해부 · 생물인류학 제 36 권 제 3 호
Anat Biol Anthropol Vol. 36, No. 3 (2023) pp. 111~126
https://doi.org/10.11637/aba.2023.36.3.111

Original Article

C4orf46 is a Potential Prognostic Biomarker for Liver Hepatocellular Carcinoma

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Abstract : Chromosome 4 includes genetic factors involved in various cancers, such as liver hepatocellular carcinoma (LIHC), and those related to the diagnostic biomarkers of human diseases. However, chromosome 4 open-reading frame 46 (*C4orf46*), a part of chromosome 4, has not yet been evaluated as an LIHC biomarker. In this study, we investigated the potential of *C4orf46* as a prognostic biomarker for LIHC and analyzed its correlations with immune cells and molecular functions. Data analysis using open online databases, including the Tumor Immune Estimation Resource, University of ALabama at Birmingham CANcer, Kaplan-Meier plotter, LinkedOmics, and Gene Expression Profiling Interactive Analysis version 2, revealed that *C4orf46* was highly expressed in LIHC tissues than in normal tissues. Moreover, survival rate analyses, such as disease-specific, overall, progression-free, and relapse-free survival analyses, revealed that high expression of *C4orf46* was associated with a poor prognosis in LIHC. *C4orf46* expression was positively correlated with various tumor-infiltrating immune cells, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells. *C4orf46* was also involved in chromosome segregation, DNA replication, cell cycle G2/M phase transition, and microtubule cytoskeleton organization during mitosis, mitotic cell cycle phase transition, and cell cycle checkpoints. In conclusion, *C4orf46* is a potential prognostic biomarker that is correlated with immune infiltration in LIHC.

Keywords : Liver hepatocellular carcinoma, *C4orf46*, Prognosis, Biomarker

INTRODUCTION

Liver cancer has a high mortality rate worldwide. Liver hepatocellular carcinoma (LIHC) is the most common histological subtype of liver cancer [1]. Moreover, LIHC is one of the most common malignant tumors worldwide that is the leading cause of death in patients with cirrhosis [2]. It is caused by various factors, including hepatitis virus infection, diabetes, and metabolic syndrome [3]. Despite the increase in the survival rate of patients with LIHC due to advances in diagnostic methods, treatment efficacy remains limited. In addition, LIHC is difficult to diagnose in the early stages, with most patients having a poor prognosis [4]. Therefore, LIHC, which has a high mortality rate owing to early detection failure, is recommended to increase the survival rate during the health checkups of patients with

The author(s) agree to abide by the good publication practice guideline for medical journals.
The author(s) declare that there are no conflicts of interest.
Received: August 31, 2023; **Revised:** September 15, 2023; **Accepted:** September 20, 2023
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ISSN 2671-566X (Online)

chronic liver disease. Moreover, new strategies for early detection and prognosis improvement are needed [5]. Therefore, identification of new prognostic biomarkers is necessary to improve the early diagnosis and survival rates of patients with LIHC.

Immune system plays an important role in the progression of cancer [6]. Tumor immune microenvironment (TIME) and tumor-infiltrating immune cells (TIICs) also play important roles in the body [7]. TIME refers to the microenvironment surrounding the tumor cells; when TIME is formed, immune cells gather at the tumor site. Immunocytes and substrate cells, the main components of TIME, prevent immune monitoring, inhibit apoptosis, and mediate various tumor growth processes. These TIMEs were correlated with immunotherapy and clinical outcomes. In LIHC, TIME is composed of immune cells, such as neutrophils, T cells, and B cells, and plays an important role in tumor progression [8]. TIME is controlled by TIICs and related to cancer; therefore, TIICs are used as predictors of cancer treatment.

Chromosome 4 encodes 757 proteins and is associated with various diseases, including cancer [9]. However, functions of various chromosome 4 open-reading frame (*C4orf*) genes remain unknown. Therefore, potential disease-related functions of *C4orf* genes require further investigation. Kim et al. confirmed *C4orf47* as a biomarker of LIHC [10]. These findings highlight the importance of *C4orf* genes for the prognosis and diagnosis of LIHC. However, the efficacy of *C4orf46* as a biomarker for LIHC remains unknown.

In this study, we performed bioinformatics analysis to determine the potential functions and prognostic value of *C4orf46* and explore whether its expression is related to immune infiltrates in LIHC. The findings of this study can aid in understanding the role of *C4orf46* in LIHC development.

MATERIALS AND METHODS

1. Tumor Immune Estimation Resource (TIMER) database analysis

TIMER (https://cistrome.shinyapps.io/timer/) was used to confirm the expression of *C4orf46* in various cancer and normal tissues, including LIHC [11]. TIMER is an online database including The Cancer Genome Atlas (TCGA), which contains more than 10,000 tumor samples. Here, we analyzed the expression of *C4orf46* in various cancers and determined its association with immune cells.

2. University of ALabama at Birmingham CANcer (UALCAN) database analysis

UALCAN (http://ualcan.path.uab.edu) is a database that uses TCGA level 3 RNA sequencing and clinical data from 31 cancer types [12]. Here, UALCAN was used to analyze the relative expression levels of query genes in tumor and normal samples and various tumor subgroups based on histological subtypes, individual cancer stage, patient age, tumor grade, sex, *TP53* mutation, nodal metastasis status, and sample type.

3. Kaplan-Meier (KM) plotter database analysis

KM plotter (http://kmplot.com) was used to estimate the efficacy of the genes identified via cancer prognostic analysis. We confirmed the prognostic value of *C4orf46* in LIHC [13]. Using KM plotter, survival rates, such as overall survival (OS), relapse-free survival (RFS), progression-free survival (PFS), and disease-specific survival (DSS), and clinicopathological characteristics, such as sex, stage, grade, and race, were confirmed in LIHC. It represents a hazard ratio (HR) with a 95% confidence interval and log-rank *P*-value.

4. LinkedOmics database analysis

LinkedOmics database is an online platform for TCGA cancer data analysis [14]. It can be used to identify biological processes using Kyoto Encyclopedia of Genes and Genomes (KEGG) via gene set enrichment analysis (GSEA). The gene expressing *C4orf46* is indicated by a scatterplot. The rank criterion was false-discovery rate <0.05, and 500 simulations were identified.

5. Gene Expression Profiling Interactive Analysis version 2 (GEPIA2)

GEPIA2 database is an interactive website that includes $>9,000$ cancer samples and $>8,000$ normal samples from TCGA and Genotype-Tissue Expression projects [15]. GEPIA2 was used to determine the gene expression levels and survival rates, including OS and DFS, in 33 cancers. Survival results are shown as KM curves with HR and *P*-values from a log-rank test. $P<0.05$ using the Student's *t*-test.

6. Statistical analysis

All results of this study were derived from an open database, and all analyses were conducted using web tools. All results were expressed as the *P*-values of the log-rank test, and the statistical significance was set at $P<0.05$.

RESULTS

1. mRNA expression levels of *C4orf46* in LIHC

Expression levels of *C4orf46* in various types of tumors, including LIHC, were compared to those in normal tissues using the TIMER database. *C4orf46* expression levels were upregulated in the LIHC, bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell?carcinoma (KIRP), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC) tissues than in the normal tissues (Fig. 1A). Moreover, significant correlations were observed between *C4orf46* expression levels and the histological subtype, stage (I, II, and III), age, tumor grade, sex, *TP53* mutation, metastasis, and sample type in LIHC (Fig. 1B).

2. Prognostic value analysis of *C4orf46* in LIHC

We analyzed the correlations of *C4orf46* expression levels with the survival rates, including DSS, OS, PFS, and RFS, of patients with LIHC. Upregulated *C4orf46* expression was associated with a poor prognosis in LIHC (DSS: HR = 1.65, $P=0.028$; OS: HR = 1.44, $P=0.039$; PFS: HR = 1.53, $P=0.0045$; RFS: HR = 1.43, $P=0.032$; Fig. 2A). Moreover, high *C4orf46* expression was associated with poor OS (HR = 1.5, $P=0.023$) and DFS (HR = 1.2, $P=0.15$; Fig. 2B). These findings demonstrate the prognostic value of *C4orf46* in LIHC.

3. Correlations between *C4orf46* expression levels and clinicopathological characteristics of patients with LIHC

We determined the correlations between *C4orf46* expression levels and clinicopathological characteristics of patients with LIHC. High *C4orf46* expression levels were correlated with poor OS in females, stage (II, II + III, III and III + IV), grade III, AJCC_T (II and III), vascular invasion, alcohol consumption (Yes and No) and hepatitis virus (No). In addition, high *C4orf46* expression levels were correlated with poor RFS in females, stage (I and II + III), grade (II and III), AJCC_T I, vascular invasion (No), Whites, Asians, alcohol consumption (Yes and No) and hepatitis virus (Yes and No). High *C4orf46* expression levels were correlated with poor PFS in males, females, stage (I, I + II and II), grade (II and III), AJCC_T (I and II), vascular invasion (No), Whites, Asians, alcohol consumption (Yes and No) and hepatitis virus (Yes and No). Finally, high *C4orf46* expression levels were correlated with poor DSS in males, stage (II, II + III, III and III + IV), grade (I, II and III), AJCC_T (II and III), vascular invasion (No), Whites, Asians, alcohol consumption (No), and hepatitis virus (No) These results indicate that *C4orf46* expression is correlated with the prognosis of LIHC (Table 1). Supplementary figures were provided below.

4. Correlations between *C4orf46* expression levels and infiltrating immune cells in LIHC

We explored the correlations between *C4orf46* expression levels and infiltrating immune cells in LIHC using the TIMER database. *C4orf46* expression levels were significantly and positively correlated with the infiltration levels of neutrophil (R = 0.324, $P=7.08e-10$), myeloid dendritic cells (R = 0.543, $P=7.74e-28$), macrophages (R = 0.419, $P=3.91e-16$), B cells (R = 0.375, $P=7.97e-12$), CD4+ T cells (R = 0.3, $P=1.25e-08$), and CD8+ T cells (R = 0.188, $P=4.48e-04$; Fig. 3A). We also investigated the relationship between each infiltrating immune cell type and LIHC prognosis. High *C4orf46* expression and cell infiltration levels were associated with a worse LIHC prognosis (Fig. 3B). Therefore, high *C4orf46* expression is related to immune cell infiltration and prognosis in LIHC.

5. *C4orf46* co-expression network in LIHC

We assessed the biological functions of *C4orf46* in LIHC using LinkedOmics. In total, 13711 genes were positively correlated, whereas 6203 genes were negatively correlated with *C4orf46* (Fig. 4A). Top 50 genes positively and negatively correlated with *C4orf46* are shown in

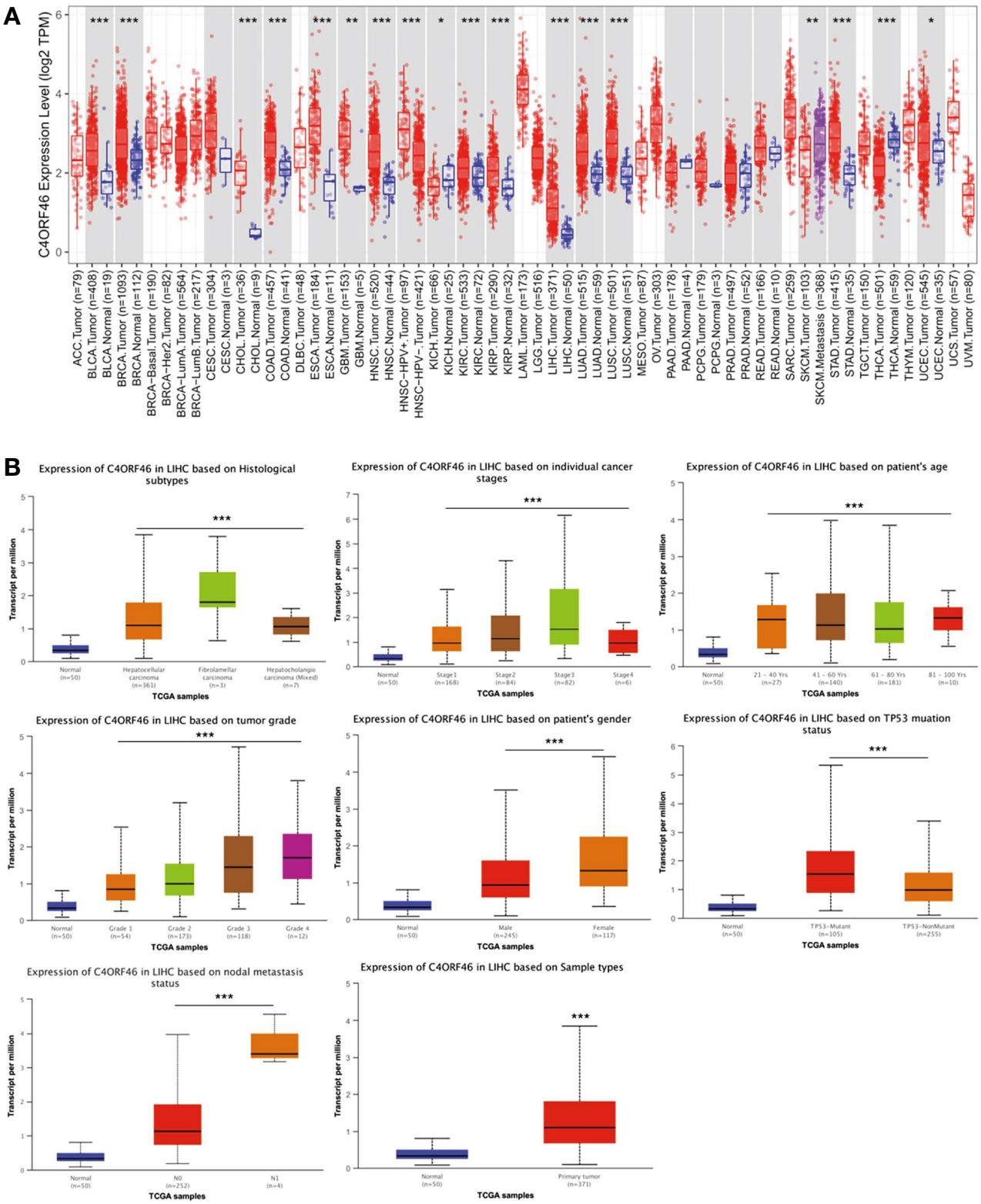

Fig. 1. Expression levels of chromosome 4 open-reading frame 46 (*C4orf46*) in various types of cancer. (A) Expression levels of *C4orf46* in human tumor tissues compared with those in normal tissues. (B) Histological subtype, stage, age, tumor grade, sex, *TP53* mutation, metastasis, and sample types in patients compared with those in the control group (***$P < 0.005$).

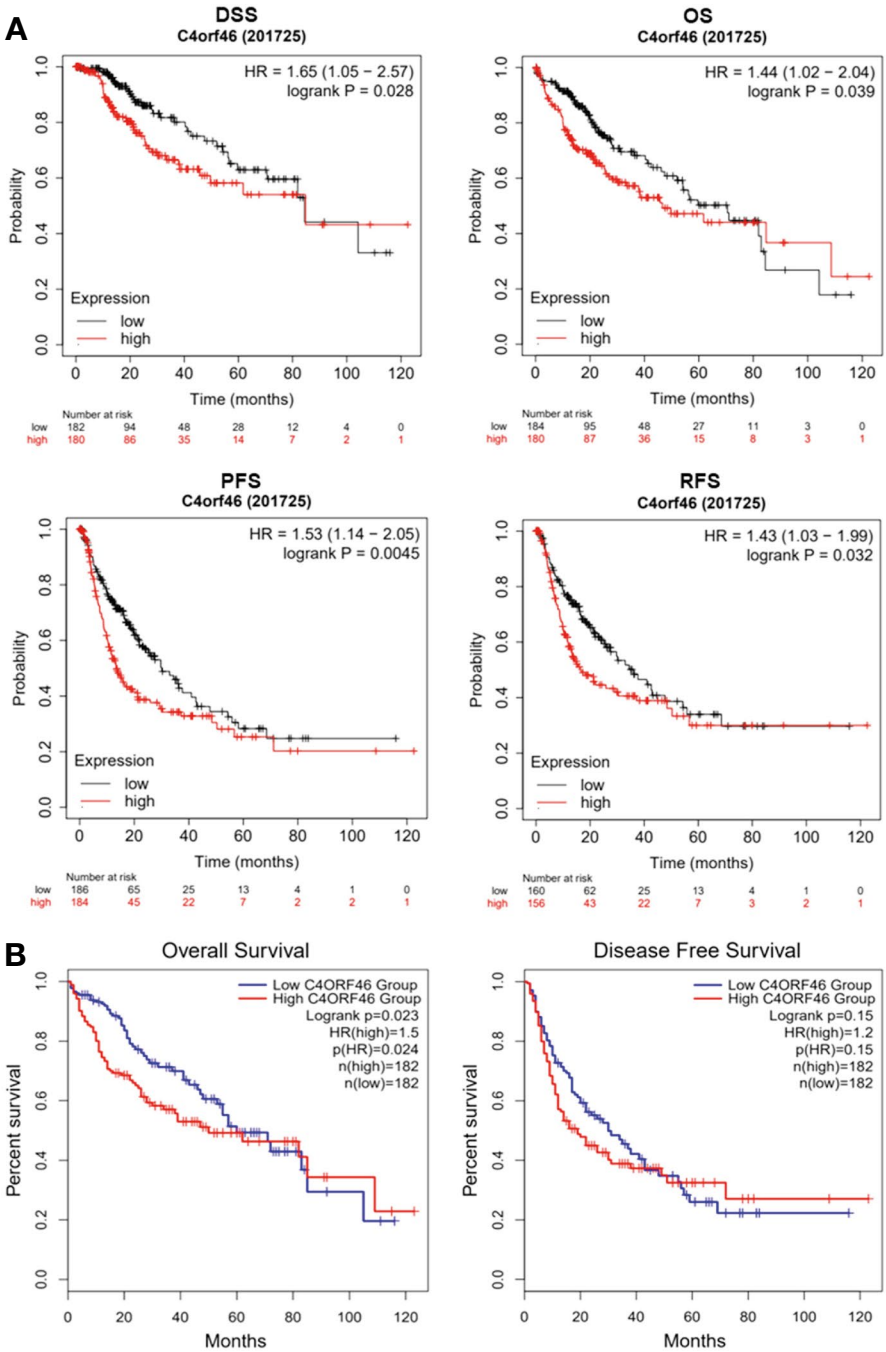

Fig. 2. Prognostic significance of the overexpression of *C4orf46* in liver hepatocellular carcinoma (LIHC). Prognostic significance of *C4orf46* was analyzed using the Kaplan-Meier plotter (A) and Tumor Immune Estimation Resource (TIMER) (B) databases. Abbreviations: DSS, disease-specific survival; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival.

Fig. 4B. *C4orf46* was positively correlated with epithelial cell transforming 2 (*ECT2*; R = 0.7556, *P* = 5.891e-68), *KIAA1524* (R = 0.7628, *P* = 5.843e-70), kinesin family member 23 (*KIF23*; R = 0.7592, *P* = 6.275e-69), polo-like kinase 4 (*PLK4*; R = 0.8119, *P* = 6.083e-86), and WD repeat and HMG-box DNA-binding protein 1 (*WDHD1*; R = 0.7732, *P* = 5.409e-73; Fig. 4C). *C4orf46* was negatively correlated with abhydrolase domain containing 14B

Table 1. Correlations between chromosome 4 open-reading frame 46 (*C4orf46*) expression levels and clinicopathological characteristics of patients with liver hepatocellular carcinoma (LIHC)

Clinicopathological characteristics	Overall survival ($n=3218$)			Relapse-free survival ($n=2809$)			Progression-free survival ($n=3162$)			Disease-specific survival ($n=3189$)		
	N	Hazard ratio	*P*-value	N	Hazard ratio	*P*-value	N	Hazard ratio	*P*-value	N	Hazard ratio	*P*-value
Sex												
Male	246	1.15 (0.74-1.79)	0.53	210	1.03 (0.69-1.53)	0.88	149	1.2 (0.84-1.72)	0.31	244	1.45 (0.82-2.56)	0.2
Female	118	1.39 (0.79-2.43)	0.25	106	1.44 (0.8-2.6)	0.22	121	1.55 (0.93-2.58)	0.093	118	1.18 (0.58-2.43)	0.64
Stage												
I	170	0.96 (0.52-1.76)	0.89	153	1.28 (0.75-2.2)	0.37	171	1.38 (0.84-2.27)	0.2	168	1.02 (0.42-2.46)	0.97
I + II	253	1.11 (0.69-1.78)	0.67	228	1.14 (0.75-1.72)	0.55	256	1.38 (0.94-2.01)	0.096	251	1.24 (0.63-2.47)	0.53
II	83	1.48 (0.67-3.26)	0.33	75	0.89 (0.45-1.75)	0.74	85	1.46 (0.81-2.63)	0.2	83	1.96 (0.64-6.01)	0.23
II + III	166	1.66 (1.03-2.67)	0.035	145	1.18 (0.76-1.83)	0.47	170	1.48 (0.99-2.2)	0.055	166	1.97 (1.07-3.63)	0.026
III	83	1.68 (0.92-3.06)	0.087	70	1.12 (0.61-2.04)	0.72	85	1.1 (0.64-1.88)	0.74	83	1.64 (0.8-3.35)	0.17
III + IV	87	1.47 (0.82-2.61)	0.19	70	1.12 (0.61-2.04)	0.72	90	1.17 (0.69-1.98)	0.56	87	1.44 (0.72-2.87)	0.3
IV	4	–	–	0	–	–	5	–	–	3	–	–
Grade												
I	65	0.92 (0.35-2.41)	0.86	55	0.87 (0.33-2.31)	0.79	55	0.96 (0.43-2.12)	0.91	55	0.58 (0.15-2.28)	0.43
II	174	1.02 (0.61-1.7)	0.95	149	1.41 (0.87-2.31)	0.16	177	1.66 (1.07-2.58)	0.023	171	1.49 (0.76-2.91)	0.24
III	118	1.54 (0.84-2.83)	0.16	107	1.46 (0.85-2.5)	0.16	121	1.63 (0.99-2.69)	0.052	119	1.53 (0.72-3.28)	0.27
IV	12	–	–	11	–	–	12	–	–	12	–	–
AJCC_T												
I	180	0.98 (0.55-1.76)	0.96	160	1.34 (0.79-2.27)	0.28	181	1.37 (0.85-2.22)	0.19	178	1.05 (0.47-2.36)	0.9
II	90	1.48 (0.7-3.11)	0.3	80	1.2 (0.64-2.23)	0.57	93	1.49 (0.86-2.58)	0.15	91	2.16 (0.79-5.86)	0.12
III	78	1.51 (0.82-2.78)	0.18	67	1.13 (0.6-2.12)	0.7	80	1.16 (0.66-2.04)	0.61	77	1.51 (0.72-3.17)	0.27
IV	13	–	–	6	–	–	13	–	–	13	–	–
Vascular invasion												
No	203	1.36 (0.82-2.28)	0.23	175	1.46 (0.9-2.36)	0.12	205	1.7 (1.08-2.66)	0.019	201	1.45 (0.71-2.95)	0.31
Micro	90	1.12 (0.53-2.39)	0.77	82	1 (0.54-1.89)	0.99	92	1.18 (0.67-2.09)	0.56	90	1.33 (0.44-3.97)	0.61
Macro	16	–	–	14	–	–	16	–	–	14	–	–
Race												
White	181	1.03 (0.65-1.62)	0.91	147	1.37 (0.87-2.15)	0.17	184	1.43 (0.97-2.12)	0.073	179	1.23 (0.7-2.15)	0.47
Asian	154	2.87 (1.52-5.44)	0.00071	145	1.87 (1.12-3.12)	0.015	157	2.03 (1.26-3.28)	0.0029	154	2.79 (1.23-6.34)	0.011
Alcohol consumption												
Yes	115	0.72 (0.38-1.36)	0.31	99	1.45 (0.81-2.61)	0.21	117	1.39 (0.83-2.33)	0.21	117	0.91 (0.45-1.88)	0.81
No	202	1.63 (1.02-2.59)	0.038	183	1.57 (1.01-2.44)	0.045	205	1.88 (1.25-2.82)	0.0019	199	2.02 (1.07-3.8)	0.027
Hepatitis virus												
Yes	150	1.16 (0.61-2.21)	0.66	139	1.19 (0.73-1.96)	0.48	153	1.31 (0.83-2.08)	0.24	151	1.22 (0.54-2.77)	0.64
No	167	1.47 (0.94-2.31)	0.092	133	1.67 (1-2.76)	0.046	169	2.02 (1.29-3.14)	0.0016	165	1.81 (1.02-3.22)	0.041

(*ABHD14B*; R = 0.5941, *P* = 8.025e-36), acireductone dioxygenase 1 (*ADI1*; R = 0.5994, *P* = 1.368e-36), lactate dehydrogenase D (*LDHD*; R = 0.6104, *P* = 3.169e-38), NADH:ubiquinone oxidoreductase complex assembly factor 1 (*NDUFAF1*; R = 0.6153, *P* = 5.548e-39), and thiosulfate sulfurtransferase (*TST*; R = 0.5945, *P* = 7.036e-36; Fig. 4D). These results indicate that *C4orf46* expression has a significant effect on LIHC prognosis.

6. The gene set enrichment analysis of *C4orf46* in LIHC

We identified the biological process categories of GSEA-GO. Co-expressed genes were involved in chromosome segregation, DNA replication, cell cycle G2/M phase transition, microtubule cytoskeleton organization during mitosis, mitotic cell cycle phase transition, and cell cycle checkpoints (Fig. 5A). Furthermore, GSEA-KEGG

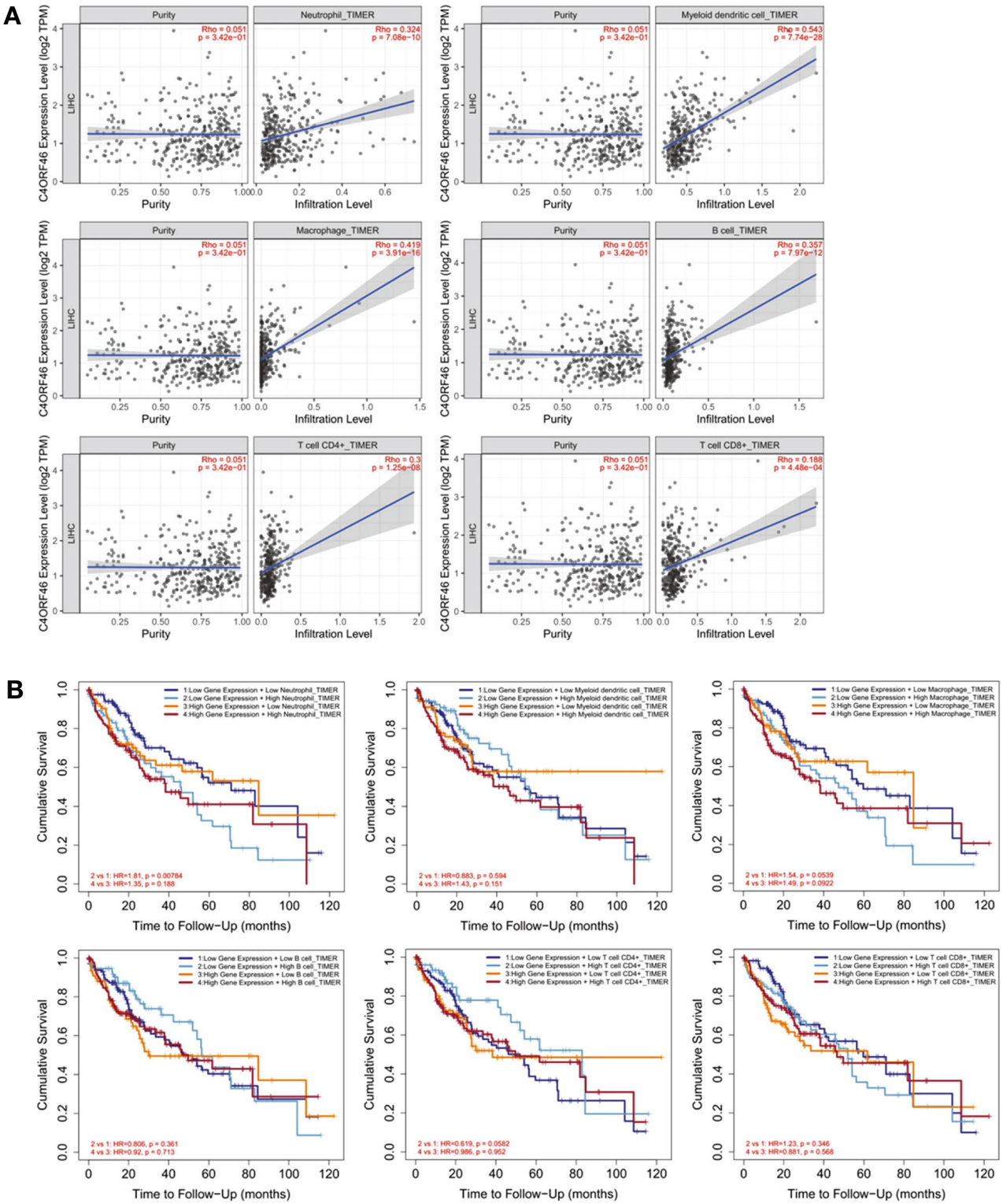

Fig. 3. Correlations between *C4orf46* expression levels and infiltrating immune cells in LIHC. (A) Correlations between *C4orf46* levels and infiltrating immune cells were analyzed. (B) Prognostic value of *C4orf46* and its correlation with infiltrating immune cells were assessed.

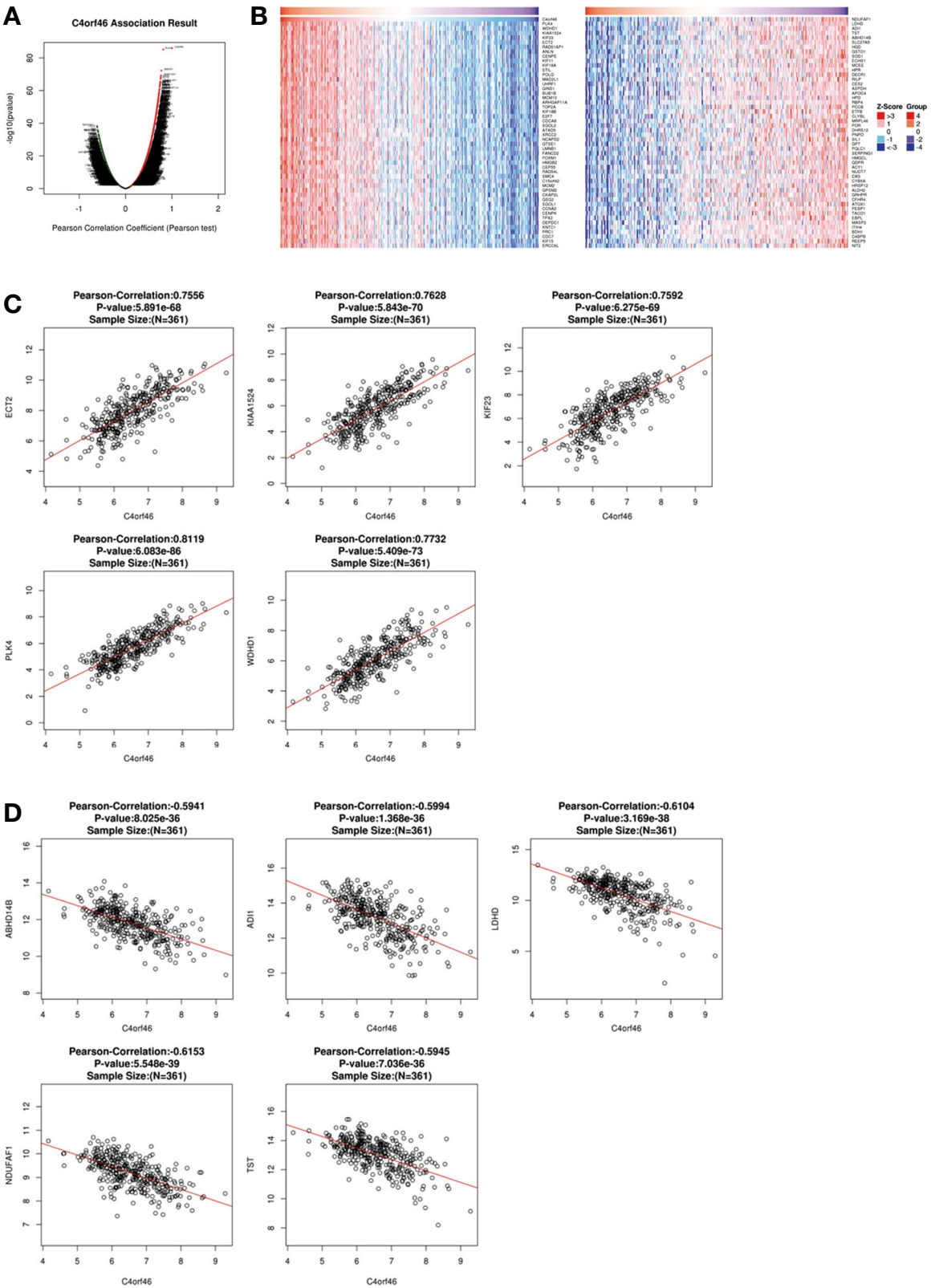

Fig. 4. Co-expression genes of *C4orf46* in LIHC. Co-expression genes of *C4orf46* were analyzed (A). Red and blue indicate the positively and negatively correlated genes of *C4orf46*, respectively (B). Correlations with positively (C) and negatively (D) related genes of *C4orf46* in LIHC.

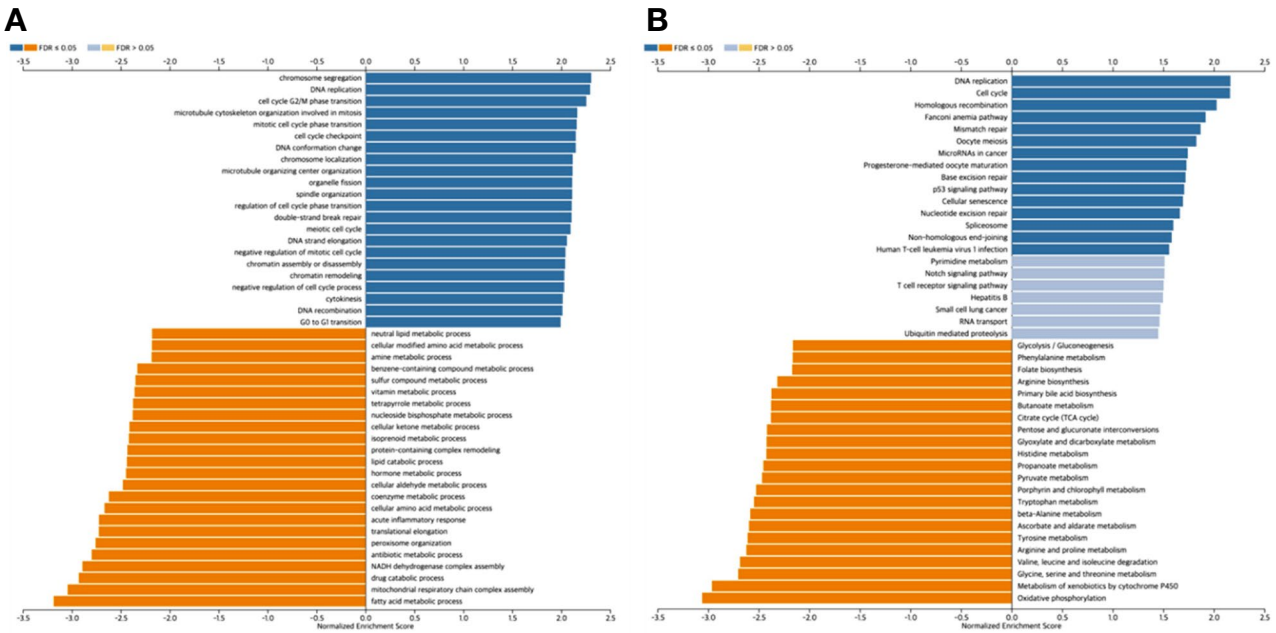

Fig. 5. The gene set enrichment analysis of *C4orf46* in LIHC. Bar charts of biological process (A) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (B).

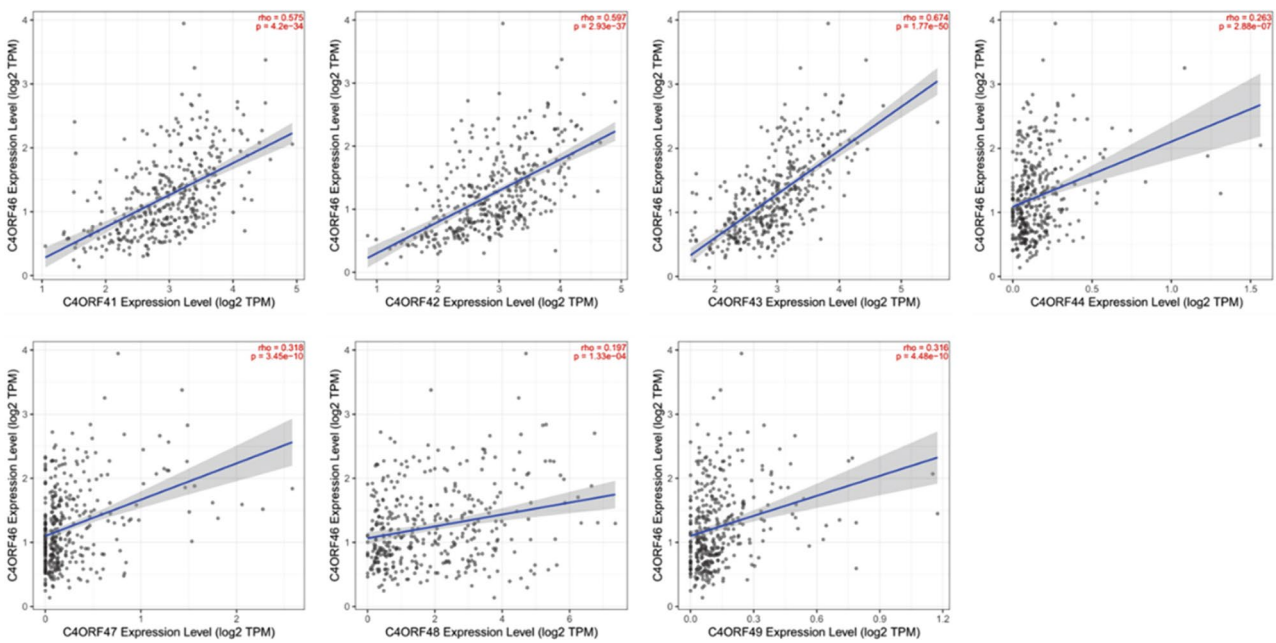

Fig. 6. Correlations between *C4orf46* and *C4orf* genes in LIHC.

pathway analysis revealed that the co-expressed genes were involved in DNA replication, cell cycle, homologous recombination, Fanconi anemia pathway, and mismatch repair (Fig. 5B). These findings suggest that *C4orf46* influences the LIHC prognosis by controlling the global transcriptome process.

7. Correlations between *C4orf46* and *C4orf* genes in LIHC

We explored the correlations between *C4orf46* and

C4orf in LIHC using the TIMER database. *C4orf46* was significantly and positively correlated with *C4orf41* (R = 0.575, *P* = 4.2e-34), *C4orf42* (R = 0.597, *P* = 2.93e-37), *C4orf43* (R = 0.674, *P* = 1.77e-50), *C4orf44* (R = 0.263, *P* = 2.88e-07), *C4orf47* (R = 0.318, *P* = 3.45e-10), *C4orf48* (R = 0.197, *P* = 1.33e-04), and *C4orf49* (R = 0.316, *P* = 4.48e-10; Fig. 6). Therefore, *C4orf46* was confirmed to be correlated with various *C4orf* genes.

DISCUSSION

LIHC, the most common form of primary liver cancer, is a major cause of cancer-related deaths worldwide. Viral infections and drugs are risk factors for patients with underlying liver diseases. Surgical treatment is effective in the early stages of LIHC; however, the 5-year survival rate is only approximately 50% in advanced stages. Therefore, identification of new biomarkers is necessary to improve the diagnosis and prognosis of LIHC [16,17].

Immune cells play important roles in cancer progression [18]. They even facilitate cancer cell growth in some cases [19]. Therefore, TIME, which refers to the microenvironment surrounding the tumor cells, is used as a predictor of cancer treatment efficacy using immune cells mediated by various tumor growth processes. The prognostic value of LIHC-related immune cells has been demonstrated in many studies [20-23].

Chromosome 4 includes genetic factors associated with various cancers, including LIHC, and factors related to the diagnosis of human diseases. However, *C4orf46*, a member of chromosome 4, has not yet been reported as an LIHC biomarker. Here, we determined the expression levels of *C4orf46* in LIHC using online databases and observed its high expression in tumor tissues than in normal tissues. In addition, we identified the correlation between *C4orf46* expression and cancer histological subtype, stage, age, tumor grade, sex, *TP53* mutation, metastasis, and sample types. These factors were confirmed as predictors of high *C4orf46* levels in LIHC. In addition, KM plotter and GEPIA analyses revealed that high expression of *C4orf46* was correlated with poor prognosis in LIHC. High *C4orf46* expression was correlated with a high HR for low survival in patients with LIHC. In addition, high *C4orf46* expression was correlated with several clinicopathological factors. Our results suggest *C4orf46* as a prognostic biomarker for LIHC.

Next, we confirmed the correlation between *C4orf46* expression and infiltration levels of immune cells in LIHC. High *C4orf46* expression and immune cell infiltration were associated with worse prognosis in LIHC. These results were consistent with those reported for *C4orf47* in *C4orf* gene [10]. Taken together, these results suggest that high expression of *C4orf46* and infiltration of immune cells are related to poor prognosis in LIHC.

Determination of the expression levels of various genes aids in the classification of cancer patients and assessment of their prognoses as well as their correlations with related genes. *C4orf46* is positively correlated with genes, such as *ECT2*, *KIF23*, *PLK4*, and *WDHD1*. *ECT2* has been suggested as a biomarker for lung adenocarcinoma [24]. *KIF23* and *PLK4* act as biomarkers for renal cell carcinoma [25,26]. Moreover, *WDHD1* acts as a prognostic biomarker for LIHC [27]. In this study, we evaluated the correlations between *C4orf46* and various other biomarkers.

We identified biological process categories and found that *C4orf46* mainly participated in chromosome segregation, DNA replication, cell cycle G2/M phase transition, and microtubule cytoskeleton organization during mitosis, and mitotic cell cycle phase transition. Moreover, GSEA-KEGG pathway analysis showed that the co-expressed genes were involved in DNA replication, cell cycle, homologous recombination, Fanconi anemia pathway, and mismatch repair. These results highlight the diverse functions of *C4orf46*.

In addition, the correlation between *C4orf46* and *C4orf* genes (*C4orf41*, *C4orf42*, *C4orf43*, *C4orf44*, *C4orf47*, *C4orf48* and *C4orf49*) was confirmed. These results suggest the possibility as a prognostic factor for *C4orf* genes, and further research is considered necessary to predict the prognosis of LIHC.

In conclusion, we assessed the value of *C4orf46* as a prognostic biomarker for LIHC and determined its correlations with immune cells and molecular functions in this study. Our results can facilitate the identification of potential targets for LIHC immunotherapy and provide insights into the roles of *C4orf46* in various cancers, including LIHC. However, the results of this study present the online database, and it is necessary to confirm the expression and mechanism of *C4orf46* genes in liver cancer cell lines through further research.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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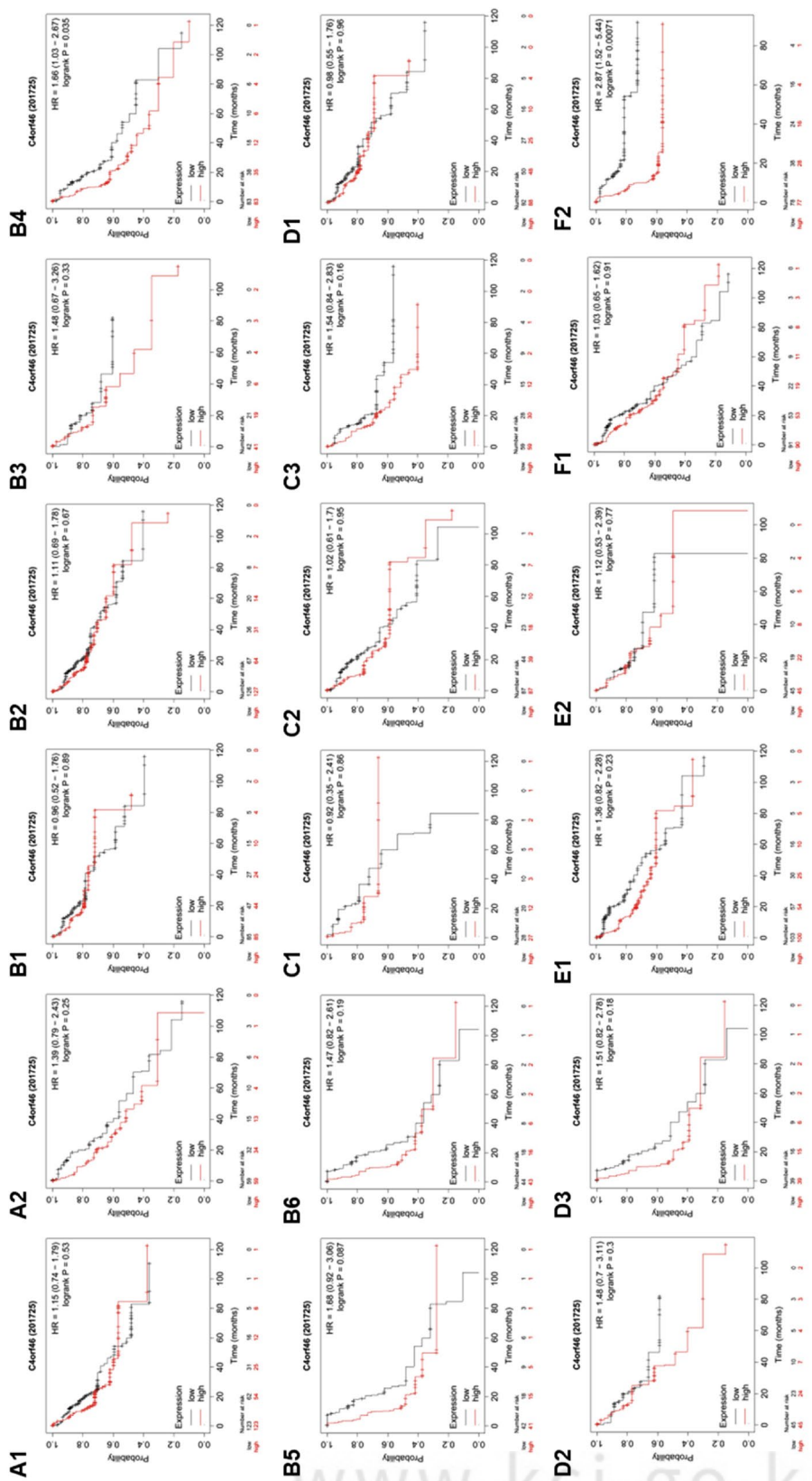
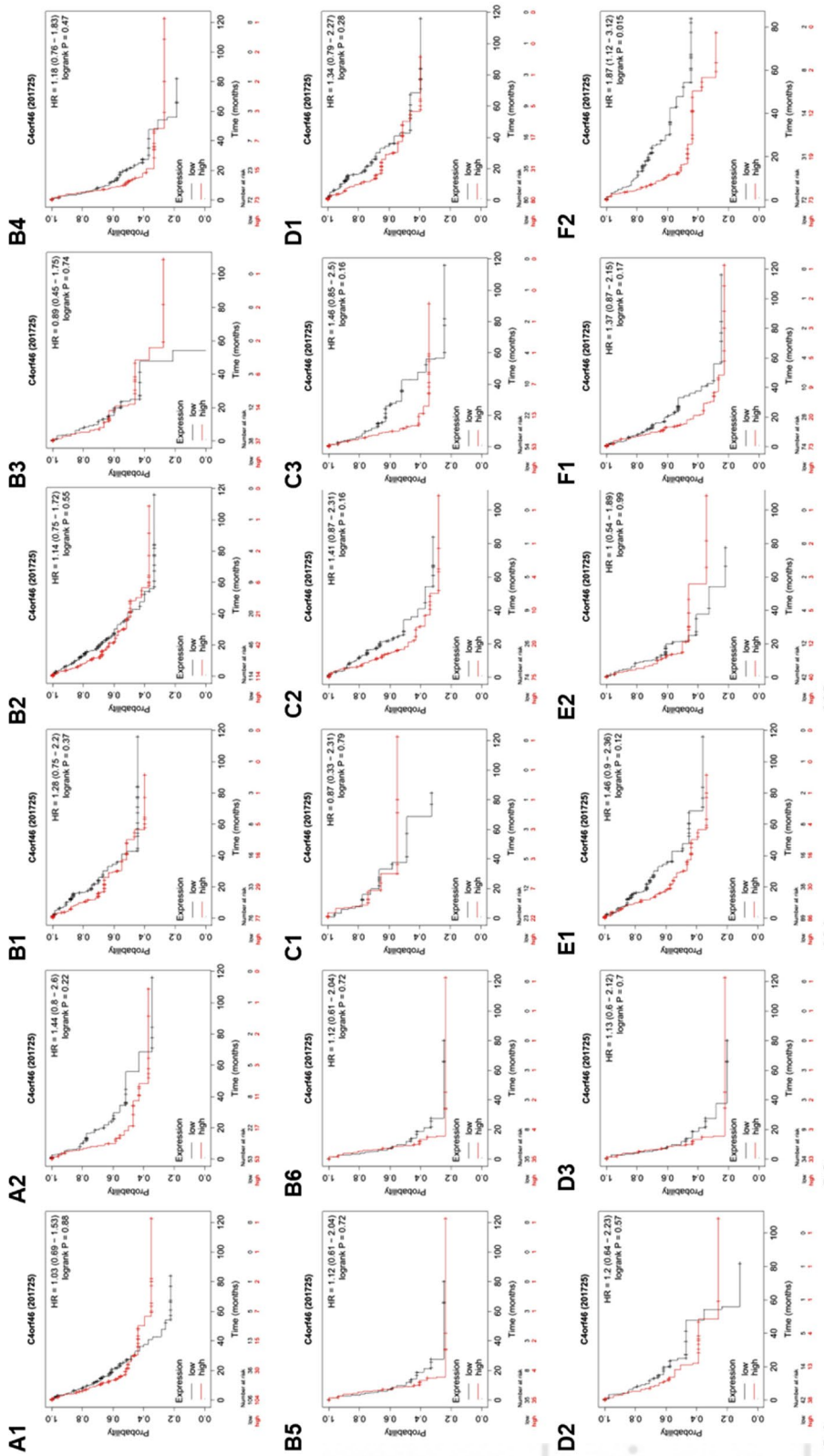
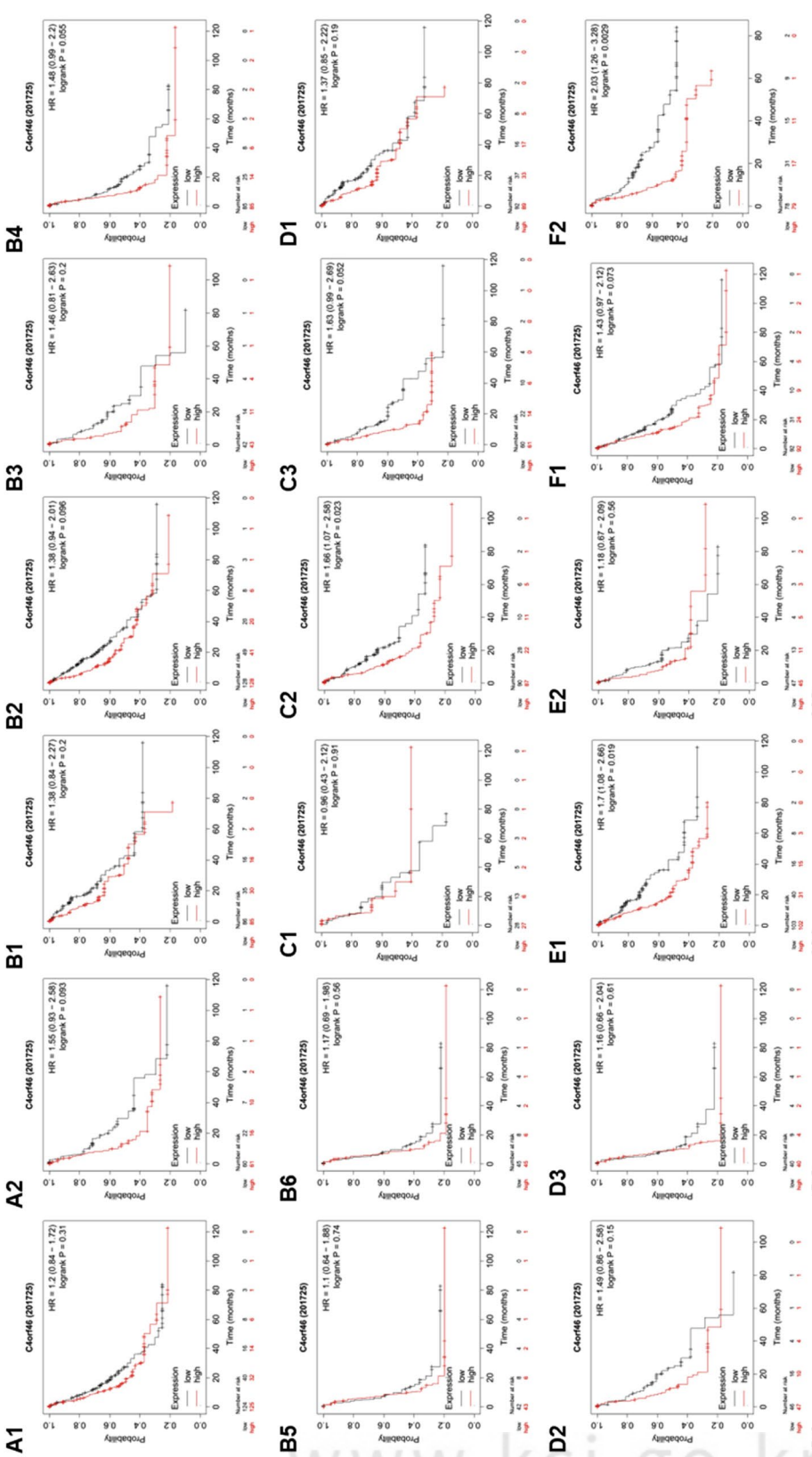


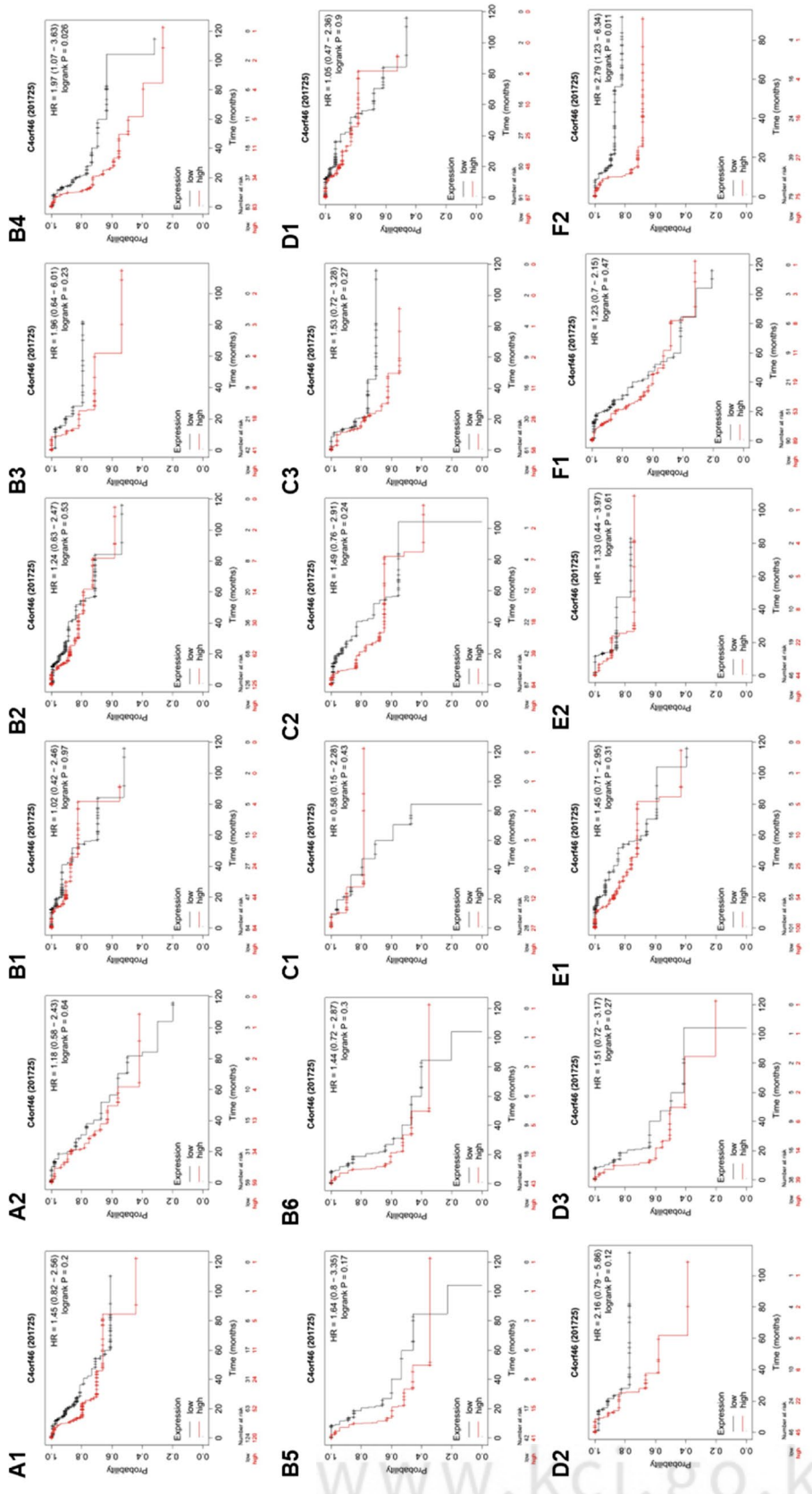
Supplementary Fig. 1. Correlation between *C4orf46* and clinicopathological characteristics in OS. (A) Sex: male, female, (B) Stage: I, I+II, II, II+III, III, III+IV, IV, (C) Grade: I, II, III, (D) AJCC_T: I, II, III, (E) Vascular invasion: no, micro, (F) Race: white, asian, (G) Alcohol consumption: yes, no, (H) Hepatitis virus: yes, no.



Supplementary Fig. 2. Correlation between *C4orf46* and clinicopathological characteristics in RFS. (A) Sex: male, female, (B) Stage: I, I+II, II, II+III, III, III+IV, IV, (C) Grade: I, II, III, (D) AJCC_T: I, II, III, (E) Vascular invasion: no, micro, (F) Race: white, asian, (G) Alcohol consumption: yes, no, (H) Hepatitis virus: yes, no.



Supplementary Fig. 3. Correlation between *C4orf46* and clinicopathological characteristics in PFS. (A) Sex: male, female, (B) Stage: I, I+II, II, II+III, III, III+IV, IV, (C) Grade: I, II, III, (D) AJCC_T: I, II, III, (E) Vascular invasion: no, micro, (F) Race: white, asian, (G) Alcohol consumption: yes, no, (H) Hepatitis virus: yes, no.



Supplementary Fig. 4. Correlation between *C4orf46* and clinicopathological characteristics in DSS. (A) Sex: male, female, (B) Stage: I, I+II, II, II+III, III, III+IV, IV, (C) Grade: I, II, III, (D) AJCC_T: I, II, III, (E) Vascular invasion: no, micro, (F) Race: white, asian, (G) Alcohol consumption: yes, no, (H) Hepatitis virus: yes, no.