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ORIGINAL RESEARCH

Elevated Expression of CHAF1A in Hepatocellular Carcinoma Progression and Immune Modulation

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Purpose: This study aimed to explore the role of CHAF1A in hepatocellular carcinoma (HCC) progression, focusing on its expression, co-expression genes, genomic alterations, promoter methylation, clinical relevance, prognostic value, and immune associations.

Methods: CHAF1A mRNA expression was analyzed using UALCAN. Co-expression genes and functions were explored via LinkedOmics. Genomic alterations were assessed using cBioPortal. Promoter methylation and clinical correlations were examined using GEO datasets. Prognostic significance was evaluated via Kaplan-Meier analysis. Immune cell infiltration and checkpoint gene associations were investigated.

Results: CHAF1A was significantly upregulated in HCC and involved in cancer-related pathways. Genomic alterations were prominent in T1-stage tumors, often linked to alcohol-related liver disease. Promoter methylation influenced HCC progression and prognosis. CHAF1A expression correlated with clinical characteristics (gender, stage, grade, etc.) and showed diagnostic potential (AUC = 0.795). High CHAF1A expression predicted poor prognosis across various subgroups and was positively associated with immune cell infiltration and checkpoint genes.

Conclusion: CHAF1A plays a critical role in HCC progression, with elevated expression linked to poor prognosis and immune modulation. These findings highlight its potential as a therapeutic target for HCC.

Keywords: CHAF1A, HCC, expression patterns, immune cell infiltration, gene

Introduction

Hepatocellular carcinoma (HCC), the most prevalent form of primary liver cancer, represents a significant global health burden, particularly in regions with high incidences of hepatitis B and C virus infections, alcohol abuse, and non-alcoholic fatty liver disease.[1,2] With an annual incidence exceeding 800,000 cases worldwide and a five-year survival rate hovering around 18%, HCC poses a formidable challenge to healthcare systems and underscores the urgent need for better understanding of its molecular mechanisms and the development of more effective therapeutic strategies. HCC progression is a multi-step process, involving complex interactions between genetic alterations, epigenetic modifications, and the tumor microenvironment, including immune cells and factors.[3–5]

In recent years, research efforts have intensified to unravel the intricate molecular landscape of HCC, aiming to identify novel biomarkers and therapeutic targets.[6,7] Among the myriad of genes implicated in HCC, CHAF1A (Chromatin Assembly Factor 1 Subunit A) has garnered significant attention due to its emerging role in cancer progression.[8,9] CHAF1A, a component of the histone chaperone complex CAF-1, plays a pivotal role in chromatin assembly and epigenetic regulation, processes that are often dysregulated in cancer. While previous studies have hinted at CHAF1A's involvement in various cancer types, including HIV-1 infection, gastric cancer, epithelial ovarian cancer and B cell lymphoma,[10–13] its specific role in HCC remains largely unexplored. Given the critical function of chromatin dynamics and epigenetic regulation in HCC development and progression,[14,15] investigating the expression patterns, functional roles, and underlying mechanisms of CHAF1A in HCC is of paramount importance.

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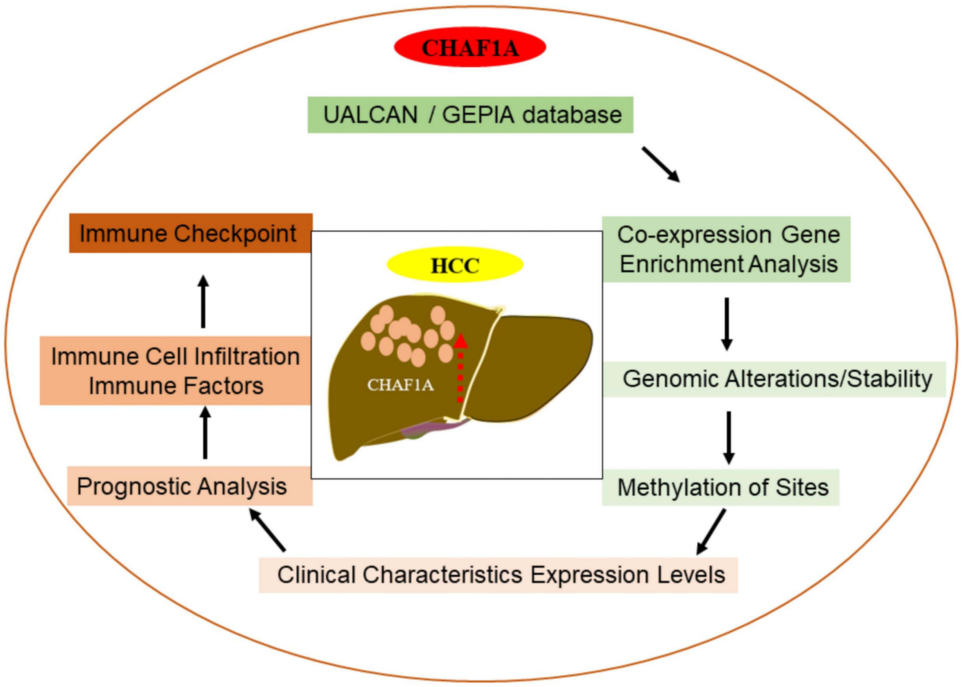
Graphical Abstract



In this study, we first analyzed the expression level of CHAF1A and its prognostic value in various types of human cancers, especially in HCC. Subsequently, genomic alterations of CHAF1A were predominantly detected in the early T1 stage of tumor development and expression levels were analyzed with clinical characteristics such as gender, tumor stage, grade, obesity, age, and ethnicity, which showed good sensitivity and specificity in the clinical diagnosis of HCC. Finally, High CHAF1A expression was associated with poor prognosis in HCC patients and was positively correlated with immune cell infiltration and immune checkpoint genes in HCC. Together, this study provides potential biomarkers and therapeutic targets for improving HCC diagnosis, prognosis, and treatment.

Materials and Methods

UALCAN Database

The UALCAN platform comprehensively analyzes transcriptomic data from The Cancer Genome Atlas (TCGA) (http://ualcan.path.uab.edu/index.html) (doi: 10.1016/j.neo.2022.01.001). This database is used to study the expression levels of CHAF1A in relation to various clinical and pathological parameters of HCC (such as gender, age, tumor grade, cancer staging, etc), as well as its expression in various human tumors.

GEPIA Database

To further confirm the expression of CHAF1A in HCC, we compared the expression levels of CHAF1A mRNA between normal liver tissue and HCC tissue using the GEPIA database (doi.org/10.1093/nar/gkx247) (http://gepia.cancer-pku.cn/index.html) (an online analysis tool that can use TCGA and GTEx data for gene expression analysis). Meanwhile, this database is used to explore the correlation between the expression of CHAF1A and HCC related factors.

GEO Database

The GEO database (doi.org/10.1093/nar/gks1193) (https://www.ncbi.nlm.nih.gov/geo/) is a gene expression database created and maintained by the National Center for Biotechnology Information (NCBI) in the United States. Use it to

screen for liver cancer expression data induced by various factors and visualize it using GraphPad Prism 6.01. Meanwhile, the data in this database can also be used for ROC analysis.

Human Protein Atlas Database

The Human protein Atlas database (https://www.Proteinatlas.org) provides immunohistochemical data on protein expression in 20 different types of cancer, which can be used to explore different tumor type specific proteins that exhibit differential expression (doi: 10.1126/science.aay5947, doi: 10.1126/science.aan2507, doi: 10.1126/science.aan2507). This study selected the expression results of CHAF1A protein detected by specific antibody CAB015186. Compare the expression levels of CHAF1A protein in normal and HCC tissues using immunohistochemistry images.

LinkedOmics Database

LinkedOmics (http://www.linkedomics.org/login.php) is a public portal website that contains multi omics data from all 32 TCGA cancer types and 10 Clinical Proteomics Tumor Analysis Alliance (CPTAC) cancer cohorts (doi.org/10.1093/nar/gkx1090). The web application has three analysis modules: LinkFinder LinkInterpreter and LinkCompare. We have chosen the TCGA-LIHC project for analysis, The differentially co expressed genes of CHAF1A were obtained from the LinkFinder module. Evaluate using Pearson correlation coefficient and display the results through heat maps and volcano maps. In addition, Gene Ontology (GO) andKyoto Encyclopedia of Genes and Genomes (KEGG) gene set enrichment analysis (GSEA) was performed using the LinkInterpreter module.

cBioPortal Database

The cBioPortal database (https://www.cbioportal.org/) is an international public database that includes large tumor research projects such as TCGA and ICGC, and integrates cancer gene data and clinical data. Its integrated genomic data types include somatic mutations, DNA copy number changes (CNA), mRNA and microRNA (miRNA) expression, DNA methylation, protein abundance, and phosphoprotein abundance (doi: 10.1126/scisignal.2004088, doi: 10.1158/0008-5472.CAN-23-0816). Select two liver cancer datasets, Firehost Legacy and PanCancer Atlas, from this database, and use OncoPrint, Cancer Type Summary, Mutations, Comparison/Survival modules to analyze the mutation, amplification, and prognosis of CHAF1A in liver cancer tissue, exploring whether its expression abnormalities in liver cancer are related to its own mutations. Meanwhile, it is inferred that the expression stability of CHAF1A in liver cancer is stable.

MethSurv Database

MethSurv (https://biit.cs.ut.ee/methsurv) is a network tool for survival analysis based on CpG methylation patterns, with 7358 methylation data from 25 different human cancers (doi.org/10.2217/epi-2017-0118). An interactive network tool for survival analysis was developed using the Cox proportional risk model. Exploring the possible methylation sites of CHAF1A and its relationship with the prognosis of liver cancer patients through this database, in order to further understand the role of CHAF1A in the occurrence and development of HCC.

Kaplan-Meier Database

Kaplan Meier is a comprehensive database (http://kmplot.com/analysis/) It can be used to evaluate the impact of miRNA or genes on the survival outcomes of different tumor types (doi. org/10.1016/j.xinn. 2024100625). Based on this database, 364, 370, 316, and 362 samples were used to analyze the survival of CHAF1B expression in terms of overall survival (OS), recurrence free survival (RFS, also known as DFS), disease-specific survival (DSS), and progression free survival (PFS). In addition, the database was used to explore the relationship between CHAF1A and some clinical pathological features in HCC (gender, AJCC T-stage, race, grade, staging, alcohol consumption, vascular invasion, sorafenib treatment, and hepatitis virus).

TISCH2 Database

Tumor Immune Single Cell Center 2 (TISCH2) is an online database (http://tisch.comp-genomics.org) Integrated single-cell transcriptome profiles of approximately 2 million cells from 76 high-quality cancer datasets (doi.org/10.1093/nar/gkac959). Use it to explore the distribution of CHAF1A in immune cells.

TIMER2.0 Database

TIMER2.0 is a comprehensive software tool (doi: 10.1158/0008-5472.CAN-17-0307) (http://timer.comp-genomics.org/) to facilitate systematic analysis of immune infiltration in various types of cancer, we utilized its online analysis to investigate the correlation between CHAF1A and tumor immune cell infiltration, and selected the tumor type as LIHC. The immune cells involved include CD8+T cells, CD4+T cells, B cells, macrophages, neutrophils, and dendritic cells.

TISIDB Database

TISIDB database (http://cis.hku.hk/TISIDB) is a tumor immunoassay database that integrates massive tumor immunology resources (including TCGA pan cancer multi omics data), which can predict responses to immunotherapy by predicting interactions between the immune system and tumor tissue (doi.org/10.1093/bioinformatics/btz210). We used RNA Seq data from a cohort of 373 TCGA-LIHC patients obtained from the TISIDB database to investigate the relationship between CHAF1A expression levels and immune agonists, immune modulators, chemokines, and their receptors.

Networkanalyst

Networkanalyze (https://www.networkanalyst.ca/) Created an interaction network between gene miRNA and gene transcription factors (doi.org/10.1093/nar/gkz240). Using it to predict possible miRNAs of key genes and transcription factors that regulate each other, set the parameters as follows: organism: human; ID type: Official Gene Symbol; Gene TF interaction database: ENCODE and ChEA databases. Intersect the predicted transcription factors from the two databases. Gene miRNA interaction database: TarBase v8.0 database.

StarBase Database

StarBase is a database that integrates miRNA target interactions from various sources (http://starbase.sysu.edu.cn/) (doi.org/10.1093/nar/gkt1248). We used it to predict the upstream regulation of CHAF1A miRNA and summarized the predicted results with the TarBase v8.0 database. In addition, the StarBase database was used to predict the relationship between the expression of miRNAs shared by the two databases and the survival time of HCC patients.

Results

Elevated Expression of CHAF1A in Hepatocellular Carcinoma Tissues

Utilizing the UALCAN database, we first investigated the expression levels of CHAF1A mRNA in various human tumor types. The results revealed a significant upregulation of CHAF1A expression in multiple tumors, including hepatocellular carcinoma (HCC), cervical squamous cell carcinoma, cholangiocarcinoma, and lung squamous cell carcinoma (Figure 1A). Crucially, the GEPIA database further confirmed the high expression of CHAF1A in HCC tissues (Figure 1B). Moreover, CHAF1A exhibited differential expression patterns in HCC tissues arising from diverse etiologies, such as hepatitis B virus (HBV), hepatitis C virus (HCV), and alcohol consumption (Figure 1C). Subsequently, an assessment of CHAF1A protein levels in HCC using the Human Protein Atlas revealed low expression in normal liver tissues and high expression in HCC tissues (Figure 1D). Additionally, we examined the correlation between CHAF1A expression and HCC-related genes such as TP53, AXIN1, and CTNNB1 (Figure 1E). The findings indicated a positive correlation between CHAF1A expression and TP53 (r=0.33, p=8.4e-11), AXIN1 (r=0.48, p=0), and CTNNB1 (r=0.28, p=4.1e-8), suggesting a pivotal role of CHAF1A in the development and progression of HCC.

Co-Expression Gene Enrichment Analysis of CHAF1A in Hepatocellular Carcinoma Tissues

Utilizing the LinkedOmics database, we explored the co-expression genes of CHAF1A and their biological functions and pathways in HCC. The results showed that CHAF1A positively correlated with 12,091 genes and negatively correlated with 7,831 genes (Figure 2A). Heatmaps of the top 50 positively and negatively correlated genes with CHAF1A expression are presented in Figure 2B and C. Functional enrichment analysis revealed that these differentially expressed genes primarily impact biological processes such as DNA replication, chromosome segregation, protein activation cascade, and antibiotic metabolic

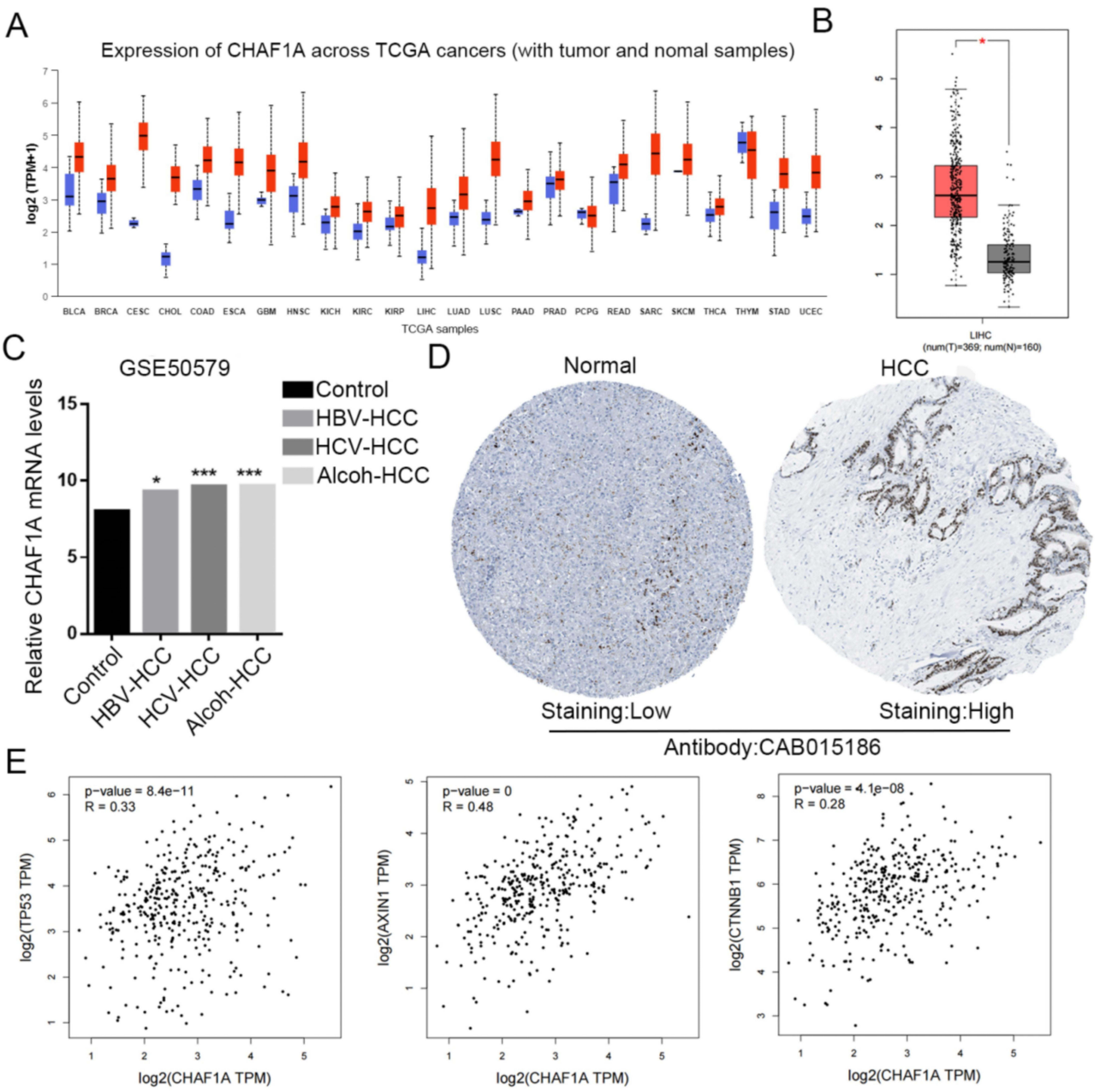


Figure 1 Expression of CHAF1A in Hepatocellular Carcinoma. (**A**) Expression of CHAF1A in various tumors detected by the UALCAN database; (**B**) Expression of CHAF1A in HCC detected by the GEPIA database; (**C**) Expression patterns of CHAF1A in HCC caused by different factors; (**D**) Expression of CHAF1A protein in normal and HCC tissues; (**E**) Correlation between CHAF1A expression and HCC-related genes. *p<0.05, ***P<0.001.

process (Figure 2D). In cellular components, they are primarily associated with the composition of condensed chromosomes, chromosomal regions, blood microparticles, and microbodies (Figure 2E). Regarding molecular functions, these genes primarily affect catalytic activity acting on DNA, damaged DNA binding, nucleosome binding, and electron transfer activity (Figure 2F). KEGG pathway enrichment analysis indicated that they are primarily enriched in pathways such as cell cycle, DNA replication, and drug metabolism (Figure 2G).

Analysis of Genomic Alterations and Stability

Genomic strategies offer effective insights into cancer analysis. To assess the potential genomic-level alterations of CHAF1A in HCC, we analyzed its genetic alterations using the cBioPortal database. The results revealed that the copy number variation

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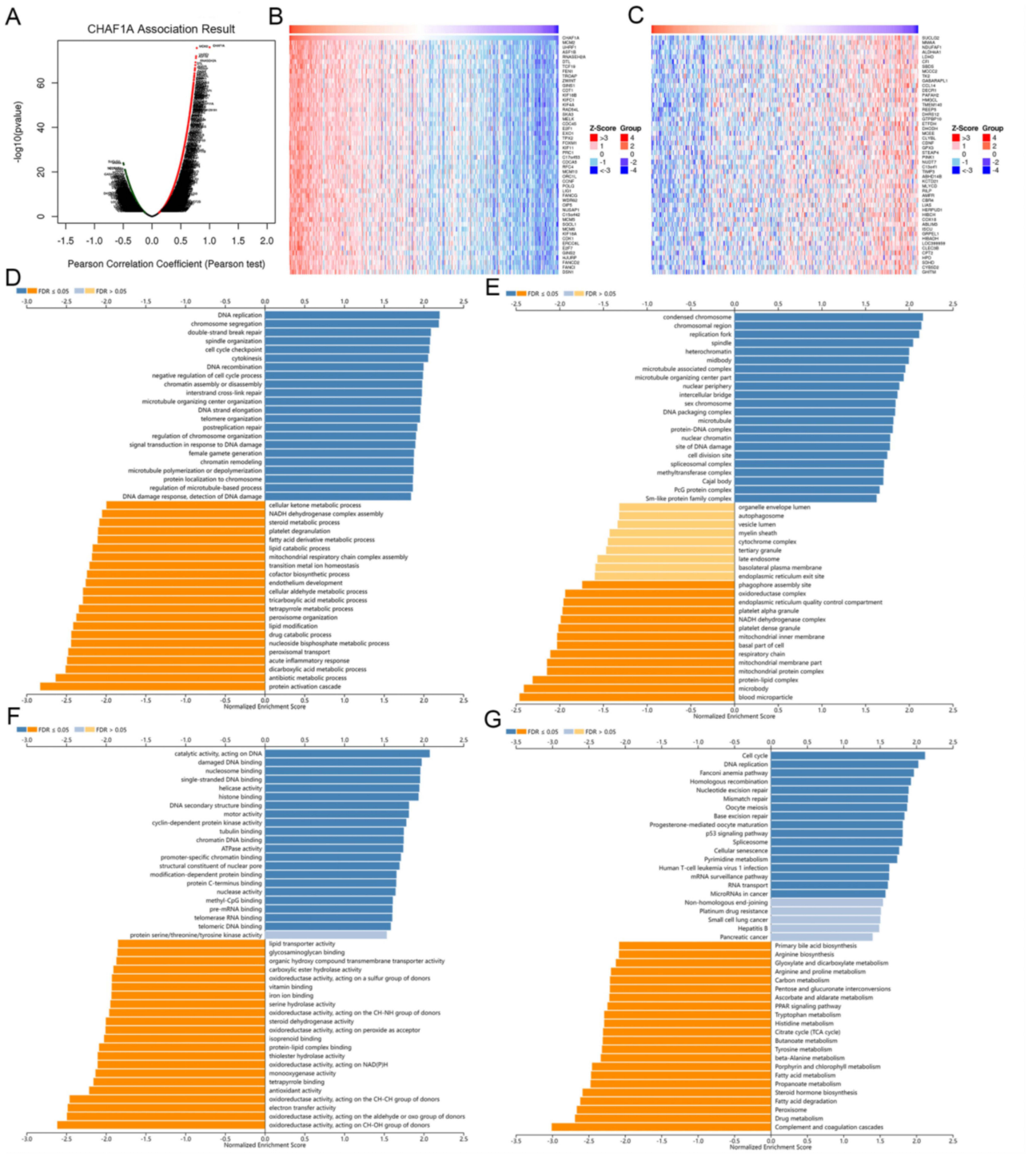

Figure 2 CHAF1A Co-expression Gene Enrichment Analysis. (**A**) Volcano plot of CHAF1 co-expression genes; (**B**) Heatmap of the top fifty positively correlated gene with CHAF1; (**C**) Heatmap of the top five negatively correlated genes with CHAF1A; (**D**) Biological processes; (**E**) Cellular components; (**F**) Molecular functions; (**G**) KEGG signaling pathways.

of CHAF1A in liver cancer, matched with TCGA data, was merely 1.7%, indicating its relative stability. The primary types of alterations were gene amplification, mutations, and deep deletions (Figure 3A and B), with mutations primarily occurring at amino acid positions 480, 482, and 790 (Figure 3C). Our investigation into the impact of these alterations on HCC patient outcomes revealed that patients with genomic alterations had shorter overall survival times compared to those without alterations (Figure 3D), suggesting an adverse prognosis associated with CHAF1A gene alterations. Furthermore, we

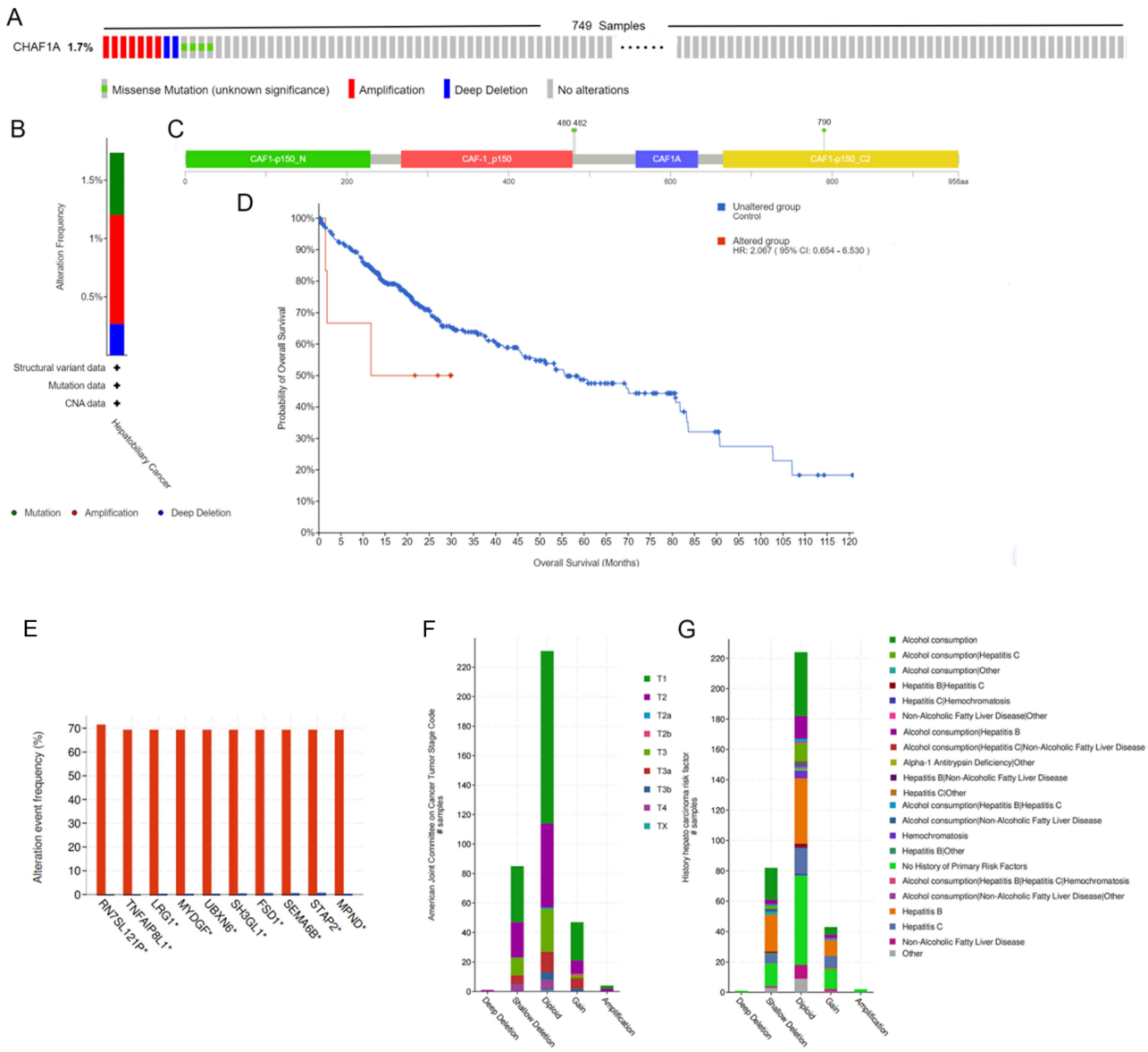

Figure 3 Genetic Variations of CHAF1A. (**A**) Copy number variations of CHAF1 in liver cancer; (**B**) Genomic alterations of CHAF1; (**C**) Common mutation sites of the CHAF1A gene; (**D**) Survival curve of patients with CHAF1A variations; (**E**) Genes with significant changes in the mutation group; (**F**) Relationship between tumor staging and copy number variations; (**G**) Association between risk factors for liver cancer and copy number status.

observed a significantly higher frequency of other gene alteration events in the CHAF1A genetic alteration group compared to the normal group, with the top 10 genes being RN7SL121P, TNFAIP8L1, LRG1, MYDGF, UBXN6, SH3GL1, FSD1, SEMA6B, STAP2, and MPND (Figure 3E). Additionally, according to the GISTIC database, alterations in CHAF1A, such as Shallow Deletion, Diploid, and Gain, were primarily observed in the T1 stage of tumor progression, and most patients with these alterations had various liver diseases caused by alcohol consumption (Figure 3F and G).

Methylation of Sites in the CHAF1A Promoter Region and HCC Patient Outcomes

Alterations in the expression status of epigenetically regulated genes can affect cell growth, differentiation, and proliferation, thereby influencing tumor development. To further explore the role of CHAF1A epigenetics in HCC, we investigated potential methylation sites in the CHAF1A promoter region and their relationship with patient outcomes. The results suggested that the methylation sites of CHAF1A could be cg03006338, cg03741332, cg04346879, cg05297461, cg06727521, cg07783855, cg09700233, cg18804734, cg19682666, cg23811775, and cg24085258 (Figure 4A and B). The relationship between

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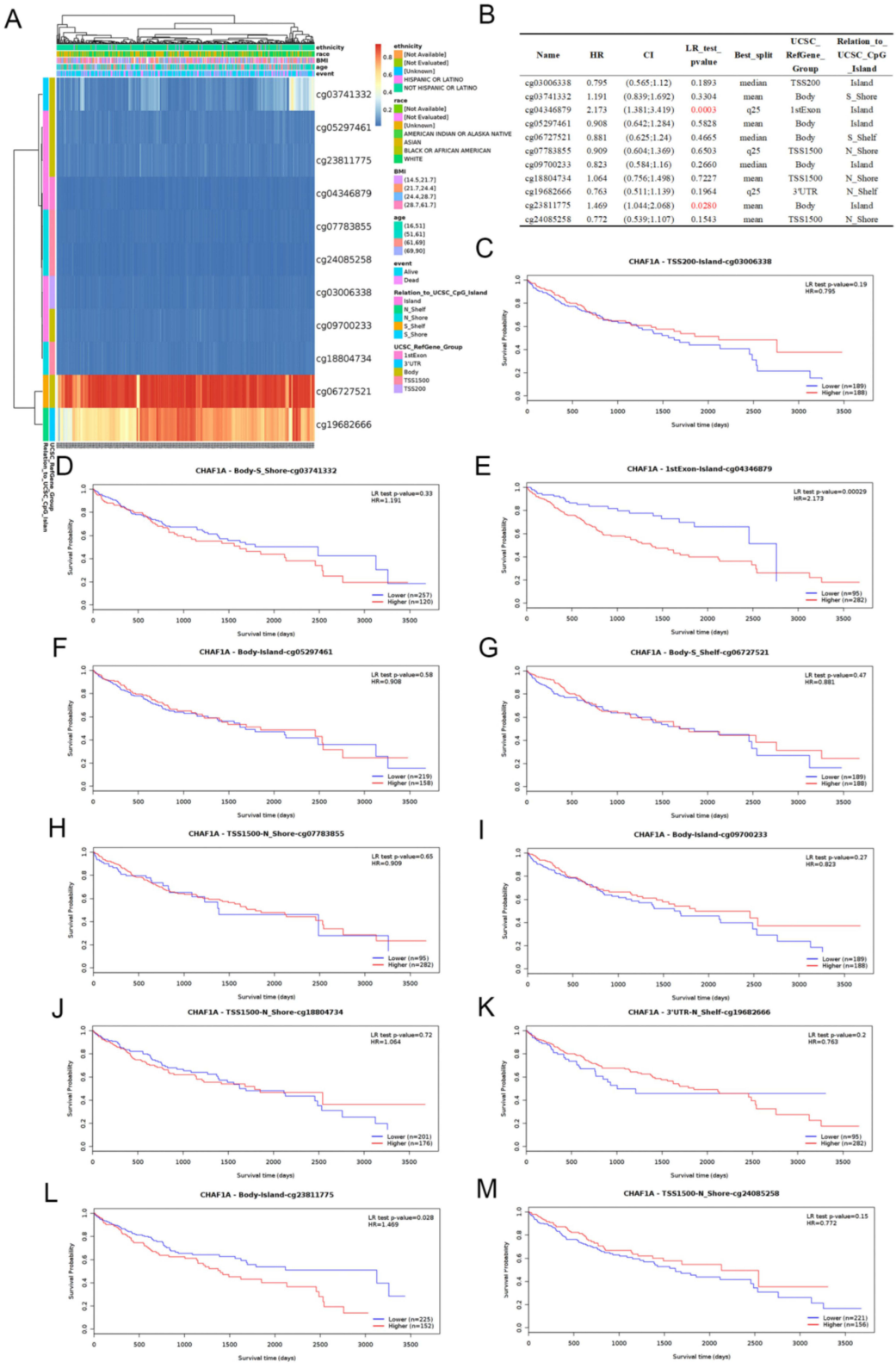


Name	HR	CI	LR_test_pvalue	Best_split	UCSC_RefGene_Group	Relation_to_UCSC_CpG_Island
cg03006338	0.795	(0.565;1.12)	0.1893	median	TSS200	Island
cg03741332	1.191	(0.839;1.692)	0.3304	mean	Body	S_Shore
cg04346879	2.173	(1.381;3.419)	0.0003	q25	1stExon	Island
cg05297461	0.908	(0.642;1.284)	0.5828	mean	Body	Island
cg06727521	0.881	(0.625;1.24)	0.4665	median	Body	S_Shelf
cg07783855	0.909	(0.604;1.369)	0.6503	q25	TSS1500	N_Shore
cg09700233	0.823	(0.584;1.16)	0.2660	median	Body	Island
cg18804734	1.064	(0.756;1.498)	0.7227	mean	TSS1500	N_Shore
cg19682666	0.763	(0.511;1.139)	0.1964	q25	3'UTR	N_Shelf
cg23811775	1.469	(1.044;2.068)	0.0280	mean	Body	Island
cg24085258	0.772	(0.539;1.107)	0.1543	mean	TSS1500	N_Shore

Figure 4 Methylation Sites in the CHAF1A Promoter and Their Prognostic Implications. (**A**–**D**) Depict the methylation sites, degrees of methylation, and related information within the DNA sequence of CHAF1,in (**B**), the red-marked sites represent the modified sites with significant differences. (**C**–**M**) Illustrate the correlation between the methylation status of the CHAF1 promoter and the prognosis of HCC patients.

methylation at these sites and HCC patient outcomes is depicted in Figure 4C–M, with cg04346879 (p=0.0003) and cg23811775 (p=0.0280) standing out as significant and meaningful. Higher methylation levels at these two sites were associated with shorter survival times for patients (Figure 4E and L), suggesting that CHAF1A promoter methylation plays a crucial role in liver cancer progression and that the level of CHAF1A promoter methylation may influence the prognosis of HCC patients.

Clinical Characteristics of CHAF1A Expression Levels

To investigate the clinical role of CHAF1A, we leveraged the UALCAN online platform to assess the expression patterns of CHAF1A in HCC across various parameters. The results revealed that both male and female HCC patients exhibited higher CHAF1A expression levels compared to the control group, with females showing a slightly higher expression (Figure 5A). Irrespective of tumor stage or grade, CHAF1A expression was significantly upregulated in HCC patients compared to normal counterparts (Figure 5B and C). Additionally, obese HCC patients displayed relatively high CHAF1A expression (Figure 5D). From an age perspective, CHAF1A expression was relatively high in HCC patients aged 21–60 years, while it slightly declined in patients aged 61–100 years (Figure 5E). Compared to the control group, CHAF1A expression was significantly upregulated in HCC patients of Caucasian, African American, and Asian ethnicities (Figure 5F). Furthermore, HCC patients with TP53 mutations exhibited significantly higher CHAF1A expression levels compared to both the normal and non-mutated groups (Figure 5G). Subsequently, to validate the diagnostic efficacy of CHAF1A in HCC, ROC regression analysis was performed based on GSE46408 and GSE115018 datasets. An AUC value > 0.50 was considered diagnostically valuable. The results indicated an AUC value of 0.795 for CHAF1A, suggesting its good sensitivity and specificity in clinical diagnosis of HCC (Figure 5H).

Prognostic Analysis of CHAF1A Expression

Utilizing the Kaplan-Meier database, we explored the prognostic significance of CHAF1A expression in HCC patients. Our findings revealed that patients with high CHAF1A expression exhibited lower OS (HR = 1.68, P = 0.016) (Figure 6A), DFS (HR = 1.5, P = 0.023) (Figure 6B), DSS (HR = 1.76, P = 0.041) (Figure 6C), and PFS (HR = 1.41, P = 0.024) (Figure 6D) compared to those with low expression, all with statistical significance. This suggests that the upregulation of CHAF1A expression is associated with poor prognosis in HCC patients. Moreover, the upregulated expression of CHAF1A in HCC patients correlated with OS, DFS, DSS, and PFS (Figure 6E–H), exhibiting a certain degree of correlation regardless of tumor grade, stage, gender, vascular invasion, ethnicity, sorafenib treatment, alcohol consumption, and hepatitis virus status.

The Association of CHAF1A Expression with Immune Cell Infiltration and Immune Factors in HCC

Moving forward, our aim was to identify potential mechanisms underlying CHAF1A's role in HCC development. Understanding the interplay between tumor immune cells and key genes involved in cancer progression is crucial for improving cancer treatment strategies. In this study, we conducted a correlation analysis to explore the relationship between CHAF1A and tumor immune cell infiltration and immune checkpoints.

Within the HCC transcriptome dataset, we found that CHAF1A is expressed in various immune cells, including T cells and B cells (Figure 7A). Subsequently, we analyzed the GSE140228 single-cell transcriptome data further, revealing that CHAF1A is primarily expressed in T immune cells and dendritic cells (DCs), but also exhibits expression in most immune cell types (Figure 7B). Furthermore, analysis using the TIMER 2.0 database showed that CHAF1A expression positively correlates with cell purity and the immune infiltration levels of B cells (r = 0.437, p = 1.82e-17), CD8+ T cells (r = 0.311, p = 4.13e-9), CD4+ T cells (r = 0.355, p = 1.27e-11), macrophages (r = 0.382, p = 2.57e-13), neutrophils (r = 0.385, p = 1.29e-13), and dendritic cells (r = 0.454, p = 1.15e-18) (Figure 7C). These findings may provide insights into understanding the immune microenvironment of hepatocellular carcinoma.

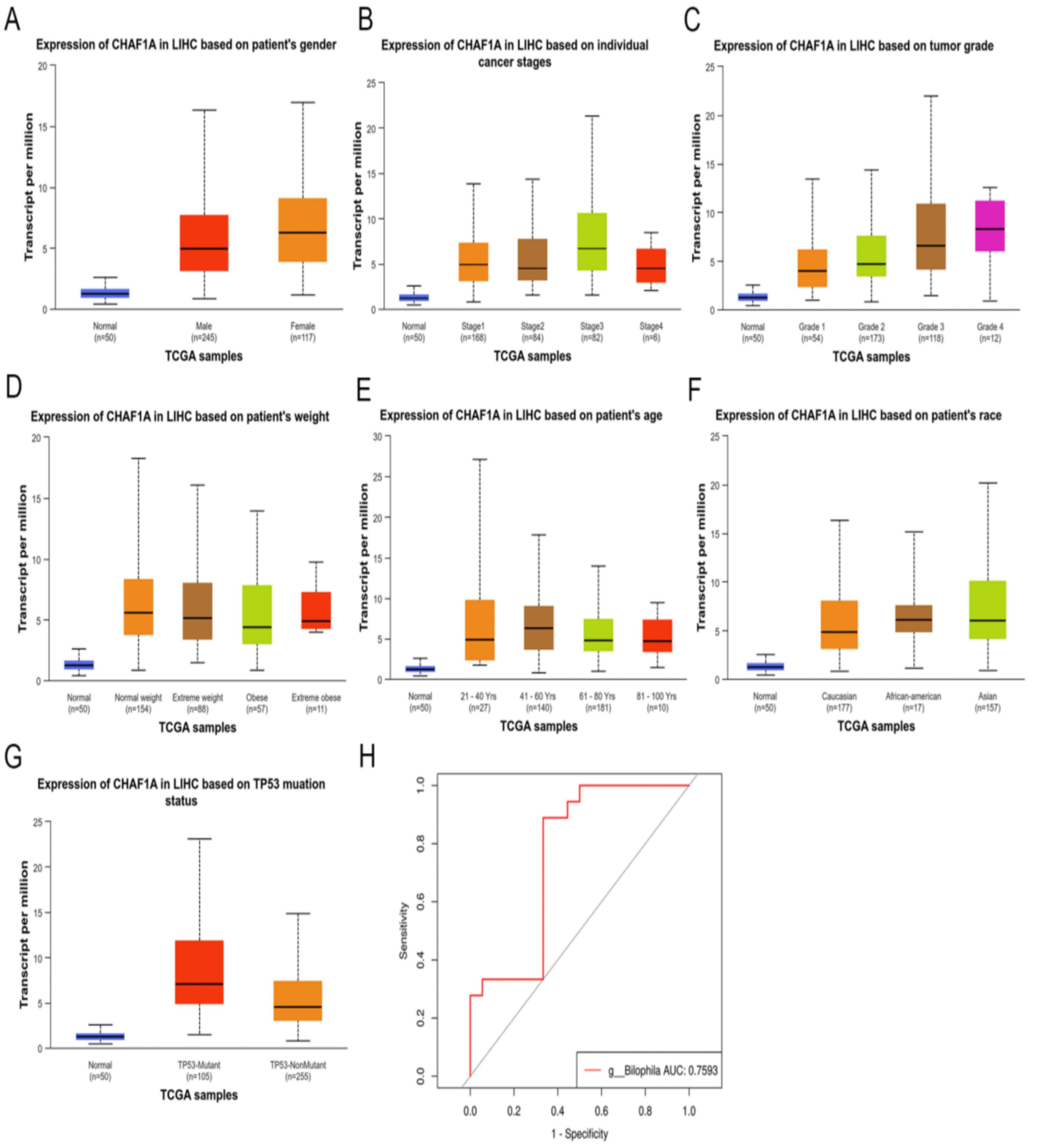
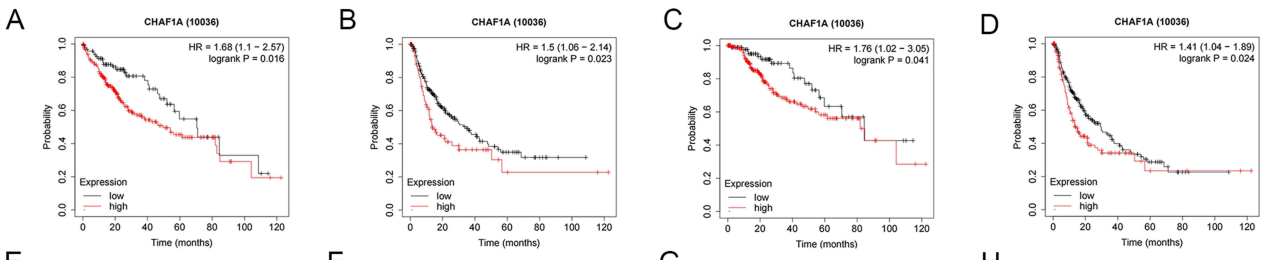


Figure 5 Quantitative Analysis of CHAF1A Expression in Different Patient Groups Using the UALCAN Database. (**A**–**G**) Expression of CHAF1A in relation to gender, tumor stage, tumor grade, body weight, age, race, and the presence of TP53 mutations; (**H**) ROC Diagnostic Curve for CHAF1A.

The Association Between CHAF1A Expression and Immune Checkpoint Genes in HCC

Next, we explored the relationship between CHAF1A expression and immune-related factors using the TISIDB database. The results revealed a correlation between CHAF1A expression in various tumors and immune suppressors, enhancers, and chemokines. Specifically, in HCC, CHAF1A expression exhibited a significant correlation with immune suppressors (Figure 8A), such as LAG3 (r=0.196, p=1.42e-4) and KDR (r=−0.326, p=1.49e-10). Furthermore, CHAF1A expression



E OS

Characteristics	N%	HR(95% CI)	P value
Stage			
1	170	1.47（0.77-2.79）	0.2421
2	83	2.59（0.88-7.56）	0.0719
3	83	1.63（0.89-3）	0.11
4	4	/	/
Grade			
1	55	2.34(0.9-6.08)	0.0732
2	174	1.73(1.03-2.92)	0.0367
3	118	1.92(0.98-3.74)	0.0515
4	12	/	/
AJCC Stage T			
1	180	1.52(0.82-2.8)	0.1774
2	90	2.73(0.95-7.88)	0.053
3	78	1.47(0.78-2.74)	0.227
4	13	/	/
Vascular invasion			
None	203	1.99(1.12-3.55)	0.017
Micro	90	1.99(1.12-3.55)	0.3419
Macro	16	/	/
Gender			
Male	246	2(1.14-3.51)	0.0138
Female	118	1.57(0.89-2.76)	0.1195
Race			
White	181	2.18(1.22-3.92)	0.0073
Black or African American	17	/	/
Asian	155	2.13(1.17-3.86)	0.0109
Sorafenib treatment			
Treated	29	3.29(0.94-11.44)	0.0484
Alcohol Consumption			
Yes	115	1.46(0.72-2.93)	0.2895
None	202	1.75(0.98-3.15)	0.0575
Hepatitis Virus			
Yes	150	1.32(0.64-2.73)	0.4521
None	167	2.61(1.4-4.85)	0.0018

F DFS

Characteristics	N%	HR(95% CI)	P value
Stage			
1	153	1.38(0.79-2.41)	0.2565
2	74	1.9(0.91-3.96)	0.0834
3	68	0.62(0.31-1.25)	0.1767
4	0	/	/
Grade			
1	45	0.55 (0.2-1.49)	0.2318
2	147	2.04(1.23-3.36)	0.0045
3	106	1.58(0.92-2.71)	0.0967
4	11	/	/
AJCC Stage T			
1	160	1.5(0.88-2.58)	0.1368
2	79	1.81 (0.9-3.65)	0.0903
3	65	0.72 (0.38-1.36)	0.046
4	6	/	/
Vascular invasion			
None	175	1.39(0.85-2.27)	0.193
Micro	81	2.26(1.17-4.35)	0.0127
Macro	14	/	/
Gender			
Male	208	1.35 (0.88-2.07)	0.1626
Female	105	2.47(1.33-4.57)	0.0031
Race			
White	147	1.68(1.04-2.71))	0.0334
Black or African American	13		
Asian	143	1.91(1.15-3.19)	0.0111
Sorafenib treatment			
Treated	22	2.78(0.93-8.29)	0.0568
Alcohol Consumption			
Yes	98	1.94 (0.92-4.06)	0.076
None	182	1.99(1.25-3.16)	0.0031
Hepatitis Virus			
Yes	138	0.61(0.36-1.03)	0.0624
None	142	2.36(1.42-3.94)	0.0007

G DSS

Characteristics	N%	HR(95% CI)	P value
Stage			
1	170	1.61(0.86-3.02)	0.1341
2	84	1.85(0.95-3.58)	0.0644
3	83	1.4(0.8-2.43)	0.232
4	5	/	/
Grade			
1	55	1.49(0.67-3.3)	0.3215
2	175	2(1.29-3.11)	0.0016
3	119	1.45(0.85-2.48)	0.1645
4	12	/	/
AJCC Stage T			
1	180	1.63(0.89-3)	0.1098
2	92	1.64(0.9-2.99)	0.0995
3	78	1.32(0.75-2.33)	0.3321
4	13	/	/
Vascular invasion			
None	204	1.3(0.77-2.21)	0.3231
Micro	91	1.8(1-3.24)	0.0465
Macro	16	/	/
Gender			
Male	246	1.39(0.96-2)	0.0787
Female	120	1.74(1.01-2.99)	0.0417
Race			
White	183	1.53(1.04-2.27)	0.0315
Black or African American	17		
Asian	155	1.86(1.14-3.01)	0.0111
Sorafenib treatment			
Treated	30	2.56(1.08-6.1))	0.0282
Alcohol Consumption			
Yes	115	1.92(0.99-3.7	0.0483
None	204	1.69(1.1-2.6)	0.0155
Hepatitis Virus			
Yes	152	0.65(0.4-1.06)	0.0839
None	167	2.3(1.48-3.58)	0.0001

H PFS

Characteristics	N%	HR(95% CI)	P value
Stage			
1	167	0.59(0.23-1.48)	0.2522
2	82	2.51(0.56-11.34)	0.215
3	81	1.95(0.94-4.05)	0.0682
4	3	/	/
Grade			
1	55	2.03(0.57-7.16)	0.2643
2	169	1.94(0.99-3.79)	0.0496
3	116	2.05(0.96-4.36)	0.0578
4	12	/	/
AJCC Stage T			
1	177	1.57(0.62-3.96)	0.3364
2	89	2.96(0.68-12.97)	0.13
3	75	1.95(0.91-4.15)	0.0799
4	13	/	/
Vascular invasion			
None	200	2.23(0.91-5.44)	0.0719
Micro	88	0.55(0.18-1.64)	0.2779
Macro	14	/	/
Gender			
Male	241	2.05 (0.99-4.23)	0.0481
Female	116	1.84(0.89-3.79)	0.0954
Race			
White	177	2.6(1.22-5.56)	0.0103
Black or African American	17		
Asian	152	3.08(1.4-6.78)	0.0033
Sorafenib treatment			
Treated	29	3.29(0.94-11.44)	0.0484
Alcohol Consumption			
Yes	115	1.38 (0.61-3.11)	0.4338
None	197	2(1.08-3.71)	0.0247
Hepatitis Virus			
Yes	149	0.65 (0.28-1.46)	0.2917
None	163	3.06(1.37-6.84)	0.0041

Figure 6 Relationship between CHAF1A Expression and Prognosis of HCC Patients. (**A**–**D**) Associations between CHAF1A expression levels and patient outcomes, including OS, DFS, DSS, and PFS; (**E**–**H**) Correlation analysis between CHAF1A expression and clinicopathological parameters in HCC patients.

also showed a notable association with immune agonists like MICB (r=0.296, p=5.64e-9) and CXCL12 (r=−0.313, p=7.83e-10) (Figure 8B). More importantly, a strong correlation was observed between its expression and chemokines CCL14 (r=−4.18, p<2.2e-16) and CXCL12 (r=−0.313, p=7.83e-10) (Figure 8C). Finally, there was a certain relationship between CHAF1A and chemokine receptors (Figure 8D), particularly CXCR1 (r=0.225, p=1.19e-5) and CXCR2 (r=−0.205, p=6.94e-5). In summary, these data suggest that CHAF1A may influence the development and progression of HCC by modulating immune checkpoint-related factors.

Discussion

HCC is a complex disease driven by multiple factors, including viral infections such as hepatitis B and C, aflatoxin exposure, and lifestyle choices. Despite advancements in treatment modalities, HCC prognosis remains poor, emphasizing the need for novel therapeutic targets and biomarkers.[4,5] In this study, we explored the expression and functional significance of CHAF1A in HCC. We found that CHAF1A is dysregulated in HCC tissues, contributing to tumor progression and aggressiveness. Our data suggest that CHAF1A promoter methylation played a crucial role in liver cancer progression, and CHAF1A expression levels were associated with clinical characteristics such as gender, tumor stage, grade, obesity, age, and ethnicity. Furthermore, we uncovered that CHAF1A expression was positively correlated with immune cell infiltration and immune checkpoint genes in HCC, providing insights into the development of targeted therapies.

CHAF1A, as a component of the histone chaperone complex CAF-1, has garnered significant attention in recent years for its role in chromatin assembly and epigenetic regulation, processes that are frequently dysregulated in cancer.[16,17] Prior studies have

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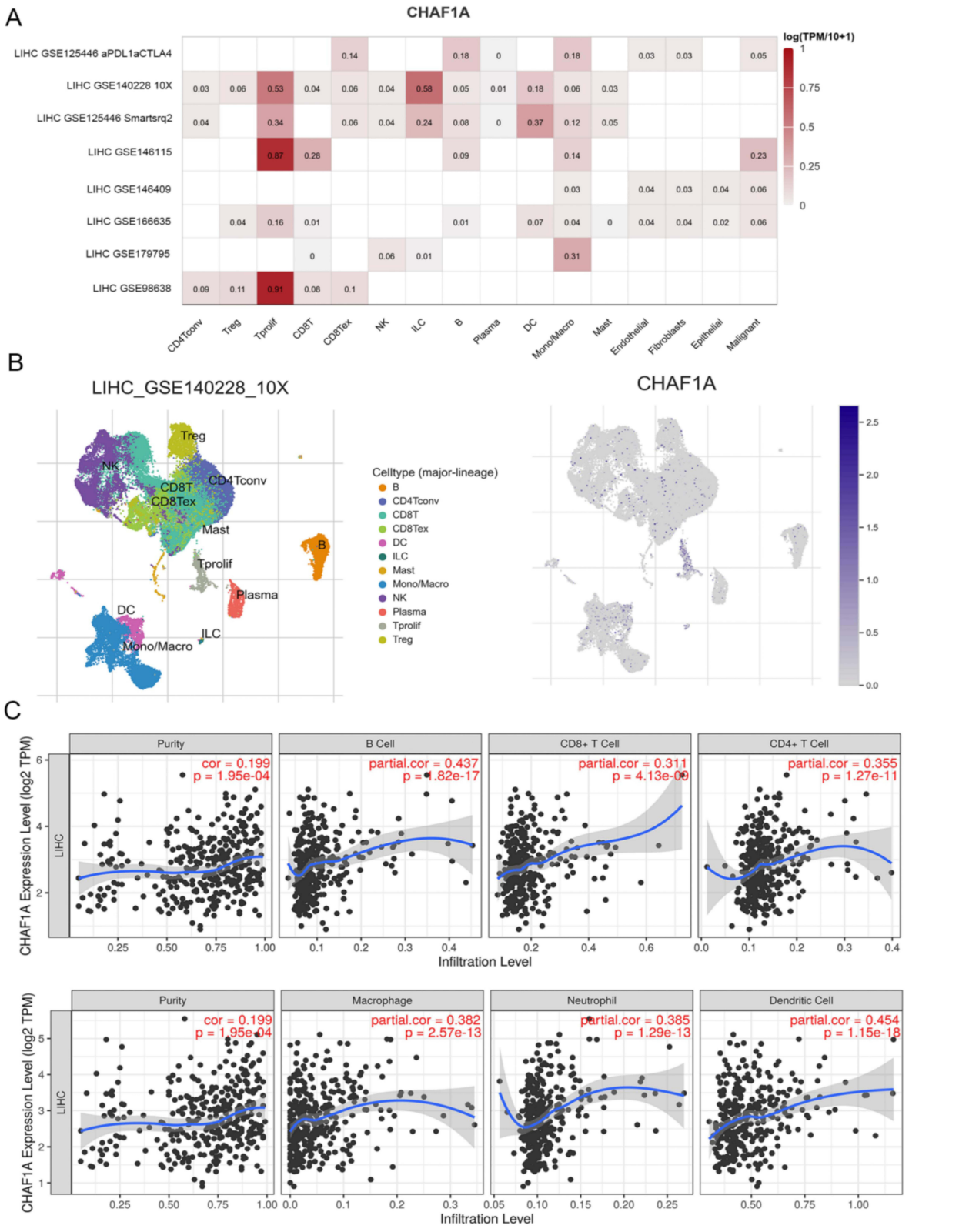

Figure 7 Relationship between CHAF1A Expression and Immune Cell Infiltration. (**A**) Heatmap of CHAF1A expression across various immune cell types in different datasets; (**B**) Distribution of CHAF1A expression in the GSE140228 dataset; (**C**) Correlation plot depicting the association between CHAF1A expression and the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells.

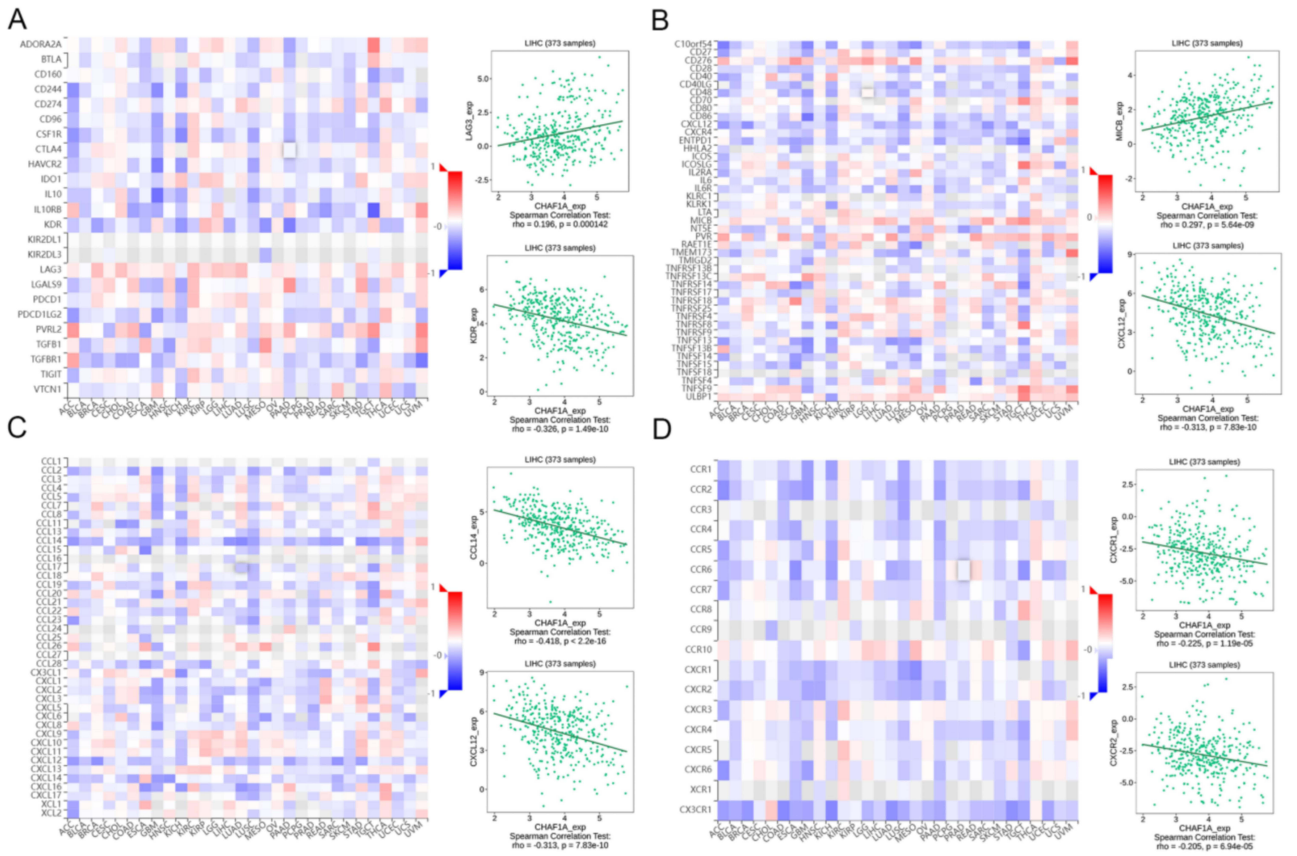

Figure 8 Relationship between CHAF1A Expression and Immune Checkpoints. (**A**–**D**) Depicts the correlations between CHAF1A expression and immune suppressors, agonists, chemokines, and their receptors.

implicated CHAF1A in the progression of various cancer types, including breast, ovarian, and lung cancers.[8,18–20] These findings highlight the potential significance of CHAF1A in cancer biology and suggest its involvement in tumorigenesis and metastasis. However, the specific mechanisms underlying CHAF1A's function in HCC remain largely unexplored. Our study provides a comprehensive investigation into the role of CHAF1A in HCC progression. The significant upregulation of CHAF1A expression in HCC tissues compared to normal liver tissues, as observed using the UALCAN and GEPIA databases, underscores its potential as a biomarker for HCC. Furthermore, the analysis of CHAF1A's co-expression genes revealed its involvement in crucial biological processes such as DNA replication, chromosome segregation, and protein activation cascades, further emphasizing its functional importance in HCC.

Genomic and genetic alterations influence the progression.[21,22] Notably, our findings on genomic alterations in CHAF1A reveal a relatively low frequency of copy number variations but significant associations with patient outcomes. The predominance of genomic alterations in the early T1 stage of tumor progression, coupled with the adverse prognosis observed in patients with CHAF1A alterations, highlights the potential significance of these genetic changes in HCC initiation and progression. In addition, DNA methylation patterns in circulating tumor DNA hold great promise for minimally invasive cancer detection and classification.[23,24] The analysis of CHAF1A promoter methylation further underscores the epigenetic regulation of this gene and its impact on HCC patient outcomes.

Immune cell infiltration into the tumor microenvironment is closely related to the therapeutic efficiency and prognosis of multiple human cancers.[25–27] The positive correlation between CHAF1A expression and immune cell infiltration, as well as immune checkpoint genes, observed in our study, suggests a potential role for CHAF1A in modulating the tumor microenvironment. This finding has important implications for the development of novel immunotherapeutic strategies targeting CHAF1A. By modulating CHAF1A expression, it may be possible to influence the immune response against HCC, thereby enhancing the efficacy of immunotherapy.

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In conclusion, our study provides novel insights into the role of CHAF1A in HCC progression, including its expression patterns, genomic alterations, and epigenetic regulation. The associations observed between CHAF1A expression and clinical characteristics, prognosis, and immune modulation suggest its potential as a biomarker and therapeutic target in HCC. However, further studies are needed to fully elucidate the molecular mechanisms underlying CHAF1A's function in HCC and to evaluate the efficacy of CHAF1A-targeted therapies in clinical settings. These efforts will pave the way for the development of more effective diagnostic and therapeutic approaches for HCC patients.

Data Sharing Statement

The data that support the findings of this study are openly available in at http://ualcan.path.uab.edu/index.html, http://gepia.cancer-pku.cn/index.html, https://www.ncbi.nlm.nih.gov/geo/, https://www. Proteinatlas.org,http://www.linkedomics.org/login.php,https://www.cbioportal.org/,https://biit.cs.ut.ee/methsurv, http://kmplot.com/analysis/, http://tisch.comp-genomics.org, http://timer.comp-genomics.org/, http://cis.hku.hk/TISIDB, https://www.networkanalyst.ca/, http://starbase.sysu.edu.cn/.

Ethics and Consent to Participate Declarations

Our organization supports the relevant provisions of the "Ethical Review Measures for Life Science and Medical Research Involving Human Beings" and the use of legally obtained public data can be exempted from ethical review. The details contents are as follows:

Using human information data or biological samples to carry out life science and medical research involving humans in the following circumstances, which does not cause harm to the human body, does not involve sensitive personal information or commercial interests, can be exempted from ethical review to reduce unnecessary burdens on scientific researchers and promote the development of life science and medical research involving humans.

(i) Using legally obtained public data, or data generated by observation without interfering with public behavior for research;

(ii) Using anonymized information data for research;

(iii) Using existing human biological samples for research, the source of the biological samples used complies with relevant laws and regulations and ethical principles, the research-related content and purpose are within the scope of standardized informed consent, and do not involve the use of human germ cells, embryos and reproductive cloning, chimerism, heritable gene manipulation and other activities;

(iv) Using human cell lines or cell lines from biological sample banks for research, the research-related content and purpose are within the scope of authorization by the provider, and do not involve human embryos and reproductive cloning, chimerism, heritable gene manipulation and other activities.

Consent for Publication

All authors agree to publish the final manuscript.

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Disclosure

The authors declare no conflicts of interest in this work.

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