Original Article

Identification of potential prognostic biomarkers among gene models for coiled-coil domain-containing family members in hepatocellular carcinoma elucidates their influence on the hypoxia pathway and immune microenvironment

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Background: The family of coiled-coil domain-containing (CCDC) proteins participates in a wide range of physiological functions and plays a pivotal role in governing the invasion and metastasis of malignant tumor cells. Nonetheless, the precise mechanism governing the interaction among the immune microenvironment, hypoxia pathway, and proliferation in hepatocellular carcinoma (HCC) remains elusive. In this study, our objective was to identify the prognostic significance of CCDC family genes in HCC.

Methods: We conducted an analysis of RNA-seq data from HCC patients sourced from The Cancer Genome Atlas (TCGA) database. Our analysis involved comparing the expression profiles of 168 CCDC family genes between tumor and normal tissues to identify differentially expressed genes (DEGs). The prognostic value of these genes was verified using overall survival (OS) data from TCGA-LIHC patients, employing Univariate and multivariate Cox proportional hazards regression models and Kaplan-Meier plots. Subsequently, we constructed a prognostic signature known as the CCDC score and validated it using additional datasets (ICGC-LIRI-JP and GSE14520). Additionally, we performed functional enrichment analysis and conducted an assessment of the tumor immune microenvironment (TIME).

Results: We identified 34 DEGs of the CCDC family. Among them, six DEGs (*CCDC6/22/51/59/132/134*) were upregulated and associated with poor prognosis. Higher CCDC score was an independent predictor of poor OS in TCGA-HCC patients (P<0.001, HR =2.37), which was validated in the ICGC-LIRI-JP (P=0.021, HR =2.15) and GSE14520 (P=0.002, HR =2.23) datasets. Functional enrichment analysis showed that hypoxia pathway genes were enriched in the high CCDC score group. Furthermore, immune microenvironment analysis demonstrated that high CCDC score was associated with a suppressed TIME caused by the extrinsic immune escape.

Conclusions: The CCDC score, derived from six CCDC genes, exhibits remarkable expression levels in liver cancer and holds promise as an independent prognostic indicator. Our bioinformatics analysis revealed a high CCDC score is strongly associated with activation of the hypoxia pathway and an immunosuppressive tumor microenvironment in HCC. This profound finding may serve as a cornerstone for innovative targeted drug therapies and pave the way for further investigations into the underlying mechanisms of CCDC-related carcinogenesis in liver cancer.

Keywords: Hepatocellular carcinoma (HCC); coiled-coil domain-containing (CCDC); hypoxia; tumor immune microenvironment (TIME); prognostic model

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Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer, and ranks as the sixth most prevalent cancer worldwide and the fourth leading cause of cancer-related deaths. There were 841,080 new liver cancer cases in 2018, emphasizing its global impact on mortality (1). Although early-stage HCC can be treated curatively by local ablation, surgical resection, or liver transplantation, the majority of patients are not diagnosed until later stages when curative treatment is no longer feasible (2,3). Despite advances in systemic therapies, such as immune checkpoint inhibitors (ICIs), the median life expectancy of HCC patients remains less than 2 years. Previously approved agents for first-line therapy in unresectable HCC patients, such as sorafenib, lenvatinib, and the combination of bevacizumab with atezolizumab, have been focused on angiogenesis. The HIMALAYA trial was a groundbreaking development, being the first to demonstrate the benefit of dual ICIs and thus representing a new treatment avenue in this context (4). A stage III trial demonstrated that atezolizumab (anti-PD-L1 antibody) in combination with bevacizumab (anti-VEGF antibody) as a first-line treatment resulted in better overall survival (OS) and progression-free survival (PFS) outcomes than sorafenib (tyrosine kinase inhibitor) in unresectable HCC patients (5). Given multiple different approaches including anti-VEGF and immune-checkpoint inhibitors, it is crucial to explore the molecular classification of HCC and identify more effective therapeutic targets as well as prognostic and predictive biomarkers.

The relationship between immune score and HCC prognosis is evident, suggesting that lower immune scores in HCC patients correspond to more favorable clinical outcomes. Notably, the concentration of the $CD8^+$ T cell interaction network is prominent in the C1 subtype, underscoring the potential significance of tumor-infiltrating immune cells for predicting clinical outcomes and assessing immunotherapy response in HCC patients. The C1 subtype emerges as a promising predictive factor for immunotherapy response (6). High SOX9 expression emerges as a valuable prognostic indicator specifically in non-cirrhotic HCC (NCHCC), with no significant difference between CHCC and NCHCC. SOX9 serves as a reliable diagnostic marker for both HCC types (7). Furthermore, three pivotal hub genes—BIRC5, CDC20, and UBE2C—are closely correlated with the progression and prognosis of HCC, offering substantial potential as therapeutic targets in HCC treatment (8). In parallel, REXO4 demonstrates significant overexpression in liver cancer and holds promise as a predictive marker for liver cancer prognosis and a biomarker for targeted therapeutic interventions. Elevated REXO4 expression is linked to unfavorable outcomes in HCC patients and may be connected to immune infiltration in liver cancer. A comprehensive understanding of the role of REXO4 could pave the way for targeted drug therapies and further exploration of its mechanisms in liver cancer carcinogenesis (9). In addition, PTER protein is significantly up-regulated in HCC tumors, and its high

Highlight box

Key findings

- We identified six coiled-coil domain-containing (CCDC) genes that may serve as a potential prognostic marker for hepatocellular carcinoma (HCC) survival, and a high CCDC score of these genes is linked to hypoxia pathway activation and an immunosuppressive tumor microenvironment (TME).

What is known and what is new?

- These CCDC genes were found to be highly expressed in tumor samples.
- A CCDC score was calculated based on six CCDC genes, which was associated with hypoxia pathway activation and immunosuppressive TME in HCC.

What is the implication, and what should change now?

- High CCDC score is an independent predictor of poor overall survival.

expression is associated with aggressive clinicopathological features, including advanced tumor staging, vascular invasion, recurrence, and shortened OS and disease-free survival (DFS) time. PTER protein serves as an independent predictor for OS and DFS in HCC patients, particularly in those with elevated PTER protein expression (10). Proteins belonging to the coiled-coil domain-containing (CCDC) family play important roles in various diseases, particularly in the migration, proliferation, and metastasis of different cancers, where high levels of CCDC proteins are associated with unfavorable prognoses (11). For instance, CCDC34 is upregulated in bladder cancer and regulates bladder cancer cell proliferation, apoptosis, and migration, whereas CCDC88A accumulation indicates poor OS in human pancreatic cancers and promotes the motility and invasiveness of pancreatic cancer cells (12,13).

In contrast, CCDC proteins such as CCDC50 are required for survival in mantle cell lymphoma and chronic lymphocytic leukemia, whereas CCDC62/ERAP75 functions as a coactivator in prostate cancer cells (14,15). Similarly, CCDC106 promotes non-small cell lung cancer (NSCLC) cell proliferation (16). However, some CCDC proteins may also exhibit tumor-inhibiting properties. For example, CCDC67 is downregulated in gastric tumors and papillary thyroid cancer and functions as a tumor suppressor gene (17,18). Although CCDC family genes play important roles in various diseases, their specific roles in HCC remain unclear. The CCDC family genes play an immunosuppressive role in liver cancer, and the precise mechanisms underlying their immune regulation require further in-depth research. This is also a pressing issue and a hot topic in clinical research. The exact mechanism that governs the interaction between the immune microenvironment, the hypoxia pathway, and proliferation in liver cancer remains a mystery and a critical area of investigation. In this study, we aimed to determine the prognostic value of CCDC family genes for HCC and investigate the pathways and tumor immune microenvironment (TIME) changes that are regulated by these genes. The CCDC score based on six CCDC genes is a potential prognostic indicator for HCC survival. The results show that patients with high CCDC score in HCC have poor prognosis, and it is associated with the activation of the hypoxia pathway and immune-suppressed tumor microenvironment (TME), suggesting the existence of an immune-suppressed TME. We present this article in accordance with the TRIPOD reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-652/rc).

Methods

Study design and datasets

A total of three independent datasets of HCC were included in this study. Gene expression profiles of 371 HCC patients were downloaded and merged using the GDC-RNASeq-tool (https://gdc.cancer.gov/content/gdc-rnaseq-tool) from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) portal. The University of California, Santa Cruz (UCSC) Xena website (https://xenabrowser.net/datapages/) was accessed to download the raw read counts and clinical information, including age, gender, stage, and OS. To further validate the OS status of the candidate gene set, the microarray data and clinical information of ICGC-LIRI-JP (n=212) and GSE14520 (n=209) were downloaded from the International Cancer Genome Consortium Data Portal (ICGC; https://dcc.icgc.org/) and the Gene Expression Omnibus Database (GEO; https://www.ncbi.nlm.nih.gov/geo/) (*Figure 1A*). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Protein-protein interaction (PPI) analysis

GeneMANIA is a flexible web tool used to construct PPI networks, generate hypotheses on gene roles, explore gene lists, and prioritize genes (19). In this study, we utilized GeneMANIA to visualize the gene network of CCDC co-expression genes. The six candidate genes were entered as a set to produce a network map that depicts physical interactions, co-localization, co-expression, and predicted genes and interactions. Scores for predicted genes and the number of gene interactions were used to identify the strongest predicted genes in the network.

Differentially expressed genes (DEGs) analysis

DEGs between normal and tumor samples were identified by R package "DEseq2". The up-regulated or down-regulated genes were defined as those with a false discovery rate (FDR) value of <0.05 and a $\log_2$(fold change) >0.5 or <–0.5 as the cutoff criteria, respectively; these genes

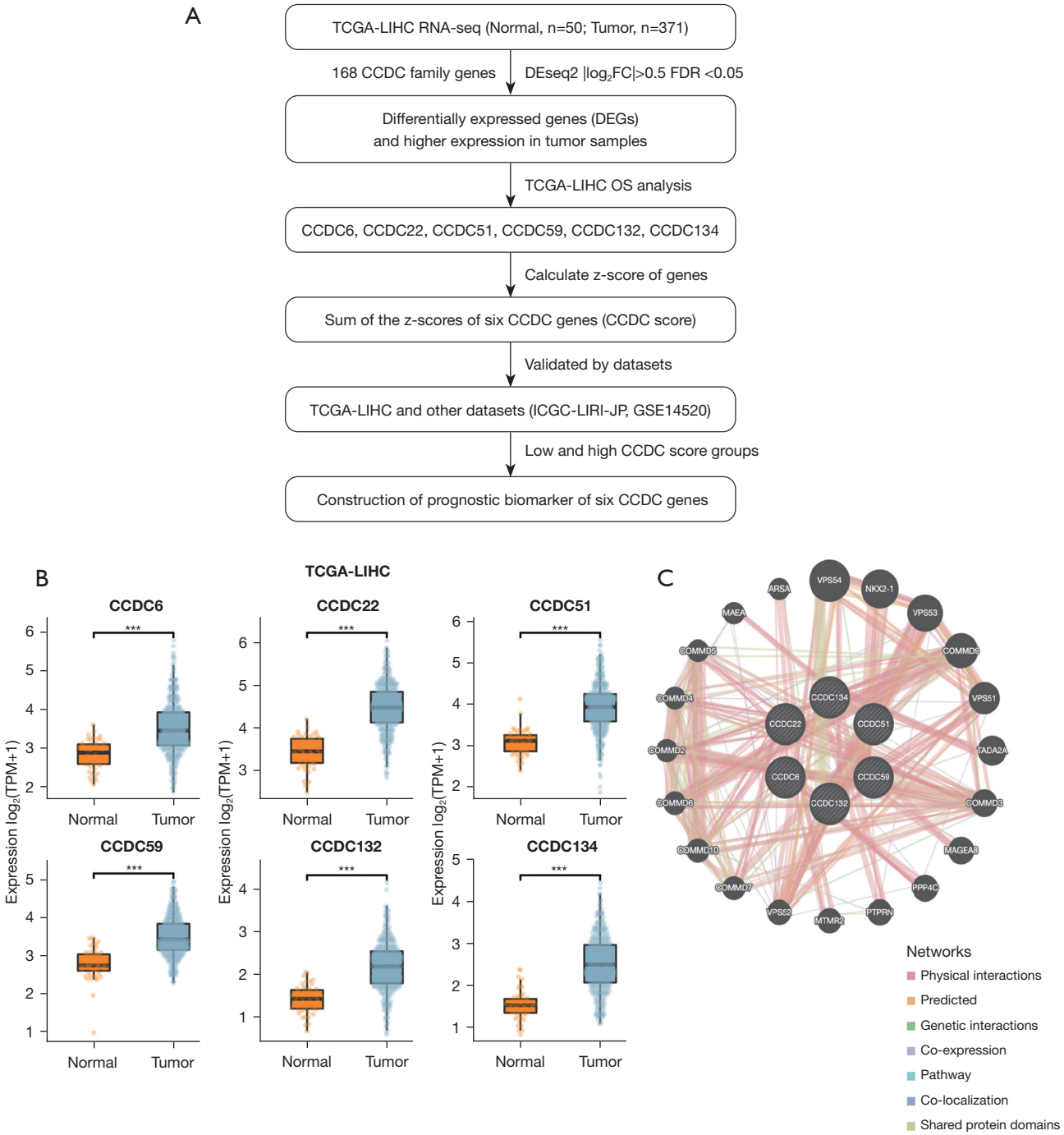

Figure 1 The workflow of the study and identification of CCDC DEGs. (A) The workflow of the study. (B) Comparison of *CCDC6*, *CCDC22*, *CCDC51*, *CCDC59*, *CCDC132*, *CCDC134* expression in paired HCC tumor (n=371) and normal tissues (n=50). Data shown as mean ± SD. (C) Protein-protein interaction network of CCDC gene family members and their related genes was analyzed by GeneMANIA. The area of the gray circle represents prediction scores and thickness of line represent interaction scores, with a larger area/thicker line representing stronger prediction/interaction. ***, P<0.001. TCGA, The Cancer Genome Atlas; HCC, hepatocellular carcinoma; CCDC, coiled-coil domain-containing; FC, fold change; FDR, false discovery rate; DEGs, differentially expressed genes; OS, overall survival; ICGC, International Cancer Genome Consortium Data Portal; SD, standard deviation.

were deemed to be the DEGs. The candidate gene expression profiles of the heatmap were generated after z-score normalization of transcripts per million (TPM) by "pheatmap" package of R.

Prognostic model construction using CCDC score

A CCDC score was generated by summing the z-scores of six *CCDC* genes (20). TCGA-HCC patients were classified as high or low CCDC score group. The log rank test was used to compare survival times by Kaplan-Meier survival curve analysis. Multivariate analysis with the Cox proportional hazards model was performed to predict the OS for HCC. This analysis was performed with R packages ("survival", "survminer", and "forestplot"). The ICGC and GSE14520 datasets were used for independent validation also using multivariate Cox proportional hazards regression models and Kaplan-Meier plots.

Pathway enrichment analysis

The enrichment scores of the gene set in each TCGA-HCC sample were quantified by the single sample gene set enrichment analysis (ssGSEA) algorithm using the R package "GSVA". GSEA analysis was performed to detect significant enrichment pathways with hallmark gene sets (c2.cp.v7.4.symbols.gmt) from the molecular signature database (MSigDB; http://software.broadinstitute.org/gsea/msigdb/). Gene sets for hypoxia activity pathway analysis of high and low CCDC score groups were selected from a previously published study (21). The gene set for $CD8^+$ T effector cells was selected from Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT). Gene Ontology (GO) annotations, including biological process (BP), cellular component (CC), and molecular function (MF) analysis, were employed by R package "clusterProfiler" to handle the DEGs and visualize the enriched GO terms between the two groups.

Immunotherapy response prediction

Tumor immune dysfunction and exclusion (TIDE) (22) is a new computing architecture that integrates data on two tumor immune escape mechanisms. Based on tumor expression profiles, TIDE can score multiple transcriptomic biomarkers of several immune features, including immune system dysfunction, T cell exclusion, and myeloid-derived suppressor cells (MDSCs). We calculated the estimated score of the immune features for each patient based on their mRNA expression profiles from TCGA.

The prediction of response to targets agents

The response of each sample to targeted agents was evaluated by determining the half-maximal inhibitory concentration (IC50) using the R package "pRRophetic", based on the GDSC (Genomics of Drug Sensitivity in Cancer, https://www.cancerrxgene.org/) database. An investigation was undertaken to identify the differences in sensitivity to targeted agents between the groups with high and low CCDC scores.

Statistics

Statistically significant differences were assessed using 2-tailed Student's *t*-test with the R platform (R v4.0.3; The R Foundation for Statistical Computing, Vienna, Austria). Pearson correlation coefficient was performed with the R platform (R v4.0.3) to evaluate the relevant correlation between two datasets. The FDR was used to correct the P value. P values or FDR <0.05 were considered to be significant. The adjusted P<0.05 was set as the cutoff criterion.

Results

Differentially expressed CCDC family genes in HCC

We analyzed the expression patterns of 168 CCDC family genes in the TCGA-HCC dataset by comparing gene expression levels between HCC tumors and normal liver tissue. Our analysis identified 34 DEGs, consisting of 26 upregulated genes and 8 downregulated genes. Notably, we found that the expression of *CCDC6*, *CCDC22*, *CCDC51*, *CCDC59*, *CCDC132*, and *CCDC134* was significantly higher than that in HCC than in normal tissue (*Figure 1B*). Furthermore, we observed that the expression of these genes was not associated with tumor stage, age, or gender (Figure S1). To investigate the gene interaction of the six CCDC genes, we identified *VPS54*, *NKX2-1*, *VPS53*, *COMMD9*, and *VPS51* as having the strongest physical interactions with the six CCDC genes (*Figure 1C*).

The establishment of a 6-CCDC gene prognosis model

To further explore the prognostic value of the six CCDC genes, we analyzed their association with OS in the TCGA-

HCC cohort. Our results showed that higher expression of these genes was significantly associated with poor OS (*Figure 2A*). We then developed a formula to calculate the CCDC score, which is based on the expression levels of the six genes, to predict prognosis in HCC patients. In TCGA-HCC, a high CCDC score was found to be significantly associated with poor OS (P<0.001; *Figure 2B*). Furthermore, in multivariate Cox regression analysis, high CCDC score was identified as an independent predictor of poor OS in TCGA [hazard ratio (HR) =2.13, 95% confidence interval (CI): 1.42–3.2, P<0.001; *Figure 2C*].

Validation of the CCDC prognosis model in the ICGC-LIRI-JP and GSE14520 datasets

To validate the prognostic value of the CCDC model, we analyzed the ICGC-LIRI-JP and GSE14520 datasets. Our results showed that a higher CCDC score was significantly associated with poor OS in the ICGC-LIRI-JP dataset (P=0.021; *Figure 3A*). Furthermore, in the validation set GSE14520, the CCDC score high group population has a worse prognosis, and it is significantly related to the low group population (P=0.002; *Figure 3B*).

Functional annotation of CCDC prognosis model

To investigate the pathways that were activated or suppressed in the high CCDC score group compared to the low CCDC score group, we performed a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Our results showed that the DEGs were mainly enriched in signaling pathways closely related to hypoxia (*Figure 4A*). Additionally, GSEA indicated that the hypoxia pathway was significantly enriched in the high CCDC score group (*Figure 4B*). These findings suggest that the hypoxia pathway may play a critical role in patients with a high CCDC score. Furthermore, we conducted a GO term enrichment analysis and found that small molecule catabolic process, collagen-containing extracellular matrix, and channel activity were the top BP, CC, and MF in the DEGs between the high and low CCDC score groups (*Figure 4C-4E*).

We then explored the relationship between hallmark hypoxia pathway genes in the high and low CCDC score groups. Our analysis revealed that 14 hypoxia genes were significantly enriched in the high CCDC score group in the TCGA-HCC cohort (*Figure 4F*). Moreover, a high CCDC score was associated with higher EHD2 and HIF1A in the TCGA-HCC cohort (*Figure 4G*).

Immune suppressive microenvironment in the CCDC score high group

Hypoxia has been shown to act on various immune cells, leading to the promotion of an immunosuppressive TME (23). To further explain the difference in survival between the two groups, we investigated the relationship between CCDC genes and TME. Schreiber *et al.* introduced the concept of tumor immune editing and described two potential pathways involved in tumor immune escape (24). The first pathway is intrinsic immune escape, which involves the loss of tumor antigen expression and tumor antigen-presenting capacity. The second pathway is extrinsic immune escape, which involves T cell exhaustion, high infiltration of immunosuppressive cells such as regulatory T cells (Tregs) and MDSCs, and elevated levels of immune checkpoint molecules (24).

To explore the potential mechanisms of intrinsic immune escape in HCC progression, we compared immunogenicity indicators, including the fraction of genome altered (FGA) and tumor mutation burden (TMB), between the two groups. The results indicated similar levels of FGA and TMB in both groups (*Figure 5A*).

Hypoxia can impact the recruitment and function of immune cells within the TME. For instance, it can enhance the recruitment of tumor-associated macrophages (TAMs), which can have both pro-tumoral and anti-tumoral functions, depending on their polarization state. We compared the estimated levels of tumor-infiltrating lymphocytes (TILs) in the TCGA-HCC cohort to further investigate the potential mechanisms of extrinsic immune escape in HCC progression. In the high CCDC score group, we observed a significant decrease in the abundance of effector $CD8^+$ T cells (P=0.0027; *Figure 5B*), as well as a lower abundance of $CD8^+$ T cells overall (P=0.038; *Figure 5C*). Conversely, Treg cells, M0, and M2 showed higher abundance in the high CCDC score group, whereas M1 showed no significant change (*Figure 5C*). Additionally, we evaluated the TIDE score and found that the high CCDC score group had higher levels of T cell exclusion and MDSC (P<0.0001; *Figure 5D*). We also observed higher expression of immunosuppressive molecules, including *PDCD1* (encoding *PD-1*) and *CD274* (encoding *PD-L1*), which indirectly reflected exhaustion of TILs (*Figure 5E*). Moreover, the expression of immunosuppressive cytokines interleukin 10 (*IL-10*) and inflammatory cytokines *IL1R1*, *IL2RA*, *IL2RB*, and *IL4R* was significantly higher in the high CCDC score group (*Figure 5F*). In summary,

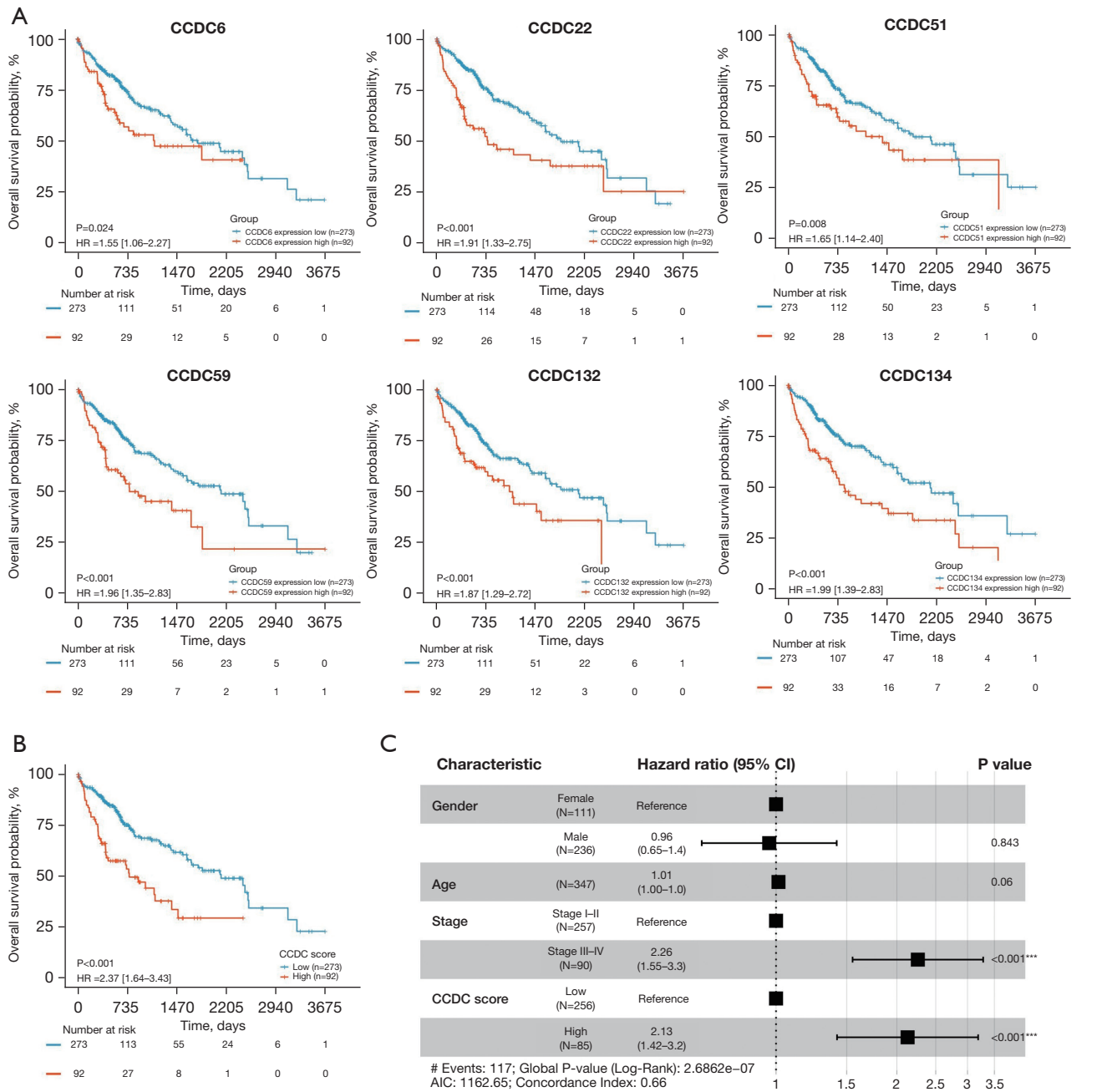

Figure 2 CCDC score construction and its prognostic role. (A) Survival curves comparing the high (top 25% patients) and low expression (bottom 75% patients) of six CCDC family genes in HCC patients. (B) Survival curves comparing the high (top 25%) and low (bottom 75%) CCDC score in TCGA-HCC patients. (C) Multivariate Cox regression analysis for OS. ***, P<0.001. P values were derived from log-rank test. HRs and 95% CI derived from multivariate Cox proportional hazard models. Number of samples between high and low expression status were presented as number. HR, hazard ratio; CI, confidence interval; AIC, Akaike information criterion; CCDC, coiled-coil domain-containing; TCGA, The Cancer Genome Atlas; HCC, hepatocellular carcinoma; OS, overall survival.

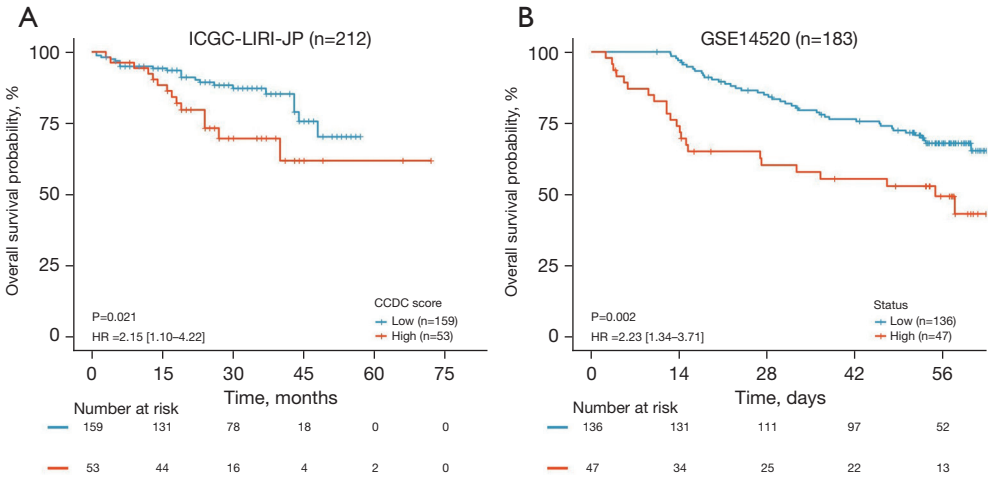

Figure 3 CCDC score prognosis model validation. Survival curves comparing the high (top 25%) and low (bottom 75%) CCDC score in ICGC-LIRI-JP patients (A) and GSE14520 patients (B). P values were derived from log-rank test. HRs and 95% CI derived from multivariate Cox proportional hazard models. Number of samples between high and low expression status were presented as number. ICGC, International Cancer Genome Consortium Data Portal; HR, hazard ratio; CCDC, coiled-coil domain-containing.

hypoxia-associated genes in HCC can contribute to an immunosuppressive microenvironment, which can hinder the body's natural immune response against the cancer. This complex relationship underscores the importance of developing therapies that target both the hypoxic aspects of the tumor and the immunosuppressive mechanisms to improve outcomes for HCC patients.

The sensitivities of patients in the CCDC score high and low group to targeted agents

Targeted therapies have become the standard of care as first-line treatment for advanced unresectable HCC patients who exhibit therapeutically targeted alterations. In this study, we investigated whether the CCDC score has an impact on the efficacy of these targeted agents. A significant difference was found in the IC50 value of gefitinib between the CCDC score high and low groups, with the high group demonstrating decreased sensitivity to sorafenib. Sorafenib is an oral multikinase inhibitor that targets several protein kinases, influencing both tumor cell proliferation and angiogenesis. The high group exhibited a diminished response to sorafenib compared to the low group (P<2.22e–16, *Figure 6A*). Similarly, the CCDC score high group also showed significantly reduced sensitivity to axitinib compared to the low group (P<2.22e–16, *Figure 6B*). These findings suggest that the CCDC score might influence the treatment efficacy of targeted agents for liver cancer patients.

Discussion

The HCC TME is a complex ecosystem within the liver where cancer cells interact with various cellular and molecular components. Key characteristics include immune cell infiltration, angiogenesis, fibrosis, hypoxia, and the presence of cancer-associated fibroblasts. Understanding these features is pivotal for devising effective therapies tailored to the unique aspects of HCC. For example, the HCC patients with lower immune scores tend to experience better clinical outcomes. Collectively, the significance of tumor-infiltrating immune cells as potential determinants of clinical outcomes in HCC patients and propose the potential use of these immune cell profiles as biomarkers to predict responses to immunotherapy (6). In terms of treatment research progress, significant strides have been made in recent years. Targeted therapies, such as sorafenib and lenvatinib, have been approved, offering hope for advanced HCC patients. Immunotherapy, particularly ICIs like nivolumab and pembrolizumab, has demonstrated promising results by harnessing the body's immune system against cancer. Combinations of these approaches and locoregional treatments are being explored. These advancements underscore the evolving nature of

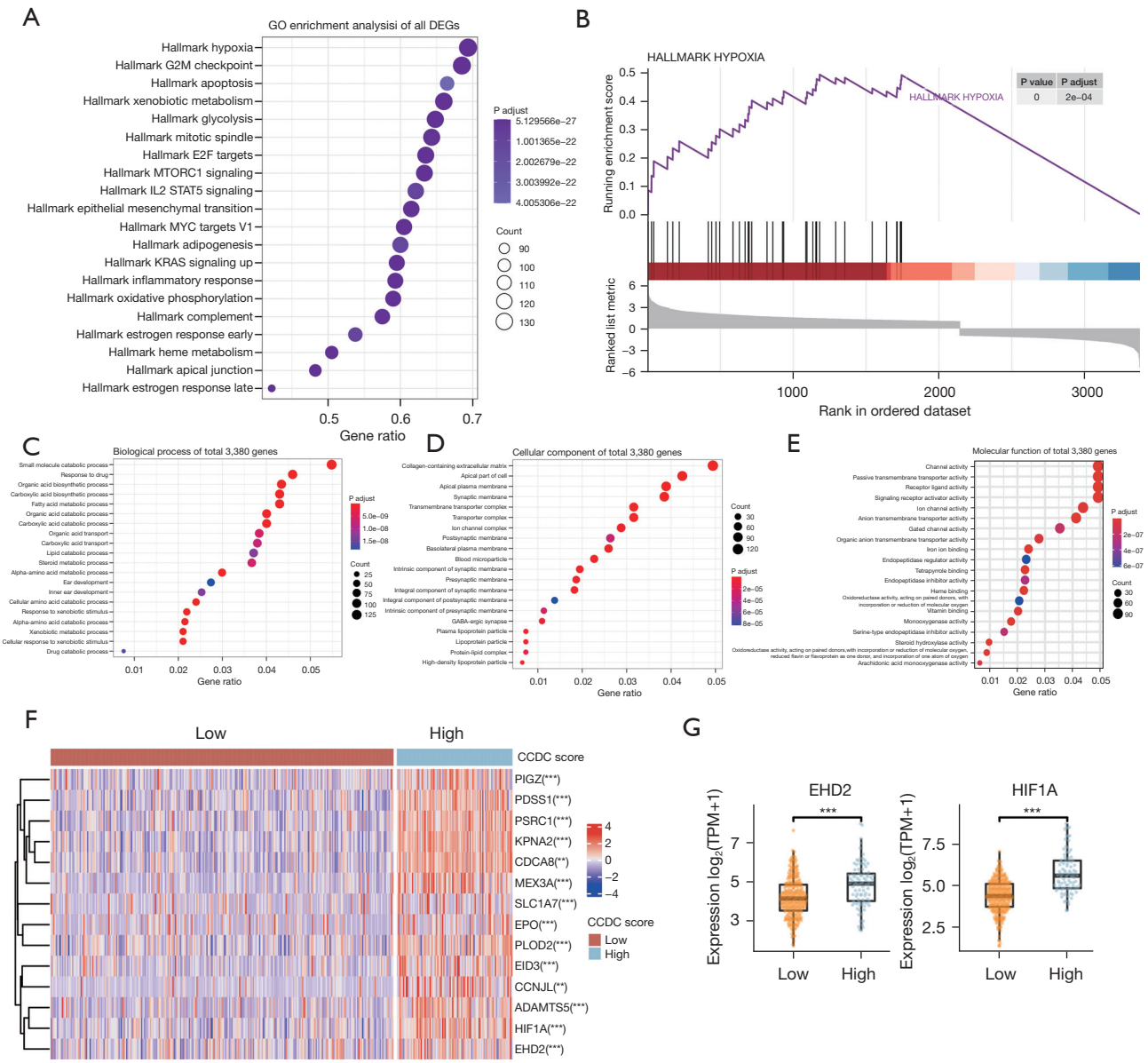

Figure 4 Function enrichment of CCDC family members in HCC. (A) Analysis of 50 hallmark gene sets from MSigDB. (B) GSEA analysis. (C) Biological processes. (D) Cellular component. (E) Molecular function. (F) The expression heatmap of 14 hypoxia genes. (G) The expression of 2 hypoxia genes between the high and low CCDC score groups. ***, P<0.001. DEGs, differentially expressed genes; CCDC, coiled-coil domain-containing; HCC, hepatocellular carcinoma; GSEA, gene set enrichment analysis.

HCC treatment and the ongoing quest for more effective interventions.

The high risk of recurrence and metastasis in patients with HCC, even after comprehensive treatment, underscores the need for reliable molecular biomarkers to improve outcomes. Although the CCDC family proteins are involved in various physiological processes, their role in HCC is not well understood (25,26). To address this gap, we analyzed the mRNA expression of 168 CCDC family genes in HCC patients from TCGA and found that 26 genes were overexpressed in HCC compared to normal tissues. Furthermore, we identified six genes (*CCDC6*, *CCDC22*, *CCDC51*, *CCDC59*, *CCDC132*, and *CCDC134*) for which high expression levels predict poor OS. We constructed a

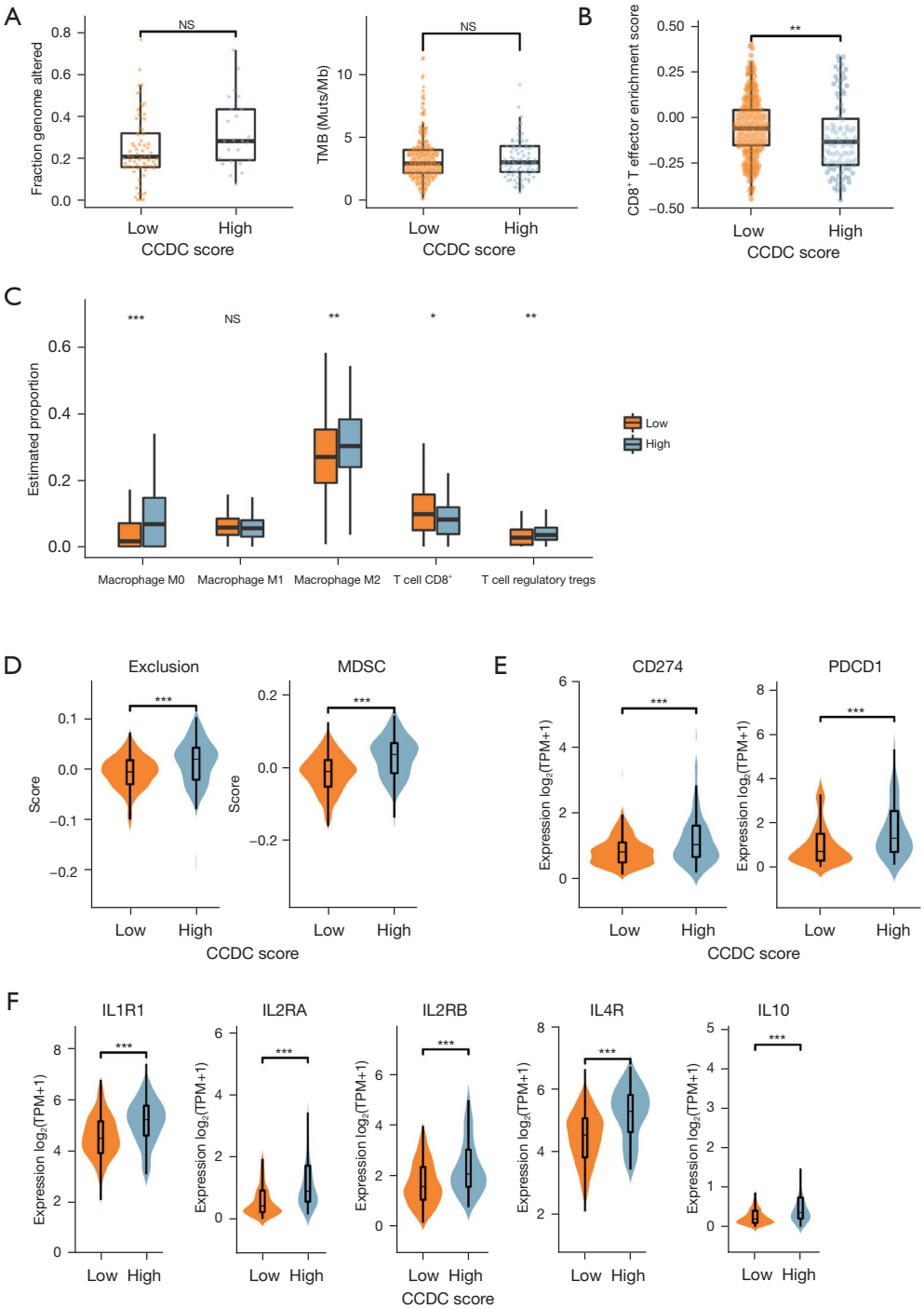

Figure 5 Immune microenvironment analysis. (A) Comparison of FGA and TMB in the high and low CCDC score group from TCGA. (B) GSVA analysis of CD8$^+$ T effector cells enrichment in two groups. (C) Immune cell score of Tregs, macrophage M0, M1, M2, and CD8$^+$ T cells by CIBERSORT. (D) The score for immune features of T cell exclusion and MDSC predicted by TIDE. (E) The expression level of CD274 and PDCD1. (F) The differences in immune inflammation gene expression between two groups. *, P<0.5; **, P<0.01; ***, P<0.001. CCDC, coiled-coil domain-containing; NS, not significant; TMB, tumor mutation burden; MDSC, myeloid-derived suppressor cell; TPM, transcripts per million; FGA, fraction of genome altered; TCGA, The Cancer Genome Atlas; GSVA, Gene Set Variation Analysis; CIBERSORT, Cell-type Identification by Estimating Relative Subsets of RNA Transcripts; TIDE, Tumor Immune Dysfunction and Exclusion.

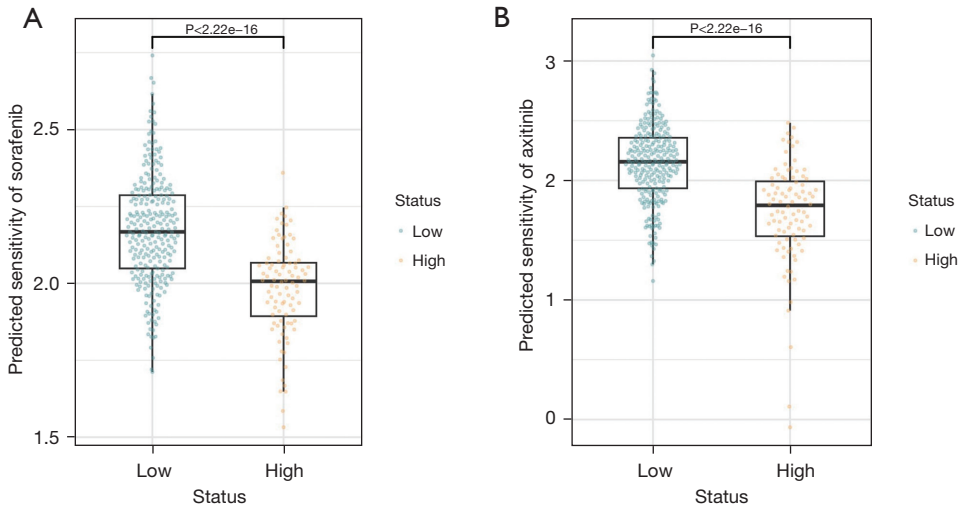

Figure 6 The sensitivities of patients in the CCDC score high group and low group to targeted agents. (A) The sensitivities to sorafenib. (B) The sensitivities to axitinib. CCDC, coiled-coil domain-containing.

prognosis model based on these six genes, and discovered that a high CCDC score is an independent predictor of poor OS. Our findings provide new insights into the molecular mechanisms of HCC and suggest that the CCDC score could be a potential biomarker for prognosis prediction in HCC.

CCDC6 is a widely expressed protein that regulates the cell cycle and DNA damage response (27,28). *CCDC22* inhibits NF-kB signaling to impact proinflammatory responses and plays a crucial role in immune-related functions (29). *CCDC51*, which encodes gene MITOK, mediates adenosine triphosphate (ATP)-dependent potassium currents in mitochondria, contributing to the homeostatic control of cellular metabolism under stress conditions (30). Single nucleotide polymorphisms in the *CCDC132* gene have been shown to be associated with the risk of IgA nephropathy (31). *CCDC134* modulates $CD8^+$ T-cell-mediated immunity and demonstrates strong antitumor effects (32). Our study's prognosis model, based on the expression levels of these six CCDC genes, was found to be a significant independent predictor of OS.

After performing KEGG pathway analysis, our study found that DEGs between high CCDC score and low CCDC score groups were primarily enriched in signaling pathways closely related to hypoxia. Specifically, in the high CCDC score group, 14 hypoxia genes were significantly enriched in the TCGA-HCC cohort, including *EHD2* and *HIF1A*. Hypoxia, which is a common feature of solid cancers due to their defective vascularization and intense metabolic activity, has been associated with poor prognosis and resistance to chemotherapeutic agents and radiation (33). It is also a key regulator in liver cancer progression (34). *HIF1A* overexpression has been linked to a poor prognosis for patients with HCC (35), and *EHD2* is a transcriptional target of HIF1, with hypoxia inducing macropinocytosis through the HIFs/EHD2 pathway in HCC cells (36). *VEGF*, a well-characterized HIF1A-regulated gene, plays a critical role in vascularization and angiogenesis. Kinase inhibitors that target vascular endothelial growth factor (VEGF) receptors, such as sorafenib and sunitinib, have been approved for the treatment of HCC. Furthermore, the development of HCC is often preceded by chronic liver inflammation, which is accompanied by increased infiltration of immune cells (37). The resulting immunosuppressive microenvironment of HCC promotes cancer metastasis, invasion, and development by establishing symbiosis with tumor cells (38). Hypoxia and hypoxia-inducible factors (HIFs) also play a crucial role in accentuating the immunosuppressive characteristics of HCC (39). In our study, we observed comparable levels of FGA and TMB in both the high and low CCDC score groups, suggesting similar tumor immunogenicity between the two groups. However, a higher CCDC score was associated with extrinsic immune escape. Notably, effector $CD8^+$ T cells and $CD8^+$ T cells were found in lower quantities in patients with higher CCDC scores. Our CIBERSORT analysis revealed increased proportions of Treg cells, M0, and M2 macrophages in high CCDC score patients compared to

the low CCDC score group. Tregs are known to promote immunosuppression in HCC and play a critical role in hindering the development of effective anti-tumor responses in HCC (40,41). Effector $CD8^+$ T cells play a crucial role in the immune response against hepatotropic viral infections. However, liver fibrosis can hamper effector $CD8^+$ T cell immune surveillance, which may lead to the development and progression of HCC (42). Our model is also associated with T cell exclusion and higher levels of MDSCs, which are potent immune modulators with inhibitory properties. Moreover, the high CCDC score group exhibited a significantly higher abundance of immunosuppressive cytokines and molecules such as *IL-10*, *IL1R1*, *IL2RA*, *IL2RB*, and *IL4R*. These inhibitory cytokines play a role in both innate and adaptive immunity in HCC (43). *PD-1* and *PD-L1* are immune checkpoint molecules that hinder anti-tumor immunity by promoting $CD8^+$ T cell exhaustion and apoptosis (44,45). The combination of VEGF and programmed cell death ligand 1 (PD-L1) inhibitors, such as bevacizumab and atezolizumab, has thus shown promising results in HCC therapy and has been included in standard guidelines (4,46). Patients with a high CCDC score may particularly benefit from ICIs therapy.

The current study has a few notable limitations. Firstly, the CCDC score prognosis model was developed and validated retrospectively using public databases, and thus requires prospective validation. Secondly, the gene set and pathway analyses, which were performed using bioinformatics tools, may provide only a suggestive conceptual rather than a literal causal relationship between the CCDC family and hypoxia in HCC. A previous study reported that the treatment of an HCC patient who received the angiogenesis inhibitor axitinib and c-Met inhibitor cabozantinib after initial treatment with sorafenib (47). The report underscores the significance of clinical management, toxicity considerations, and the judicious use of targeted therapy. Additionally, it highlights conventional first-line agents such as sorafenib, lenvatinib, and the bevacizumab-atezolizumab combination, which have been principally focused on angiogenesis (4). The study also focuses on the groundbreaking HIMALAYA trial, heralding the advent of dual ICIs as a novel therapeutic direction. Furthermore, it delves into the current literature concerning the potential mechanisms behind sorafenib resistance and presents a comprehensive overview of the biomarkers and clinicopathological indicators that might be pivotal in predicting sorafenib response and shaping personalized therapy (48). Based on this study, we found that the CCDC score high group has a lower IC50 in both Sorafenib and Axitinib, indicating that this population is more sensitive, and suggesting that they may benefit from treatment with these two agents. Therefore, this study also provides significant reference value for the treatment of the beneficiary population. One of the notable strengths of the CCDC family genes score is its potential as a prognostic marker for liver cancer outcomes. This represents a valuable tool for predicting patient prognosis, a critical factor in clinical decision-making. Additionally, the CCDC score exhibits promise as a biomarker that can guide customized therapeutic approaches—a pivotal aspect in the era of personalized medicine, where treatments are tailored to individual patient profiles. Furthermore, it has the capacity to uncover associations with immune infiltration and the activation of the hypoxia pathway, shedding light on crucial facets of the TME. This knowledge has the potential to inform targeted therapeutic strategies. However, as with many bioinformatics-based models, there is a need for validation through extensive *in vivo* and *in vitro* experiments to confirm the clinical relevance and accuracy of the CCDC score. The absence of such validation can be considered a limitation. It is also worth noting that further validation and a deeper mechanistic understanding of the CCDC family genes score are necessary to maximize its potential.

Conclusions

We have developed and validated a prognostic gene model that consists of six CCDC family genes, allowing for the prediction of OS in patients with HCC. This distinguishing feature enables the identification of high- and low-risk patients, providing guidance for personalized therapeutic approaches in clinical settings. Additionally, a high CCDC score corresponds to hypoxia and an immunosuppressive T, offering the potential to predict immunotherapy response.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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