1.88, p < 0.001) remained as the
independent prognostic indicators of LUAD (Figure 4B). The overall survival analysis of LUAD patients by nomogram showed that IGF2BP1 [C-index: 0.628 (0.578–1), \( p < 0.001 \)] had predictive values (Figure 4C). Therefore, our findings suggested that high IGF2BP1 expression was an independent risk factor of poor prognosis in LUAD.

**Variation of m\(^6\)A Modification Regulators**

We further explored the mutation rate of the significant genes (HNRNPA2B1, HNRNPC, RBM15, IGF2BP1, and IGF2BP3) using the cBioPortal database. The result showed that the five genes in LUAD samples had a low mutation rate (<10%) (Figure 5). Regarding the mutation type, amplification was the most predominant type for all samples. The result suggested that m\(^6\)A modification regulators might not only influence tumorigenesis of LUAD through gene mutation.

**Correlation Between IGF2BP1 Expression and Immune Markers**

To investigate the relationship between IGF2BP1 and the diverse immune-infiltrating cells, we focused on the correlations between IGF2BP1 and immune markers of various immune cells of LUAD in the TIMER. The results revealed that the IGF2BP1 expression level was significantly correlated with immune markers of CD4\(^+\) T cells (CD4), B cells (CD20), monocytes (CD115), M2 macrophages (CD206), and DCs (CD1C and CD141) in LUAD (Figure 7). We further analyzed the correlation between IGF2BP1 expression and the marker of B cell (CD20) in LUAD tissues by IHC. The result showed that IGF2BP1 expression was negatively correlated with CD20 expression in LUAD tissues by IHC. Together, these results suggested that IGF2BP1 played an important role in immune cell infiltration in LUAD.

**Correlation of the IGF2BP1 Gene With Immune Checkpoints, TMB, and MSI**

Immune checkpoints are the essential regulatory molecules for maintaining self-tolerance, preventing autoimmune response, and minimizing tissue damage by controlling the duration and intensity of the immune responses, which produces effective antitumor immune responses. Our result showed that IGF2BP1 was correlated with three immune checkpoint genes (SIGLEC15, CD274, and PDCD1) in LUAD (Figure 8A). TMB is defined as the total number of somatic mutations per megabase (Mb) in coding regions of an exon, which is a predictive biomarker for the efficacy of tumor immunotherapy. Our result showed that IGF2BP1 expression was positively related to TMB (\( p < 0.001 \)).
Microsatellite (MS) refers to a tandem repeat sequence (1-6 nucleotides) usually located in the intergenic region, promoter, UTR, and coding region. The changes of the MS-DNA structure (mismatches, insertions, and/or deletions) under certain pathological factors could lead to MSI, which is associated with malignant transformation of human cells. But, our result showed that there was no significant correlation between the expression of \textit{IGF2BP1} and MSI (Figure 8C). Our study suggested that IGF2BP1 might be related to immunotherapy response in LUAD, which could serve as a novel biomarker for predicting the immunotherapy response rate.

**GSEA Analysis and Functional Enrichment of the \textit{IGF2BP1} Gene**

In our study, GSEA analysis was used to analyze pathway enrichment for the \textit{IGF2BP1} gene. The result showed that \textit{IGF2BP1} was significantly related to the activation of the cell cycle-related pathway (including cell cycle checkpoint, chromosome segregation, and DNA replication) and the inhibition of the immune-related pathway (including adaptive immune response, leukocyte activation, and macrophage activation) in LUAD (Figure 9A). Meanwhile, GO enrichment analysis showed that the cell cycle had a positive correlation with the \textit{IGF2BP1} expression, and immune regulation had a negative correlation with the \textit{IGF2BP1} expression (Figure 9B), which also supported the biological functions of the \textit{IGF2BP1} gene.

**DISCUSSION**

\textit{m}^6A modification plays an important role in the tumorigenesis and metastasis of various cancers by regulating RNA stability, microRNA processing, mRNA shearing, and translation. Studies have found that \textit{m}^6A modification regulators are significantly abnormally expressed in various cancers, which lead to malignant proliferation, migration, invasion, metastasis, and drug resistance (Yang et al., 2019). Recent studies show that the levels of \textit{m}^6A-related genes are also associated with the prognosis of lung cancer patients. For instance, Zhuang et al. found that
HNRNPC played a critical role in LUAD progression (Zhuang et al., 2020). Zhang et al. also found that HNRNPA2B1 and HNRNPC were closely related to the overall survival of LUAD patients (Wang et al., 2021a). In our study, we aimed to systematically explore the biological function of m^6^A methylation regulators and the relationships with tumor immune in LUAD, which could provide a theoretical basis for making clinical treatment strategies.

We totally selected 20 m^6^A modification regulators, including two “erasers” (ALKBH5 and FTO), eleven “readers” (HNRNPA2B1, HNRNPC, IGF2BP1, IGF2BP2, IGF2BP3, RBMX, YTHDC1, YTHDC2, YTHDF1, YTHDF2, and YTHDF3), and seven “writers” (METL14, METL3, RBM15, RBMWTAP, VIRMA, WTAP, and ZC3H13) for analysis. Among 20 m^6^A modification regulators, 15 m^6^A modification regulators (ALKBH5, FTO, HNRNPC, IGF2BP1, IGF2BP2, IGF2BP3, RBMX, YTHDC1, YTHDC2, YTHDF1, YTHDF2, METL14, METL3, RBM15, VIRMA, WTAP, and ZC3H13) were significantly abnormally expressed in LUAD tissues. Previous studies have shown that m^6^A modification regulators, including m^6^A writers [METL3 (Lin et al., 2016) and METL4 (Gong et al., 2020; Liu et al., 2020; Wang et al., 2021b; Nombela et al., 2021)], m^6^A erasers [FTO (Liu et al., 2018; Chen and Du, 2019; Tang et al., 2020; Nombela et al., 2021) and ALKBH5 (Jin et al., 2020)], and m^6^A readers [YTHs (Jin et al., 2020) and IGF2BP3 (Degrauw et al., 2016; Müller et al., 2019)], play important roles in the occurrence and development of various cancers.

Then, Kaplan–Meier survival analysis showed the expressions of IGF2BP1, IGF2BP3, HNRNPC, and HNRNPA2B1 were significantly associated with the poor prognosis of LUAD. Furthermore, multivariate Cox regression analysis revealed that only IGF2BP1 remained independently associated with the prognosis of LUAD after adjusting the clinical variables (gender, age, pT/pN stage, and smoking history). The nomogram analysis also showed that IGF2BP1 had a predictive value for overall survival (1, 3, and 5 years) in LUAD patients. The result suggested that IGF2BP1 was an independent risk factor of poor prognosis in LUAD. IGF2BPs family proteins (including IGF2BP1, IGF2BP2, and IGF2BP3) are a novel discovered m^6^A-binding proteins. Studies have shown that the IGF2BPs expressions are abnormally expressed in a variety of cancers, which regulate tumor progression by a variety of molecular mechanisms (Degrauw et al., 2016). The increased expression of IGF2BP1 is significantly related to the poor prognosis of ovarian cancer, liver cancer, and lung cancer (Huang et al., 2019; Müller et al., 2019; Zhang et al., 2020). As an RNA-binding protein, IGF2BP1 can also affect the function of the target RNA by binding to the RNA. Recent studies have found that IGF2BP1 can bind to lncRNA LIN28B and activate its function to promote the proliferation and metastasis in LUAD cells (Wang et al., 2019). Currently, there are still few studies on IGF2BP1 in LUAD, and a synthetical study of IGF2BP1 in LUAD is needed to perform.

We further explored the mutation rate of m^6^A modification regulators in 1,989 LUAD samples using the cBioPortal database. Consistent with the previous studies, our result showed that the mutation rates of the m^6^A modification regulators in LUAD were significantly associated with the poor prognosis of LUAD.
not high (<10%), which suggested that m6A modification regulators might not only influence tumorigenesis of LUAD through gene mutation.

Tumor-infiltrating immune cells are closely associated with the clinical outcome of cancers. Therefore, the association between IGF2BP1 and LUAD immune infiltration were further explored. The result showed that IGF2BP1 expression was related to immune infiltration of macrophage M0/1/2, T cell CD4+ memory resting/activation, mast cell activation, monocyte, myeloid dendritic cell resting, T cell follicular helper, and B cell memory. Moreover, we found that the IGF2BP1 expression level was significantly correlated with immune markers of CD4+ T cells (CD4), B cells (CD20), monocytes (CD115), M2 macrophages (CD206), and DCs (CD1C and CD141) in LUAD. Macrophages, including macrophage M0 and macrophage M2, were shown to be the most abundant immune cells in non-small cell lung cancer (House et al., 2020). Recent studies have shown that the degree of immune cell infiltration is significantly related to the prognosis of non-small cell lung cancer. Patients with low immune cell infiltration have lower cytotoxic activity and lower expression levels of MHC-I and immune checkpoints, which may lead to the possibility of immune escape. Meanwhile, patients with a high degree of immune cell infiltration may have a better immune response (Mi et al., 2020). Taken together, our findings indicate that IGF2BP1 plays an important role in regulating tumor-infiltration of immune cells in LUAD.

Immunotherapy is a hot spot of lung cancer research studies. The roles of m6A in tumor immunity and cell cycle regulation have been highly interested by researchers. The m6A modification regulators (such as FTO) play important roles in the PD-1/PD-L1 inhibitor tumor immunotherapy (Yang et al., 2019; Li et al., 2020). Immune checkpoints are the essential regulatory molecules to control the duration and intensity of immune responses. In our study, IGF2BP1 expression was significantly associated with three immune checkpoint genes (SIGLEC15, CD274, and PDCD1). TMB can be used as a predictive biomarker for the efficacy of immune checkpoint inhibitor therapy. We found that IGF2BP1 expression was significantly correlated with the TMB of LUAD. So far, there are few studies on IGF2BP1 and immune checkpoint and TMB. Our study was the first to report the correlation of IGF2BP1 with immune checkpoints and TMB in LUAD, which might be important for immunotherapy of LUAD.

**FIGURE 8** | Association of the IGF2BP1 gene with immune checkpoints, TMB, and MSI in LUAD. (A) Correlation of IGF2BP1 expression with immune checkpoint genes. (B) Correlation of IGF2BP1 expression with tumor mutational burden (TMB). (C) Correlation of IGF2BP1 expression with microsatellite instability (MSI).
Furthermore, the GSEA showed that IGF2BP1 was significantly related to the inhibition of adaptive immune response, leukocyte activation, and macrophage activations in LUAD. At present, there are lack of studies regarding the correlation between IGF2BP1 and LUAD. Further study is necessary to explore the role of IGF2BP1 involvement in the immune response in LUAD.

There are some limitations in our study. First, the study was a retrospective bioinformatic analysis, which could have certain general bias. Second, other studies are necessary to distinguish the effects of IGF2BP1 on the tumor immune infiltration pathway. Third, further studies are necessary to verify the role played by IGF2BP1 in LUAD.

In conclusion, our work systemically elucidated the role of m^6^A modification regulators in LUAD. Among them, IGF2BP1.
was independently related to the prognosis of LUAD. Moreover, \textit{IGF2BP1} expression was significantly related to immune infiltration, TMB, and the expressions of immune checkpoints. These findings suggest that \textit{IGF2BP1} may be a potential independent biomarker for LUAD prognosis and the status of tumor immunity.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

**AUTHOR CONTRIBUTIONS**

CZ and JL designed and coordinated the study. JFL, ZL, and IC analyzed the data and edited the manuscript. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.777399/full#supplementary-material

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