



Multi-Omic Analyses of the m⁵C Regulator ALYREF Reveal Its Essential Roles in Hepatocellular Carcinoma

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The ALYREF protein acts as a crucial epigenetic regulator in several cancers. However, the specific expression levels and functional roles of ALYREF in cancers are largely unknown, including for hepatocellular carcinoma (HCC). In a pan-cancer tissue analysis that included HCC, we assessed the expression of ALYREF compared to normal tissues using The Cancer Genome Atlas database. Associations between ALYREF gene expression and the clinical characteristics of HCC patient samples were assessed using the UALCAN database. Kaplan-Meier plots were performed to assess HCC patient prognosis, and the TIMER database was used to explore associations between ALYREF expression and immune-cell infiltrations. The same methods were used to assess eIF4A3 expression in HCC patient samples. In addition, ALYREF- and eIF4A3-related differentially expressed genes (DEGs) were determined using LinkedOmics, associated protein functionalities were predicted for positively associated DEGs, and both the TargetScan and miRDB databases were used to predict potential upstream miRNAs for control of ALYREF and eIF4A3 expression. We found that ALYREF gene expression was dysregulated in several cancers and was significantly elevated in HCC patient tissue samples and HCC cell lines. The overexpression of ALYREF was significantly related to both advanced tumor-node-metastasis stages and poor HCC prognosis. Furthermore, we found that eIF4A3 expression was significantly correlated with ALYREF expression, and that upregulated eIF4A3 was significantly associated with poor HCC patient outcomes. In the protein-protein interaction network, we identified eight hub genes based on the positively associated DEGs in common between ALYREF and eIF4A3, and the high expression levels of these hub genes were positively associated with patient clinical outcomes. In addition, we identified miR-4666a-5p and miR-6124 as potential regulators of ALYREF and eIF4A3 expression. These findings suggest that increased ALYREF expression may function as a novel biomarker for both HCC diagnosis and prognosis predictions.

Keywords: ALYREF, hepatocellular carcinoma, EIF4A3, hub genes, prognosis

INTRODUCTION

It is estimated that over 18.1 million cancer patients, and 9.6 million liver cancer patients, died in 2008 globally (1), with primary liver cancer being the seventh most frequent malignancy in the world. Statistically, liver cancer has an incidence rate of 9.3 per 100,000 person-years and a mortality rate of 8.5 (2). Hepatocellular carcinoma (HCC) is the most prominent histological type of liver cancer and accounts for 75–90% of all cases (3). Due to the multifactorial, multi-stage, and complex genetic nature of HCC, its pathogenesis has not been fully elucidated. It therefore remains urgent to reveal the complicated molecular pathogenic and developmental mechanisms for HCC.

Post-transcriptional methylation modifications represent important epigenetic RNA modifications (4), and 5-methylcytosine (m^5C) additions to RNA play crucial roles in pre-mRNA splicing (5), nuclear export, transcript stability, translation initiation, RNA metabolism, tRNA recognition, and stress responses. Accumulating evidence indicates that the m^5C modification status of RNA is associated with the pathogenesis of numerous types of cancers (6–9), and the aberrant regulation of m^5C changes contributes to the pathogenesis of both tumor and non-tumor diseases (8, 10). Studies have also shown that m^5C -related regulators play essential roles in tumor progression in HCC (11, 12). Research into m^5C modifications has also provided evidence for the epigenetic m^5C regulation of lncRNA related to HCC tumorigenesis and progression (13). However, the specific genomic distribution of m^5C -related genes for HCC remains unclear.

The Aly/REF nuclear export factor (ALYREF), also known as THOC4, is an mRNA export adaptor that is part of the transcription export (TREX) complex, and in human cells it binds to a region near the 5' end of mRNA in a CBP80-dependent manner (14). Importantly, ALYREF acts as an important nuclear export factor, and is involved with the splicing of RNAs as part of an exon junction complex in a cap-dependent manner (15). ALYREF, functions as a m^5C reader, bind with lysine 171 and promote mRNA exudation in bladder cancer (16). ALYREF also contributes to alternative RNA splicing, and studies have validated that ALYREF is recruited to the 5' end of RNAs. Studies have also determined that ALYREF plays crucial roles in coordinating 3'-end processing and in the nuclear export of non-polyadenylated mRNAs (14, 17, 18). The overexpression of ALYREF is known to occur in glioblastoma, and its function is considered a putative target for glioblastoma therapy to regulate mRNA splicing (19). Aberrantly expressed ALYREF has also been shown to facilitate hypersensitivity in ovarian cancer cells to DNA-damaging chemotherapeutic agents and is correlated with poor prognosis (20). In the previous study, He et al. found that ALYREF was significantly upregulated in HCC tumor tissues compared with adjacent normal tissues. In addition, they found that the highly expressed ALYREF may involve in cell cycle, mitotic reactome and cellular nitrogen compound catabolic processes in HCC (12).

The eukaryotic translation initiation factor 4A3 (eIF4A3) is known to be involved in the stimulation of ALYREF expression (21), and eIF4A3 is also a core component of the exon junction complex (22). Importantly, eIF4A3 is also a key mediator of RNA splicing and spliced mRNA nuclear export (23) and has been shown to be involved with the coordination and regulation of the HCC cell cycle (24). However, specific mechanisms and interactions between ALYREF and eIF4A3 are currently unknown, so determining whether ALYREF and eIF4A3 interact in HCC is of great interest.

Here, we used The Cancer Genome Atlas (TCGA) database and other online tools to explore ALYREF-related gene expression profiles, immune-cell infiltrations, and HCC patient sample prognosis. Importantly, we found that eIF4A3 was a cofactor for ALYREF in HCC, and so a comprehensive analysis of the regulatory events for both ALYREF and eIF4A3 may help to better understand HCC progression.

MATERIALS AND METHODS

Patients and Online Databases

The mRNA transcription profiles for ALYREF and eIF4A3 were collected from TCGA database (<https://cancer.gov/tcga>), and the UALCAN database (<http://ualcan.path.uab.edu>) and were used to identify associations between mRNA expression levels and patient clinical characteristics (25). Patient clinical information was classified into different subgroups based on age, gender, race, tumor-node-metastasis (TNM) staging, tumor grading, metastasis status, and TP53 mutations.

Prognosis Analyses

A Kaplan-Meier analysis (<http://kmplot.com>) was used to investigate the prognostic role of ALYREF in HCC (26), including overall survival (OS), progression-free survival (PFS), relapse free survival (RFS), and disease-specific survival (DSS). We divided patient sample data into either high-expressed and low-expressed subgroups based on their sample expressions of target genes. In addition, we determined overall HCC patient survival based on their clinical characteristics, including TNM staging and grading.

Cell Culture and Quantitative Reverse-Transcription PCR (qRT-PCR)

We adopted LO2, Hep3B and Huh-7 cell lines (purchased from Chinese Academy of Sciences, Shanghai, China) for the detection of target genes expression. Cell cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and 1% penicillin as described previously (27). In addition, total RNA was extracted from cell lines using Qiagen RNeasy Mini kit according to Qiagen protocol (Qiagen, Hilden, Germany). Relative mRNA expression levels were determined by ABI7500 fast PCR instrument. GAPDH was used as the internal control. Relative expression levels of ALYREF was quantified using 2- $\Delta\Delta Ct$ method. The forward (F) and reverse (R) primer

sequences used for the amplification of ALYREF were 5'-TCTGGTCGACGCTTAGGAAC-3', and 5'-TGCCACCTCTGTTTACGCTC-3', respectively.

Associations Between ALYREF and eIF4A3, and Functional Prediction

To detect any associations between ALYREF and eIF4A3, we used the cBioPortal (<http://www.cbioportal.org/>) tool based on TCGA cohort (28, 29). In addition, we investigated the mutation data for both ALYREF and eIF4A3, and any effects of their mutations on mRNA transcription using the cBioPortal database. Correlations between ALYREF and eIF4A3 were validated using the Gene Expression Profiling Interactive Analysis (GEPIA) database (<http://gepia.cancer-pku.cn>) (30).

Differentially Expressed Genes (DEGs) and Network Analyses

To determine ALYREF- and eIF4A3-related DEGs, we used the LinkedOmics database (<http://www.linkedomics.org/>) (31), and assessed any relationships between ALYREF- and eIF4A3-related DEGs using Spearman's correlation coefficients. DEG data for ALYREF and eIF4A3 were plotted using volcano maps, and the top 10 positively-correlated DEGs were plotted using hotmaps. For known and predicted protein-protein interactions, the STRING database (<http://string-db.org/>) was used to detect interactions between the 104 positively associated DEGs in common between ALYREF and eIF4A3, and a protein-protein interaction (PPI) network was constructed based on those interactions.

Pathway Analyses

To annotate any pathway enrichments for the 104 positively associated DEGs in common for ALYREF and eIF4A3, we used both the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) tools and visualized the results using DAVID (<https://david.ncifcrf.gov/>) (32, 33). The three domains of the GO analyses, molecular functions (MF), biological processes (BP), and cellular components (CC), were visualized using the bioinformatics website (<http://www.bioinformatics.com.cn/>).

Immune-Cell Infiltrations Related to ALYREF and eIF4A3

To calculate any immune-cell infiltrates and the immune purities related to ALYREF and eIF4A3, we used the Tumor Immune Estimation Resource (TIMER) database (<https://cistrome.shinyapps.io/timer/>) and an algorithm to calculate the immune-related characteristics of ALYREF and eIF4A3 in the patient HCC samples as described previously (34).

Hub Gene Identification and Prognostic Values

To select the hub genes in the network analysis, we utilized the Cytoscape-based cytoHubba plug-in (version 0.1) to classify genes based on different criteria (K-score: 2, node score cut-off: 0.2, degree cut-off: 2, and a max depth of 100). In addition, we

used the UALCAN database to assess the prognostic values of hub gene expressions for their HCC predictive values.

MicroRNA (miRNA) Predictions and Functional Annotations

We used the TargetScan (<http://www.targetscan.org/>) (35) and miRDB (<http://mirdb.org/>) databases to both identify and predict upstream binding microRNAs for both ALYREF and eIF4A3 and for functional annotations (36, 37).

RESULTS

ALYREF Gene Expression and Clinical Characteristics

To explore the pan-cancer gene expression of ALYREF, we used the UALCAN database. The results demonstrated that ALYREF was dysregulated in tumor tissues compared to normal tissues (**Figure 1A**), including primary liver cancer (**Figure 1B**). As shown in **Figure 1C**, ALYREF expression was significantly increased in HCC tissues compared to normal tissues, and its expression in fibrolamellar carcinoma was higher compared to that of hepatocholangio carcinoma (mixed). In addition, the results showed that ALYREF expression in patients with TP53 mutations was upregulated compared to that seen in patient samples without TP53 mutations (**Figure 1D**). We also observed that ALYREF expression in Asian patient samples was elevated relative to Caucasian patient samples (**Figure 1E**), and that advanced TNM stages had higher ALYREF expressions (Stage III versus Stage I, and Stage II versus Stage I) (**Figure 1F**). Similar results were observed for tumor grading (Grade 4 versus Grades 1 and 2, and Grade 3 versus Grades 1 and 2, respectively) (**Figure 1G**). Furthermore, our evaluation of the relative mRNA expression levels of ALYREF in HCC cell lines and normal liver cell line. Results showed that the ALYREF expression was increased in the Huh-7 and Hep3B cell lines compared to LO2 cell line (both $P < 0.05$, **Figure 1H**). However, the clinical characteristics of gender, age, and lymph node metastasis status, showed no significant differences in ALYREF expression (**Supplementary Figures S1A–C**). Taken together, these findings have demonstrated that ALYREF expression was increased in HCC tissues, and that this expression may be a novel biomarker for HCC diagnosis.

The Prognostic Value of ALYREF in HCC

To comprehensively explore the prognostic value of ALYREF in HCC, we applied the Kaplan-Meier online analysis system. As shown in **Figures 2A–D**, we found that patient samples with high ALYREF expression had poor prognosis, including OS ($P = 0.0015$), DFS ($P = 0.011$), RFS ($P = 0.0022$) and DSS ($P = 0.0011$). Considering that TNM staging and tumor grading could affect patient outcomes, we further assessed OS based on TNM stages and tumor grades. Patient samples with high ALYREF expression in stage I had poor prognosis (**Figure 2E**), and a similar result was observed for stage II (**Figure 2F**). But in stage

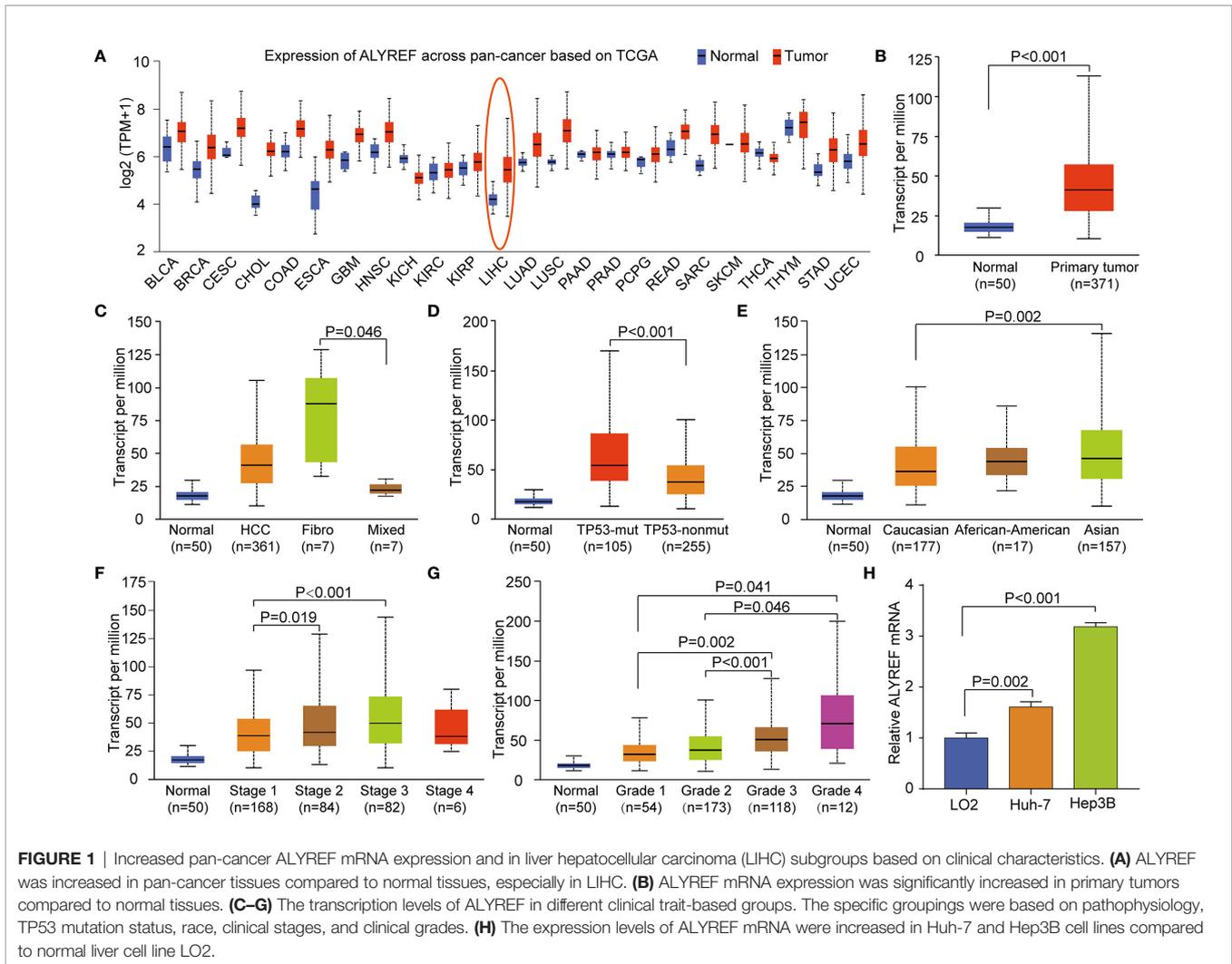


FIGURE 1 | Increased pan-cancer ALYREF mRNA expression and in liver hepatocellular carcinoma (LIHC) subgroups based on clinical characteristics. **(A)** ALYREF was increased in pan-cancer tissues compared to normal tissues, especially in LIHC. **(B)** ALYREF mRNA expression was significantly increased in primary tumors compared to normal tissues. **(C–G)** The transcription levels of ALYREF in different clinical trait-based groups. The specific groupings were based on pathophysiology, TP53 mutation status, race, clinical stages, and clinical grades. **(H)** The expression levels of ALYREF mRNA were increased in Huh-7 and Hep3B cell lines compared to normal liver cell line LO2.

III, there were no significant differences between patient samples with low or high ALYREF expressions (**Figure 2G**). Furthermore, samples with increased ALYREF expression in TNM stages I-II, and III-IV, had unfavorable prognosis (**Figures 2H, I**). In grades 1, 2, and 3, samples with high ALYREF expression had poor HCC clinical outcomes (**Figures 2J–L**). Collectively, our results indicate that patient samples with high ALYREF expression had poor prognosis, and ALYREF may be a promising prognostic biomarker for HCC.

Correlation Analysis Between ALYREF and eIF4A3

ALYREF acts as a classical m^5C regulator for RNA modifications and has a close relationship with eIF4A3 expression. However, this relationship between the two has not been fully evaluated in HCC. Using a GEPIA analysis, we observed that ALYREF expression was strongly correlated with eIF4A3 expression ($R = 0.82$, $P = 0$, **Figure 3A**). For verification, we used LinkedOmics for a further analysis, and the results indicated that eIF4A3 had a similar significant correlation with ALYREF

(**Figure 3B**). In addition, we determined both the negatively and positively associated DEGs for ALYREF, including the top 10 positively associated DEGs, using a volcano map and a heat map, respectively (**Figure 3C**). Similar results for eIF4A3 are shown in **Figure 3D**. Together, these data strongly suggest that eIF4A3 expression is closely related to ALYREF expression in HCC.

The Expression of eIF4A3 in HCC and Its Prognostic Value

A pan-cancer assessment of eIF4A3 expression indicated that it was upregulated in many cancers, including liver cancer (**Supplementary Figures S2A, B**). Similar to ALYREF, high eIF4A3 expression was significantly related to TP53 mutations, Asians and African-Americans, advanced TNM stages, and grade 3 (**Supplementary Figures S2C–F**). In addition, the beta value for eIF4A3 promoter methylation was lower for liver cancer than for normal tissue (**Supplementary Figure S2G**). The Kaplan-Meier analysis showed that patients with low eIF4A3 expression in their samples had longer survival times than those with high eIF4A3 expression (**Supplementary Figure S2H**). These results

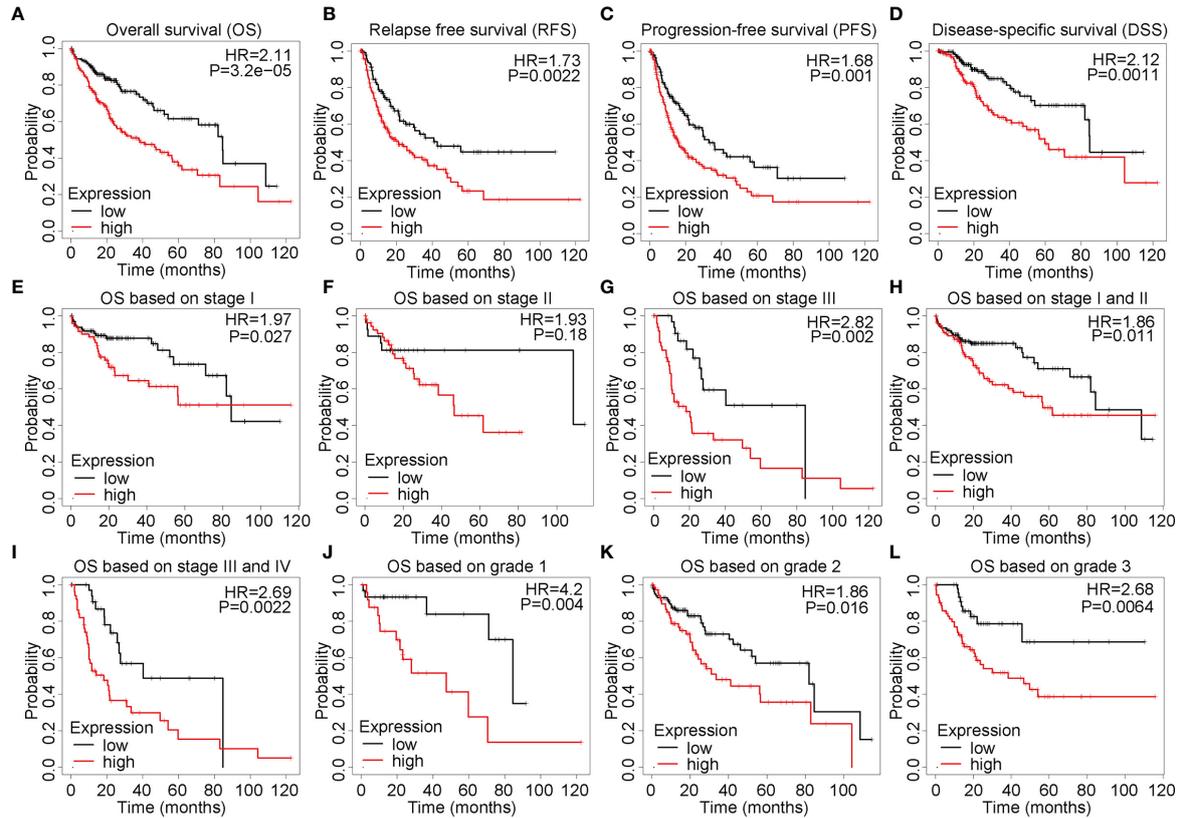


FIGURE 2 | Elevated ALYREF expression and poor prognosis in liver hepatocellular carcinoma. **(A–D)** A Kaplan-Meier analysis was used to assess ALYREF-associated prognosis, and the results indicated good prognostic values for ALYREF in OS, RFS, PFS, and DSS. **(E–L)** Kaplan-Meier analyses representing differences in OS in the various patient subgroups based on clinical staging and grading.

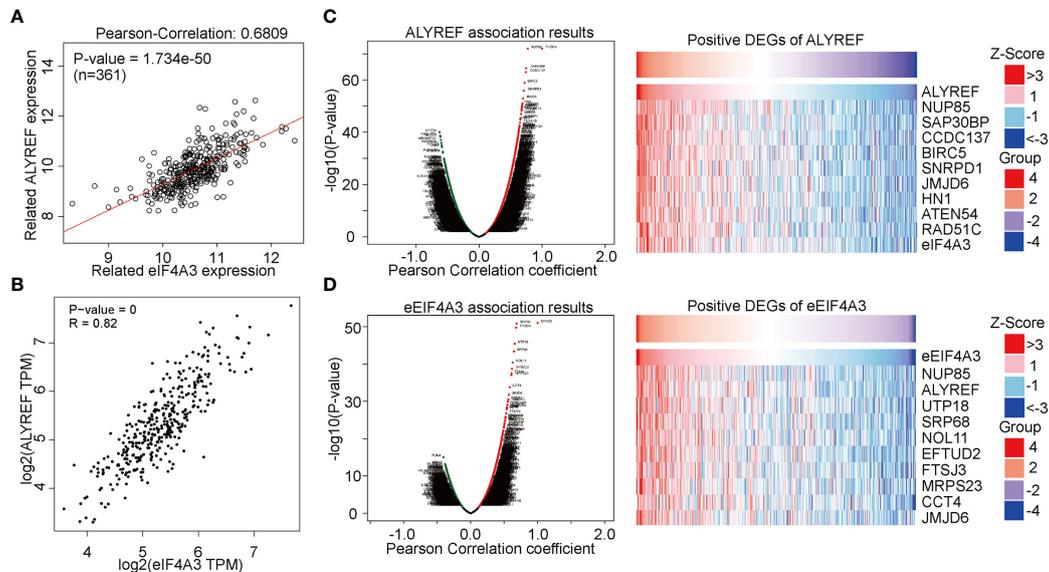


FIGURE 3 | Genomic correlations between ALYREF and eIF4A3. **(A, B)** ALYREF and eIF4A3 expressions were strongly correlated with each other. **(C, D)** ALYREF- and eIF4A3-associated differentially expressed genes (DEGs) and the top-ten DEGs.

demonstrate that eIF4A3 may have an oncogenic role in HCC, and that it may also be a potential biomarker for HCC diagnosis and for predicting prognosis.

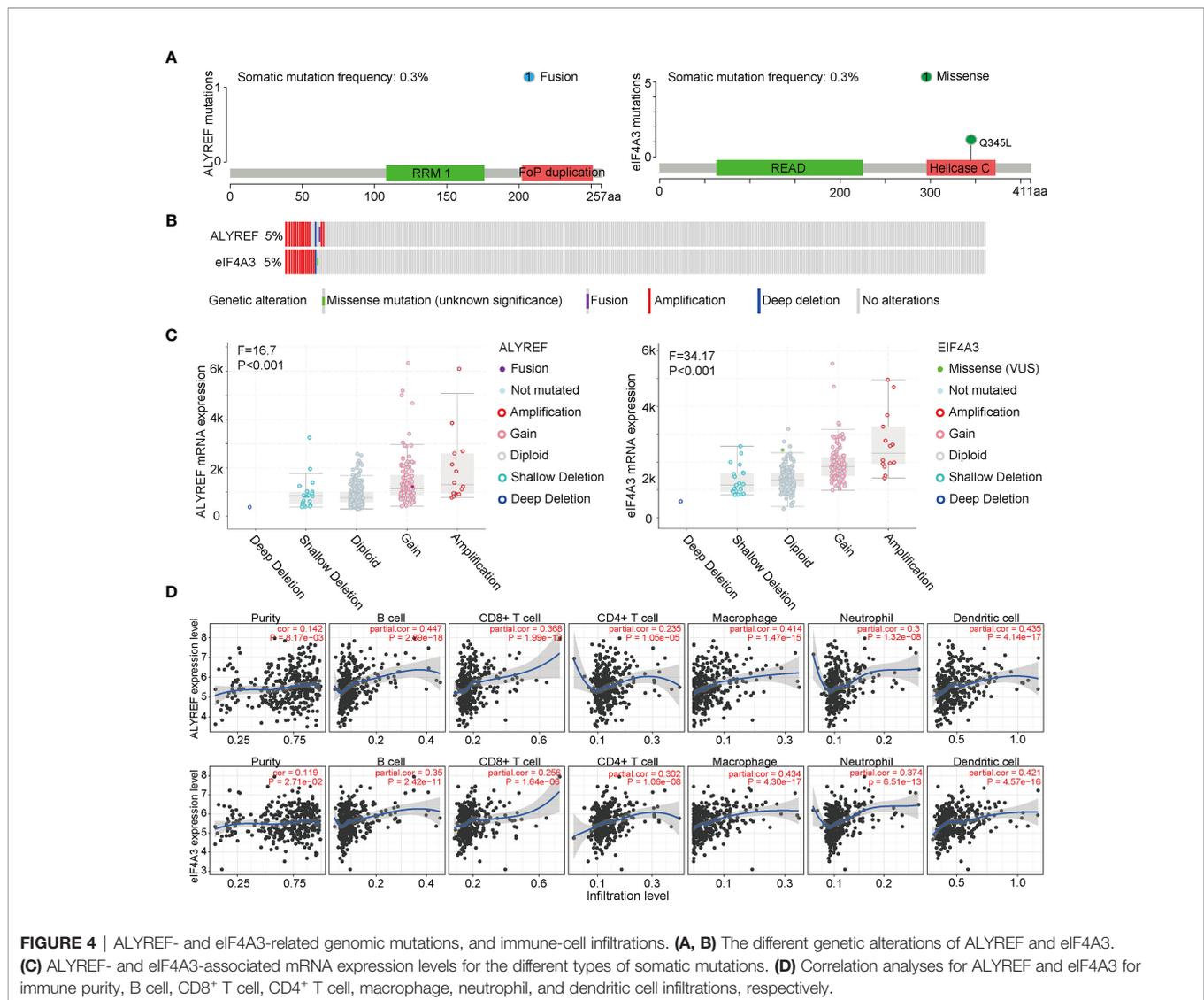
ALYREF and eIF4A3 Mutations and Immune-Cell Infiltrations

To assess the frequencies of ALYREF and eIF4A3 gene mutations, the cBioPortal genomic mutation database was used. The somatic mutation frequencies for both ALYREF and eIF4A3 were found to be 0.3%, with fusion mutations observed in ALYREF, and missense mutations found in eIF4A3 (Figure 4A). In addition, genetic alterations for both were 17 cases out of 372 cases in TCGA-LIHC dataset (approximately 5%), with details of the mutation subtypes shown in Figure 4B. An assessment of the effect of different mutation subtypes on gene expression indicated that there were significant differences between ALYREF and eIF4A3 expression levels (Figure 4C).

We next assessed any associations between immune-cell infiltrations in the patient samples and ALYREF and eIF4A3 expression levels. We found that ALYREF expression levels were significantly associated with B cells, macrophages, and dendritic cells (partial correlation >0.4, P<0.001), and that eIF4A3 expression was significantly associated with dendritic cells (partial correlation >0.4, P<0.001) (Figure 4D). These results indicate that ALYREF and eIF4A3 are closely correlated with some gene mutations and immune-cell infiltrations, providing promising clues for both HCC diagnosis and possible immune therapies.

Detection of Positively Associated DEGs in Common Between ALYREF and eIF4A3, and Pathway Enrichment Analyses

For ALYREF, we identified 835 positively associated DEGs (Spearman's correlation coefficients > 0.4), and 150 positively



associated DEGs for eIF4A3. As shown in **Figure 5A**, 104 of these DEGs were found to be in common for ALYREF and eIF4A3. For these 104 DEGs, a GO analysis for functional annotations showed that DNA replication initiation was significantly enriched in the biological processes category, and nucleoplasm and membrane were significantly enriched in the cellular component. For the molecular function component, poly (A) RNA binding and ATP binding were significantly enriched (**Figure 5B**). In addition, the KEGG analysis showed that DNA replication, cell cycle, and spliceosome were all pathways that were significantly enriched (**Table 1**).

The Protein-Protein Interaction (PPI) Network, and Identification of Hub Genes

We constructed a PPI network based on the 104 DEGs in common between ALYREF and eIF4A3 (**Figure 6A**) and found that these DEGs frequently interacted with each other. Based on Cytoscape software, we identified eight hub genes (*BIRC5*, *MCM2*, *MCM3*, *MCM6*, *NOP56*, *PCNA*, *RFC4*, and *SNRPD1*) by degree of association (**Figure 6B**). We next assessed the expression levels of these hub genes and found that all their expression levels were increased in HCC tissues

(**Figures 6C–J**), and that high hub gene expression was significantly related to poor clinical outcomes for HCC patients (**Supplementary Figure S3**). These results demonstrate that these hub genes may have oncogenic roles in HCC tumor development.

Potential Upstream miRNAs for Regulating ALYREF and eIF4A3 Expression

miRNAs can regulate target-gene expression by binding to target-gene mRNA. We explored potential upstream miRNAs for regulating ALYREF and eIF4A3 expression using the TargetScan and miRDB websites, and the details are shown in **Figure 7A**. Based on the TargetScan analysis, we identified 65 potential upstream miRNAs for ALYREF, and 435 potential upstream miRNAs for eIF4A3. In addition, based on the miRDB analysis, we identified 18 potential upstream miRNAs for ALYREF, and 24 potential upstream miRNAs for eIF4A3. Both miR-4666a-5p and miR-6124 were miRNAs that were in common for ALYREF and eIF4A3 based on these analyses. A schematic representation (**Figure 7B**) illustrates the potential binding sites for miR-4666a-5p and miR-6124 within the 3' untranslated regions (3'-UTRs) of ALYREF and eIF4A3.

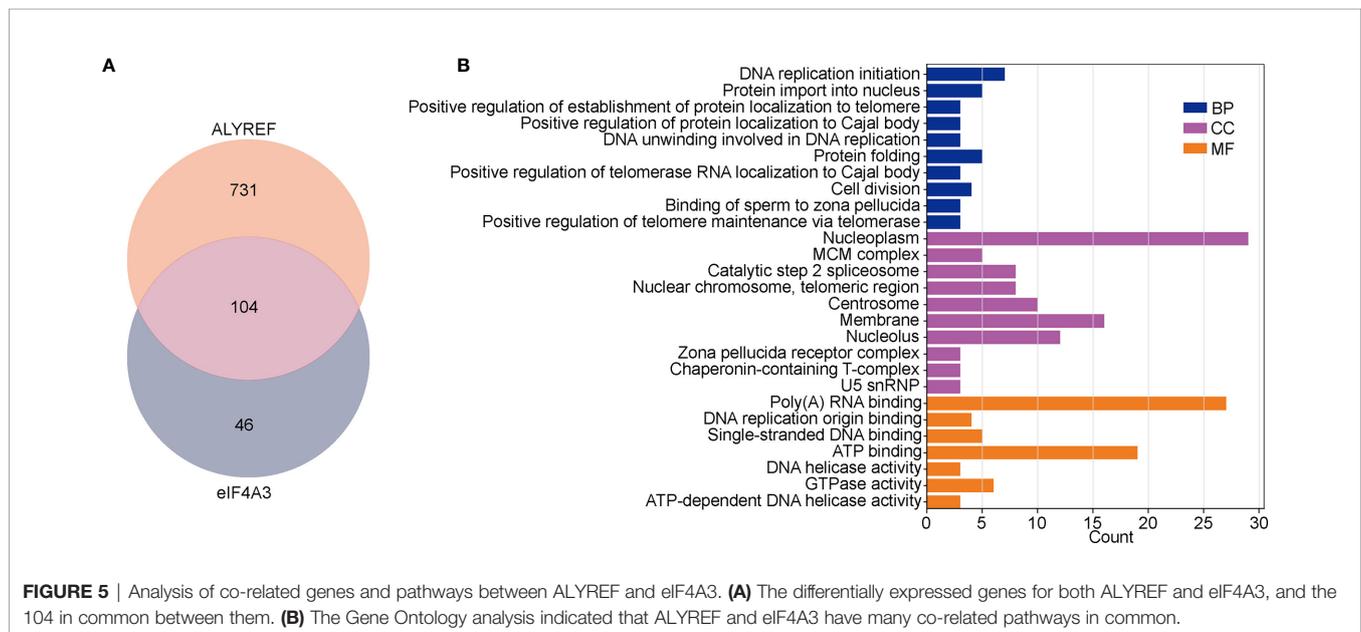
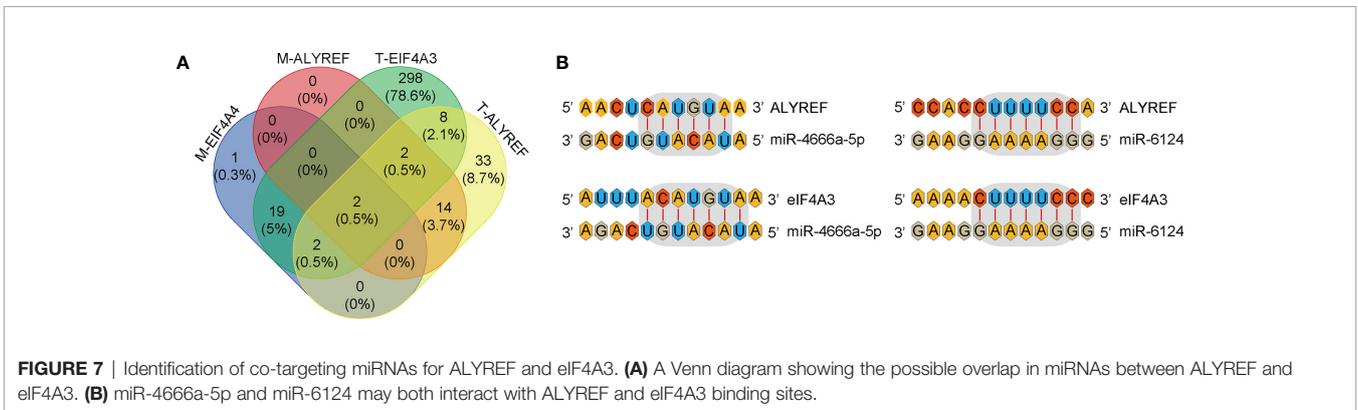
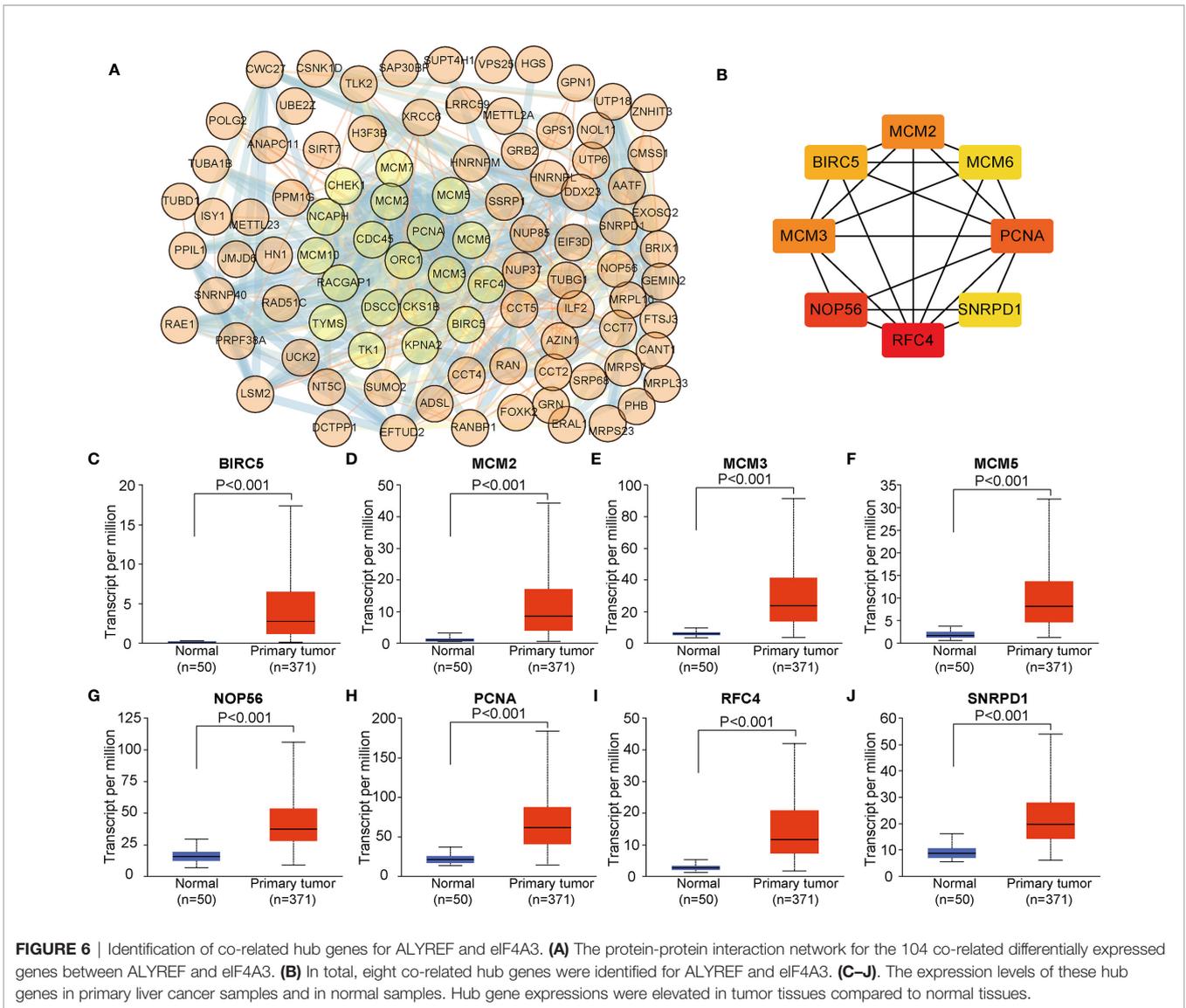


FIGURE 5 | Analysis of co-related genes and pathways between ALYREF and eIF4A3. **(A)** The differentially expressed genes for both ALYREF and eIF4A3, and the 104 in common between them. **(B)** The Gene Ontology analysis indicated that ALYREF and eIF4A3 have many co-related pathways in common.

TABLE 1 | KEGG analysis of the 104 common DEGs.

Term	Count	p-value	Genes	Fold enrichment	FDR
DNA replication	7	2.00E-07	PCNA, RFC4, MCM7, MCM3, MCM5, MCM6, MCM2	25.73529412	1.46E-05
Cell cycle	9	2.74E-06	CDC45, PCNA, MCM7, CHEK1, MCM3, MCM5, MCM6, MCM2, ANAPC11	9.684361549	9.53E-05
Spliceosome	9	3.92E-06	EFTUD2, ISY1, HNRNPM, SNRNP40, PPI1, PRPF38A, SNRPD1, DDX23, LSM2	9.233926129	9.53E-05
Pyrimidine metabolism	5	0.004612	NT5C, UCK2, TK1, TYMS, DCTPP1	7.193094629	0.084178
RNA transport	6	0.006302	NUP85, SUMO2, EIF3D, RAE1, RAN, NUP37	4.963235294	0.092004
Ribosome biogenesis in eukaryotes	4	0.020392	NOP56, UTP6, UTP18, RAN	6.701414743	0.248101



Collectively, these findings suggest that both miR-4666a-5p and miR-6124 may be potential upstream regulators of ALYREF and eIF4A3 expression.

DISCUSSION

Methylation modifications are crucial cues for malignancy initiation and progression (38, 39), and m⁵C methylation has recently attracted more research attention (40). As one of most common post-transcriptional modifications of RNA, m⁵C methylation has been found to play pivotal roles in several cancers, and has also shown great potential as a prognostic/predictive biomarker and a cancer therapy target for bladder cancer (16), glioblastoma multiforme (41), leukemia (42), and HCC (12). As m⁵C methylation is known to be involved in tumor progression, it is crucial to detect and unravel its molecular mechanisms.

Previous studies have reported that ALYREF, a reader of m⁵C methylation, could recognize and bind to m⁵C sites to exert biological functions. ALYREF has also been shown to be involved in multiple RNA processing events, and its aberrant expression has been correlated with poor cancer patient survival (14, 19, 20). Here, we have demonstrated that ALYREF expression was increased in HCC tissues, and that its high expression levels were significantly correlated with both advanced TNM staging and poor prognosis. Similar to our findings, He et al. (12) found that high ALYREF and NSUN4 expression levels were significantly associated with poor prognosis in HCC patients. Sun et al. reported that m⁵C-modified H19 promoted cancer development through the recruitment of G3BP1 in HCC (4). Taken together, both m⁵C regulators and m⁵C methylation play key roles in HCC development and progression and exploring the regulation mechanisms of m⁵C methylation may help to develop target-specific drugs and HCC gene therapies.

We also found that ALYREF expression was remarkably correlated to eIF4A3 expression, and that eIF4A3 expression was upregulated in HCC tissues compared to adjacent non-tumor tissues. Consistent with these findings, Lin et al. (22) reported that eIF4A3 mRNA was overexpressed in both cirrhosis and HCC tissue samples, and that high eIF4A3 expression was significantly correlated with shorter patient survival times. Chan et al. have reported that eIF4A3 may have a splice-dependent influence on mRNA translation (43), but more importantly, eIF4A3 has been shown to stimulate ALYREF binding not only at spliced RNAs sites, but also at single-exon transcripts sites (44). Collectively, these findings indicate that elevated eIF4A3 expression may function as an oncogene in HCC, and that it may be a novel therapeutic target for HCC.

We identified both miR-4666a-5p and miR-6124 as potential upstream regulators of ALYREF and eIF4A3 and identified the overexpression of eight hub genes (*BIRC5*, *MCM2*, *MCM3*, *MCM6*, *NOP56*, *PCNA*, *RFC4* and *SNRPD1*) in HCC tissues. In addition, the increased expressions of these hub genes were significantly associated with poor prognosis in patients with HCC. Concordant with these results, Cao et al. found that MCM2-7 expression was also both upregulated and associated

with poor patient outcomes (45), and similarly, elevated PCNA expression was reported in HCC samples and its high expression was significantly related to poor prognosis in HCC patients (46). A recent study has also reported that RFC4 expression was increased in HCC samples, and that this increased RFC4 was an indicator for poor HCC prognosis (47). These findings suggest that these identified hub genes may also be potential biomarkers and therapeutic targets for HCC.

CONCLUSIONS

Our findings have revealed that high ALYREF expression was significantly correlated with poor prognosis in HCC patients, and that the upregulation of ALYREF was significantly related to eIF4A3 upregulation. Based on these findings, the present study provides a better understanding of the function of a m⁵C regulator. ALYREF and eIF4A3 may represent novel biomarkers for HCC progression and their further study is warranted as potential targets for HCC therapy.

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 author.

AUTHOR CONTRIBUTIONS

LL defined the research theme and discussed analyses, interpretation, and presentation. And CX and YZ drafted the manuscript and analyzed the data. GL helped with references collection. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2021.633415/full#supplementary-material>

Supplementary Figure 1 | ALYREF mRNA expression based on clinical-characteristic subtypes. **(A–C)** ALYREF mRNA expression showed no significant differences based on gender, age, staging, and early metastasis status.

Supplementary Figure 2 | Increased pan-cancer expression of eIF4A3 mRNA and in clinical characteristic-based liver hepatocellular carcinoma (LIHC) subgroups. **(A)** eIF4A3 mRNA expression was increased in pan-cancer tissues compared to normal tissues, including LIHC. **(B)** eIF4A3 mRNA expression was significantly increased in primary tumor samples compared to normal tissue samples. **(C–F)** ALYREF transcription levels in different clinical trait-based groups. The

specific groupings were based on TP53 mutation status, race, clinical staging, and clinical grading. **(G)** The beta values for eIF4A3 mRNA expression in primary liver cancer and in normal samples. **(H)** A Kaplan-Meier analysis showed that high eIF4A3 expression indicated poor prognosis compared to low eIF4A3 expression.

Supplementary Figure 3 | A prognosis analysis based on the eight hub genes. **(A–H)** An overall survival analysis using the eight hub genes (*BIRC5*, *MCM2*, *MCM3*, *MCM6*, *NOP56*, *PCNA*, *RFC4*, and *SNRPD1*) showed that their high expressions were associated with poor prognosis compared to their low-expression subgroups.

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