Identification of CFHR4 Associated With Poor Prognosis of Hepatocellular Carcinoma

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Research

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the most leading causes of cancer death worldwide. The 5-year survival rate of HCC patients remains low due to the lack of early-stage symptoms. Human complement factor H-associated protein 4 (CFHR4) is a critical gene that belongs to the factor H family of plasma glycoproteins, which has not been linked to HCC development. The correlations between CFHR4 and prognosis and tumor-infiltrating lymphocytes in HCC are yet unknown. The present study demonstrated the involvement of CFHR4 in HCC via data mining approaches.

Results: A total of 18 upregulated and 67 down-regulated DEGs were identified. Importantly, CFHR4, which was screened from DEGs, was shown to express at a lower level in HCC tumor tissue than normal tissues. Western blotting (WB) and immunohistochemical (IHC) experiments of clinical samples further validated CFHR4 was aberrantly expressed in HCC patients; Data from TCGA showed that CFHR4 was inversely correlated with a cancer family history, histological grade, tumor node metastasis (TNM) stage, and serum AFP level of HCC patients; Univariate and multivariate analyses revealed that low expression of CFHR4 was an independent predictive marker in patients with HCC; Kaplan-Meier analysis showed that the lower expression of CFHR4 was significantly associated with the progression of HCC and poor prognosis rates. Furthermore, TIMER analysis indicated that CFHR4 expression levels had correlations with infiltrating levels of immune cells in HCC.

Conclusion: CFHR4 expression was low in HCC and was significantly related to the poor prognosis of HCC and the level of immune infiltration. CFHR4 played important roles in regulating the initiation and progression of HCC and could be a potential biomarker for the diagnosis and prognosis of HCC.

Methods: The expression of CFHR4 was analyzed by GEO and TCGA-LIHC database and verified by WB and IHC assay. The biological function of CFHR4 was performed by GO and KEGG enrichment analysis, and the genomic alteration of CFHR4 was investigated by cBioPortal database. The correlation between CFHR4 expression and clinical relevance was evaluated through Cox proportional hazards model, and the correlation between CFHR4 expression and tumor immune infiltrates were studied by TIMER database.

Background

Hepatocellular carcinoma (HCC) is one of the most leading causes of cancer death globally and remains second in the mortality rate of malignant tumors in China [1, 2]. Unfortunately, because of the lack of symptoms in the early stage of carcinogenesis, HCC is generally diagnosed at an advanced stage [3]. Despite significant advances in early detection, surgical surgery, and comprehensive therapy for HCC in recent years, the prognosis for HCC remains poor [4]. A correct prognosis assessment is critical in the diagnostic and therapeutic treatment of HCC patients [5]. Therefore, it is critical to discover and identify novel diagnostic and prognostic biomarkers with improved sensitivity, accuracy, and specificity of HCC [6]. Pre-mRNA processing factor 3 (PRPF3) has been found to be upregulated in LIHC tissues, which has been linked to poor Overall Survival (OS) and Disease-Free Survival (DFS) [7]; Kelch-like family member

21 (KLHL21) is upregulated in HCC, and its expression significantly inhibits cell proliferation, migration and invasion, making it the most likely target for therapeutic intervention [8]; SAC3 homology domain-containing protein 1 (SAC3D1) is related to centrosome abnormalities, a key structure of cell cycle progression and genomic stability, and can be used as a prognostic factor for HCC [9]. The high expression of nucleosome assembly proteins 1-like 1 protein (NAP1L1) in HCC is related to invasive clinicopathological features and promotes chemotherapy resistance [10]. The expression of Kinesin family member C1 (KIFC1) in HCC is upregulated, which facilitates HCC metastasis by regulating epithelial-mesenchymal transition (EMT), and is significantly related to DFS and OS [11]. These studies reveal the potential gene targets of prognostic significance in HCC and provide a reference for the investigation of CFHR4 in HCC.

CFHR4 gene is located on the human 1q32 chromosome [12] and consists of 10 coding exons [13]. The CFHR4 cDNA isolated from the human liver cDNA library is 1315bp in length and encodes an open reading frame of 331 amino acids [14]. By alternative splicing, CFHR4 obtained two different mRNA transcripts CFHR4A and CFHR4B [13]. As an amphiphilic protein, CFHR4 exists in human plasma and triglyceride-rich lipoproteins in the monomer or dimer form [14, 15] and can play a role as a component of lipoproteins [16]. CFHR4 consists of a short common repetitive sequence (SCR) domain, which binds C-reactive protein through its N-terminal SCR, activates the classical complement pathway, regulates complement-mediated foreign antigen processing [17], and enhances the regulation of phagocytic microorganisms and damaged host cells [18–20]. Studies have shown that CFHR4 shows abnormalities in various diseases, which may be connected to disease incidence and progression.

Currently, the expression of CFHR4 in HCC has not yet been investigated [21]. Bioinformatic analysis was performed in this study to address the correlation of CFHR4 with HCC. We identified the CFHR4 low expression in GEO and TCGA databases and assessed the expression of CFHR4 in HCC specimens via Western blot and immunohistochemistry [5, 21]. Meanwhile, genetic alterations of CFHR4 were analyzed by the cBio for Cancer Genomics Portal (cBioPortal) database, and immune cell enrichments in HCC were also analyzed by Tumor IMmune Estimation Resource (TIMER) database [22]. Furthermore, the CFHR4 expression on the survival for HCC patients was also assessed by Kaplan–Meier plotter. Thus, this study aimed to evaluate the potential role of CFHR4 to be a biomarker for HCC diagnosis and prognosis, with the expectation of providing novel insights into the progression of HCC [5, 23].

Results

The identification of DEGs in HCC

DEGs between HCC tumor and non-tumor samples were investigated of the GEO dataset (GSE45267, GSE45436, GSE60502, and GSE84402) by applying bioinformatics analysis. In total, 18 significantly upregulated genes and 67 significantly down-regulated genes were identified (including CFHR4) (Fig. 1) [24, 25].

The comparison of CFHR4 expression

The expression of CFHR4 in tumor samples was significantly lower than non-tumor samples in GSE45267, GSE45436, GSE60502, GSE84402, and TCGA-LIHC dataset (Fig. 2A-E)[5]. The immunohistochemistry and Western blot results of clinical samples confirmed that CFHR4 is under-expressed in HCC tissues, compared with normal tissues (Fig. 2F-G).

The correlation and GO/KEGG enrichment analysis results

The correlation between CFHR4 and CFHR4-correlated genes was identified (Fig. 3A). GO analysis results proved that CFHR4-correlated genes were significantly enriched in the drug-metabolic process, regulation of complement activation, steroid metabolic process, cytolysis, lipid transport at BP levels; organelle membrane, extracellular region, blood microparticle, endoplasmic reticulum membrane, extracellular space at CC levels, and oxygen binding, steroid hydroxylase activity, heme binding, aromatase activity, oxidoreductase activity, enzyme binding at MF levels. Additionally, the KEGG pathway enrichment of CFHR4 interactive genes showed that Retinol metabolism, Mineral absorption, Steroid hormone biosynthesis, Metabolic pathways, complement, and coagulation cascades, were the enriched pathways [5] (Fig. 3B-E).

The genomic mutation of CFHR4

The genomic mutation is closely associated with tumorigenesis[22]. The research showed about 7% of genetic alteration of CFHR4 in HCC, including amplification and missense mutation with unknown significance (Fig. 4A-B). In addition, missense mutation of CFHR4 resulted in the amino acid change, including Asparagine (N) 356 replaced by Isoleucine (I) and Lysine (K) 227 replaced by Asparagine (N) (Fig. 4C). The above results indicated that genetic alteration of CFHR4 could be found in HCC, which might play an important role in tumorigenesis of HCC [22].

The correlation of CFHR4 expression with the clinicopathological characteristics of HCC

Chi-square test was used to analyze the correlation between CFHR4 expression and clinicopathological factors (Table 3). Studies have shown that low CFHR4 expression was significantly associated with Age ($P=0.026$), BMI ($P=0.013$), Race ($P<0.001$), and Family history of cancer ($P=0.001$), Histological grade ($P<0.001$), TNM stage ($P=0.048$), and Serum AFP level ($P<0.001$) [26–28]. ($P<0.05$ *, $P<0.01$ **, $P<0.001$ ***)

The independent prognostic value of CFHR4 expression

The univariate analysis indicated that CFHR4 expression and TNM stage were directly correlated with OS in HCC. Furthermore, by Cox multivariate analysis, we found that low CFHR4 expression and TNM stage were confirmed as independent prognostic indicators influencing the prognosis of HCC patients (Fig. 5) [23].

The association between CFHR4 and survival

The results outlined that low expression of CFHR4 in tumor tissues was considerably associated with poor overall survival (OS, log rank $P = 3.1 \times 10^{-7}$, HR = 0.41 (0.29–0.59), Fig. 6A) in patients with HCC. Moreover, a subgroup analysis revealed that the down-regulation of CFHR4 in tumor tissue was a risk factor for reduced 1-year OS (log rank $P = 1.6 \times 10^{-8}$, HR = 0.25 (0.15–0.42), Fig. 6B), 3-year OS (log rank $P = 1.1 \times 10^{-7}$, HR = 0.37 (0.25–0.54), Fig. 6C), 5-year OS (log rank $P = 1.3 \times 10^{-7}$, HR = 0.39 (0.27–0.56), Fig. 6D), 5-year OS stage III-IV (log rank $P = 0.00013$, HR = 0.33 (0.18–0.60), Fig. 6E), 5-year relapse-free survival (RFS, log rank $P = 5.5 \times 10^{-5}$, HR = 0.5 (0.36–0.71), Fig. 6F), and 5-year progression free survival (PFS, log rank $P = 5.7 \times 10^{-7}$, HR = 0.47 (0.35–0.64), Fig. 6G) in HCC patients [5].

The diagnostic performance of CFHR4 mRNA Levels for the differentiation of HCC patients from healthy controls

The potential diagnostic utility of CFHR4 to differentiate between HCC and benign disease was assessed through generating a receiver operating characteristic (ROC) curve for CFHR4 expression. We found that the ROC area under the curve (AUC) of the CFHR4 was 0.72 (95% confidence interval (CI): 0.67–0.77) (Fig. 6H). However, we do not have a serum sample to verify our point, and considerable work needs to be done [24].

The correlation analysis between CFHR4 expression and tumor-infiltrating immune cells (TIICs)

We analyzed the correlation between CFHR4 expression and infiltrating immune cells (Monocyte, CD4 + T cells, Myeloid dendritic cells, CD8 + T cells, T cells regulatory (Tregs), Macrophages, Neutrophils, NK cells, Endothelial cells and Hematopoietic stem cells). The results showed that the expression of CFHR4 was positively correlated with the infiltration levels of Macrophages ($r = 0.394$, $P = 3.07 \times 10^{-14}$), Neutrophils ($r = 0.256$, $P = 1.51 \times 10^{-6}$), NK cells ($r = 0.385$, $P = 5.35 \times 10^{-4}$), Endothelial cells ($r = 0.397$, $P = 1.75 \times 10^{-14}$), and Hematopoietic stem cells ($r = 0.366$, $P = 2.19 \times 10^{-12}$) (Fig. 7A), and negatively correlated with Monocytes ($r = -0.265$, $P = 6.12 \times 10^{-7}$), CD4 + T cells ($r = -0.372$, $P = 9.66 \times 10^{-13}$), Myeloid dendritic cells ($r = -0.366$, $P = 2.18 \times 10^{-12}$), CD8 + T cells ($r = -0.329$, $P = 3.66 \times 10^{-10}$), and T cells regulatory (Tregs) ($r = -0.239$, $P = 7.00 \times 10^{-6}$) (Fig. 7B) [28].

Discussion

This study initially reported the role of CFHR4 in HCC. Compared with adjacent normal tissues, the expression of CFHR4 mRNA in HCC was significantly lower. CFHR4 mRNA levels were inversely correlated with a cancer family history, Histological grade, TNM stage, and Serum AFP level of HCC patients. The results of the database analysis were validated using our HCC samples.

Many complicated genetic disorders are caused by variations in the factor H gene family [29]. CFHR4 was maladjusted in many diseases: the absence of CFHR4 increases the risk of the atypical hemolytic uremic syndrome [30, 31], and the increase of circulation level of CFHR4 was strongly associated with age-related macular degeneration (AMD) [17, 32]. CFHR4 was significantly upregulated in the lacrimal glands

of Benign lymphoepithelial lesion (BLEL) patients, suggesting that the abnormal complement system plays a role in eye diseases [33–35]. CFHR4 was down-regulated in patients with chronic central serous chorioretinopathy (cCSC) and considered a candidate gene [36]. Deletion of CFHR4 may limit the ability of C protein to inhibit inflammation, thus promoting the development of systemic lupus erythematosus (SLE) [12, 31]. CFHR4 has been identified as one of the potential biomarkers of growth hormone (GH) deficiency in children [37]. CFHR4 as a first-line epileptic drug phenytoin hypersensitivity prognostic marker, the sensitivity is 16%, while the specificity is 97% [38]. The above results suggest that FHR4 plays a potential role in the disease occurrence and development and may represent a novel therapeutic molecule [19].

Through public database analysis, we have observed the correlation between the CFHR4 mRNA infiltrating immune cell levels and HCC. This result indicates that the CFHR4 mRNA level may reflect the infiltration of lymphocytes in HCC to a certain extent, suggesting that CFHR4 may be a potential immune biomarker of HCC. To determine the effect of specific inhibition of CFHR4 on the activity of tumor-infiltrating lymphocytes and the anti-tumor effect of CFHR4-induced expression is an interesting area for future study [28].

Conclusion

In conclusion, our findings demonstrated that the decreased expression of CFHR4 in HCC is associated with a poor prognosis. The association between CFHR4 and several clinicopathological characteristics suggests that it might be a possible predictive indicator for HCC. This work contributes to a better understanding of the possible role and prognostic significance of CFHR4 mRNA in tumor immunology. The level of CFHR4 mRNA is related to the prognosis and immune infiltration of HCC, indicating that it can be used as a biomarker to predict the prognosis [28]. Some of the findings are based on data analysis, limiting the conclusion, and we will use molecular biology to confirm the findings in the future.

Materials And Methods

Data resource and Description

Four GEO microarray series (GSE45267, GSE45436, GSE60502, GSE84402) containing HCC tumor and normal samples were downloaded from the National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>). Platforms and samples of the GEO series were summarized in Table 1 [5].

TCGA-LIHC dataset contains 424 samples with 374 tumors and 50 normal samples of clinicopathological information and genes mRNA expression data, which were downloaded from the TCGA database (<https://www.cancer.gov/aboutnci/organization/ccg/research/structural-genomics/tcga>).

Data mining in GEO and TCGA-LIHC for HCC

The raw array data of four GEO microarray series and TCGA-LIHC were subjected to background correction, quartile data normalization, and converted into gene expression values. Data were normalized using the affy package of Bioconductor R package (<https://cran.r-project.org/mirrors.html>). The differentially expressed genes (DEGs) between HCC tumor and normal samples were identified in four GEO microarray series using the limma package. The p -value < 0.05 and $|\log FC| > 2$ were chosen as cut-off criteria [25]. Volcano plot and Venn diagram of DEGs were performed with ggpubr (<https://CRAN.R-project.org/package=ggpubr>) and VennDiagram (<https://CRAN.R-project.org/package=VennDiagram>) packages of R [24]. The edgeR [39, 40] packages were used to identify CFHR4 expression between tumor and normal samples. The Spearman coefficients of the DEGs and CFHR4 were calculated, while DEGs with p -value < 0.05 were defined as CFHR4-correlated genes [5]. Correlation Heatmap of CFHR4-correlated genes was performed by R ggcorrplot (<https://CRAN.R-project.org/package=ggcorrplot>) package. ClusterProfiler [41] and GOplot [42] packages are used to draw bubble charts.

GO and KEGG enrichment analysis

Gene ontology (GO), including biological process (BP), cellular components (CC), and molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, was performed to discover the potential mechanism of the DEGs [43].

Genomic alteration analysis

Genomic alteration types, alteration frequency, and protein change in amino acids were analyzed by the cBioPortal database (<http://cbioportal.org>) to investigate the CFHR4 mutation in TCGA-LIHC [22, 44, 45].

Survival analysis.

To analyze the correlation between CFHR4 expression and clinical relevance [8], a Cox proportional hazards model was used to identify independent predictive factors for prognosis [46]. The affection of CFHR4 expression on the survival of LIHC patients was evaluated by Kaplan–Meier plotter (https://kmplot.com/analysis/index.php?p=service&cancer=liver_rnaseq) [22, 47]. Taking the median by Kaplan-Meier plotter of CFHR4 expression value as the critical point, TCGA-LIHC patients were divided into low and high expression groups to analyze the correlation between CFHR4 expression and clinicopathological characteristics [5].

Immune Infiltration Analysis

Using the TIMER database (<http://timer.cistrome.org/>) [48–50], this study analyzed the correlation between the expression of CFHR4 and many types of infiltration of immune cells in TCGA-LIHC patients [51]. P -Value < 0.05 was statistically significant.

Patients and clinical specimens

The present study was approved by the Ethics Committee of Sino-German Biomedical Center of the Hubei University of Technology, written consent was obtained from each participant, and the study was performed following the ethical standards of the Declaration of Helsinki [21]. Three pairs of fresh

samples of human HCC and corresponding paracancerous tissues (at least 2 cm away from the edge of the tumor) were obtained for immunohistochemistry (IHC) and western blot analysis from the same hospital. The samples were stored at -80°C until use [23].

Immunohistochemical assay

The expression of the CFHR4 protein was assessed by IHC. The tumor tissues excised during the operation were immediately placed in 10% formalin for fixation, followed by dehydration, paraffin embedding, and sectioning. Anti- CFHR4 (R&D Systems, Germany) antibody was used at a dilution of 1:256. These results were assessed by two neuropathologists individually. CFHR4 protein expression (semi-quantitative scoring) = expression intensity · expression area [52]. Expression intensity was scored using a 4-point scale from 0 to 3. The expression area was scored using a 5-point scale from 0 to 4 [53]. Moreover, 0–6, 7–9 and 10–12 represented +, ++, +++ respectively.

Western blot assay

According to the manufacturer's instructions, total protein was extracted from tissues using the Protein Extraction Kit (Beyotime BioTECH, Shanghai, China). Protein lysates were separated by 10% SDS-PAGE and then transferred to a PVDF membrane blocked with 5% skim milk powder at room temperature for an hour. After the membranes were blocked, they were incubated with the primary antibodies at 4°C overnight including, anti-GAPDH (Proteintech Group, China), anti-CFHR4 (R&D Systems, Germany) [26]. Then, the membranes were incubated with horseradish peroxidase-conjugated antibodies at room temperature for an hour [27]. Protein signals were detected using enhanced chemiluminescence (LI-COR Biosciences, USA). The results of western blot were quantified by densitometric analysis using ImageJ software [21].

Statistical analysis.

R software (version 3.5.1, The R Foundation), and GraphPad Prism software (version 8.0, GraphPad Software, Inc.), and SPSS software (version 24.0, SPSS, Inc.) were applied for statistical analysis and scientific graphing. Student *t*-test (and nonparametric tests) was used to compare the expression of CFHR4 between HCC tumor and normal tissues [21]; The Cox proportional hazards regression model was used to evaluate the hazard ratio (HR); The statistically significant parameters in univariate analysis were selected for Cox multivariate analysis and to confirm independent prognostic predictor factors [23]. Kaplan-Meier and Log-rank tests were employed to identify the overall survival (OS) variables in HCC patients. The chi-square test was used to determine the relationship between CFHR4 expression and the clinicopathological factors of HCC patients. $P < 0.05$ indicates that the difference was statistically significant.

Declarations

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Author's contributions

KH, QY designed the study; QD and ZX and HL performed the study; QD and HL analyzed the results; KH and QY and QD wrote the manuscript.

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Data availability

The raw data was generated and analyzed in author's organization, The data are not publicly available due to individual privacy rights. The data supporting the findings of this study can be provided at the reasonable request from the corresponding authors.

Conflict of interest

The authors declare no conflict of interest or competing interests.

Ethical approval

All the procedures performed in studies involving human participants were in accordance with the with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The database and tissue bank are approved by the Ethics Committee of Sino-German Biomedical Center of Hubei University of Technology.

Consent to participate

Informed consent was obtained from participants included in the study.

Consent for publication

All the authors consented the last version of this manuscript.

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Tables

Table 1

GEO series	Contributor(s), Year	Number of samples (Tumor/Control)	Platform
GSE45267	Chen CL, et al., 2018	87 (46/41)	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.
GSE45436	Wang HW, et al., 2013	134 (41/93)	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.
GSE60502	Kao KJ., 2014	36 (18/18)	[HG-U133A] Affymetrix Human Genome U133A Array.
GSE84402	Wang H, et al., 2017	28 (14/14)	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.

Table 2

DEGs	Number	Gene names
Up-regulated	18	MDK ASPM KIF20A HMMR MELK NCAPG PBK TOP2A CCNB1 CDC20 NDC80 PEG10 RRM2 PRC1 TTK GPC3 GINS1 SPINK1
Down-regulated	67	HAMP C9 FCN3 MT1M SLC22A1 CLEC1B CYP1A2 CRHBP NAT2 SLC01B3 APOF GYS2 GBA3 HPD MT1F CYP2A6 CFHR4 LPA AFM ADH1C C6 CYP26A1 AKR1D1 FOS CXCL14 CLEC4M KCNN2 HGFAC MT1X PCK1 MARCO SLC10A1 MT1H SRPX GLYAT LECT2 CD5L HSD11B1 FETUB MT1E SPP2 CETP RDH16 ADH1B DNASE1L3 KMO IGF1 GNMT CFP SRD5A2 C8A CYP2B6 BCHE CYP2C9 CYP39A1 MT1G CYP2A7 LYVE1 FCN2 NPY1R GSTZ1 CYP3A4 ZG16 FOSB C7 CYP1A1 OTC

Table 3

Variables	CFHR4 expression level		<i>p</i> value
	Low (n = 180)	High (n = 180)	
Age			0.026*
<55	66	47	
≥55	113	134	
Gender			0.681
Male	119	124	
Female	60	57	
BMI			0.013**
<18.5	12	9	
18.5~24.99	91	61	
25-29.99	35	52	
>30	29	38	
Race			<0.001***
Asian	103	52	
White	68	108	
Black or African American	5	12	
Family history of cancer			0.001***
No	113	89	
Yes	40	69	
Histological grade			<0.001***
G1-G2	95	129	
G3-G4	84	47	
TNM stage			0.048*
I-II	119	129	
III-IV	53	35	
AFP (ng/ml)			<0.001***
>400	44	19	
≤400	85	122	
Hepatic inflammation			0.056
None	42	72	
Mild	51	46	
Severe	6	11	

Figures

A

B

Figure 1

In total, 18 significantly upregulated genes and 67 significantly down-regulated genes were identified (including CFHR4) (Fig. 1)

Figure 2

The expression of CFHR4 in tumor samples was significantly lower than non-tumor samples in GSE45267, GSE45436, GSE60502, GSE84402, and TCGA-LIHC dataset (Fig. 2 A-E). The immunohistochemistry and Western blot results of clinical samples confirmed that CFHR4 is under-expressed in HCC tissues, compared with normal tissues (Fig. 2 F-G).

Figure 3

The correlation between CFHR4 and CFHR4-correlated genes was identified (Fig.3A) the KEGG pathway enrichment of CFHR4 interactive genes showed that Retinol metabolism, Mineral absorption, Steroid hormone biosynthesis, Metabolic pathways, complement, and coagulation cascades, were the enriched pathways [5] (Fig.3 B-E).

Figure 4

The genomic mutation is closely associated with tumorigenesis[22]. The research showed about 7% of genetic alteration of CFHR4 in HCC, including amplification and missense mutation with unknown significance (Fig. 4A-B). In addition, missense mutation of CFHR4 resulted in the amino acid change, including Asparagine (N) 356 replaced by Isoleucine (I) and Lysine (K) 227 replaced by Asparagine (N) (Fig. 4C)

Figure 5

The univariate analysis indicated that CFHR4 expression and TNM stage were directly correlated with OS in HCC. Furthermore, by Cox multivariate analysis, we found that low CFHR4 expression and TNM stage were confirmed as independent prognostic indicators influencing the prognosis of HCC patients (Fig. 5)

Figure 6

The results outlined that low expression of CFHR4 in tumor tissues was considerably associated with poor overall survival (OS, log rank P = 3.1e-07, HR = 0.41 (0.29-0.59), Fig. 6A) in patients with HCC. Moreover, a subgroup analysis revealed that the down-regulation of CFHR4 in tumor tissue was a risk factor for reduced 1-year OS (log rank P = 1.6e-08, HR = 0.25 (0.15 -0.42), Fig. 6B), 3-year OS (log rank P = 1.1e-07, HR = 0.37 (0.25 -0.54), Fig. 6C), 5-year OS (log rank P = 1.3e-07, HR = 0.39 (0.27 -0.56), Fig. 6D),

5-year OS stage III-IV (log rank $P = 0.00013$, HR = 0.33 (0.18-0.60), Fig. 6E), 5-year relapse-free survival (RFS, log rank $P = 5.5\text{e-}05$, HR = 0.5 (0.36-0.71), Fig. 6F) , and 5-year progression free survival (PFS, log rank $P = 5.7\text{e-}07$, HR = 0.47 (0.35-0.64), Fig. 6G) in HCC patients We found that the ROC area under the curve (AUC) of the CFHR4 was 0.72 (95% confidence interval (CI): 0.67–0.77) (Fig. 6H)

Figure 7

The results showed that the expression of CFHR4 was positively correlated with the infiltration levels of Macrophages ($r = 0.394$, $P = 3.07 \times 10^{-14}$), Neutrophils ($r = 0.256$, $P = 1.51 \times 10^{-6}$), NK cells ($r = 0.385$, $P = 5.35 \times 10^{-4}$), Endothelial cells ($r = 0.397$, $P = 1.75 \times 10^{-14}$), and Hematopoietic stem cells ($r = 0.366$, $P = 2.19 \times 10^{-12}$) (Fig. 7A), and negatively correlated with Monocytes ($r = -0.265$, $P = 6.12 \times 10^{-7}$), CD4⁺ T cells ($r = -0.372$, $P = 9.66 \times 10^{-13}$), Myeloid dendritic cells ($r = -0.366$, $P = 2.18 \times 10^{-12}$), CD8⁺ T cells ($r = -0.329$, $P = 3.66 \times 10^{-10}$), and T cells regulatory (Tregs) ($r = -0.239$, $P = 7.00 \times 10^{-6}$) (Fig. 7B)