Potential Prognostic and Therapeutic Target of CDC7 in Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) remains difficult to treat, owing to the lack of effective predictor and therapeutic targets. CDC7 is a critical gene that regulates cell cycle and highly expressed in patients with HCC. However, it remains unclear whether the expression levels of CDC7 predict survival for patients with HCC and direct impact on the tumor immune microenvironment (TIME).

Methods: We then downloaded CDC7 expression and clinical characteristics from public databases and used R language and tools online for statistical analysis and graphical work.

Results: Multivariate analyses showed that both high CDC7 expression and low immune scores was strongly correlated with worse disease-free survival (DFS) and overall survival (OS). CDC7 expression was positively correlated with infiltrating levels of CD4+ T cells, macrophages, dendritic cells (DCs) and M2 macrophages in HCC. Interestingly, analyses showed the high CDC7 expression was also closely related to the genes which contribute to HCC metastasis.

Conclusions: Our study now, for the first time, provides an explanation of CDC7 with clinical characteristics and immune-related factors. High CDC7 expression might promote HCC metastasis and M2-polarized macrophages resulted in a poor prognosis. The predicting nomograms may help to estimate the survival of patients.

Background

With the rapid increase in the ageing population, the prevention of cancers has emerged as an urgent challenge [1]. liver cancers are third-leading cause of cancer death worldwide, and consist of primary liver cancer and secondary liver cancer, which are highly invasive [2]. HCC is the most common type of primary liver cancers in humans and the incidence of HCC is rapidly increasing worldwide [3].

Recent studies indicated that cell division cycle 7 (CDC7) was highly expressed in many cancers by Oncomine and TCGA databases [4], including HCC [5]. CDC7 is a critical gene that regulates cell cycle [6]. It is a serine/threonine kinase that activates its downstream mini-chromosome maintenance complex (MCMs) by phosphorylation [7, 8], and this step is required for initiation of DNA replication [9]. But the capacity of CDC7 to predict DFS and OS are not fully known in HCC.

Interestingly, accumulating evidence has shown that tumor microenvironment plays a vital role in HCC epigenetics, differentiation, immune escape, and infiltration metastasis [10–12]. Meanwhile, immune cells are an important component of the tumor microenvironment [13–15]. Previous studies have demonstrated that high numbers of the tumor-infiltrating lymphocytes, such as, tumor associated macrophages (TAMs), M2 anti-inflammatory macrophages, correlate with poor prognosis even though using conventional chemotherapy [16, 17]. Furthermore, immune scores which summarise the density of CD3 + and CD8 + T-cell effectors in the tumor and its invasive margin are used to reveal immune

signatures [18, 19]. And the higher the immune scores, lower is the risk of recurrence [18]. But the effects of the CDC7 expression upon TIME are also unknown in HCC.

Consequently, we sought to evaluate the expression levels of CDC7 have what effects on survival and TIME in HCC. Utilizing CDC7 expression, immune scores and other factors to build comprehensive nomograms for predicting survival of patients with HCC. To identify factors that predict the outcome, we further explored the association of the CDC7 with immune cells, and phenotype of cells in tumor microenvironment by TIMER 2.0 database.

Methods

Materials.

This study involved data being downloaded from the public domain. TCGA's clinical information was downloaded from an open-access resource (http://www.cbioportal.org/), which included unique encoded identifier of the patients, age, sex, race, Z-scores of mRNA and tumor node metastases (TNM). The information of immune scores was download from ESTIMATE (https://bioinformatics.mdanderson.org/estimate/disease.html).

Database Search and Analysis Online.

We followed the methods of Pan et al. 2019 [20]. Increased or decreased CDC7 in data sets of different cancers compared with normal tissues in the Oncomine database (https://www.oncomine.org/resource/login.html) in Fig. 1a. The expression levels of the CDC7 gene in various types of cancers in Fig. 1b was identified in the TCGA database by tumor immune estimation resource (TIMER) (https://cistrome.shinyapps.io/timer/). The threshold was determined according to the following values: P-value of 0.001, fold change of 2, and gene ranking of top 10%. Gene set enrichment analysis (GSEA) was performed using GSEA v4.1.0 to find enriched KEGG pathways.

We analyzed CDC7 expression in HCC and the correlation of CDC7 expression with the abundance of immune infiltrates, including B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, dendritic cells, M1 macrophages and M2 macrophages, via TIMER (https://cistrome.shinyapps.io/timer/). Moreover, to investigate the effects of CDC7 on HCC metastasis, further analysis focuses on whether metastasis biomarkers in HCC patients is associated with CDC7 expression.

The survival curves were shown in Fig. 1e and 1f using the Kaplan–Meier curve, which were applied to explore OS and DFS of patients with HCC in different CDC7 expression group via the GEPIA database (http://gepia.cancer-pku.cn/index.html).

Data Preprocessing.

The data were collected for the further analysis, after excluding the duplicated and incomplete cases. In total, 277 cases were accepted. Each patient corresponding to a single immune score.

Statistical Analysis.

Primary end points were OS and DFS. The OS was defined as death from any cause, and DFS was defined as the time prior to relapse of the primary tumor. We followed the methods of Wang et al. 2019 [21]. The cut-off point for immune scores was selected using X-tile 3.6.1 software (Yale University School of Medicine, New Haven, CT, USA). Based on stratification according to immunological profiles, we provided a more comprehensive and objective outcome of this research. Categorical data were analyzed using Chi-square test or Fisher's exact test. Predictive factors were evaluated using univariate and multivariate analysis.

The prognostic nomograms were constructed based on the results of multivariate analysis using R version 4.0.3 (http://www.r-project.org). Performance of nomograms was assessed using the concordance index (C-index). We further used ROC analysis and its AUC value for measuring the performance of nomograms.

The survival curves were shown in Fig.S2 using the Kaplan–Meier curve via R version 4.0.3 (http://www.r-project.org).

Results

The Analyses Online and Characteristics of Patients.

The expression levels of CDC7 in different cancers in humans were showed in Fig. 1a and 1b. GSEA results in Fig. 1c and 1d showed that cell cycle pathway was closely correlated with high CDC7 expression, while fatty acid metabolism was closely correlated with low CDC7 expression.

A total of 277 patients from initial screening were included in our analyses. The mean age of patients was 59.06 years (SD = 12.94, range 16 - 85). Of the 277 patients,139 with stage I, 70 with stage II, 67 with stage III, 1 with stage IV, 98 (35.37%) patients were CDC7 positive. Based on the immune signature score, we divided the patients into three subtypes with high, medium and low immune scores. The cut-off points of immune scores were − 786.8 and − 427.1(X-tile plots are shown in the Fig. S1). Totally, 28 (10.11%) patients were lower than or equal to − 786.8 (low immune scores subcohorts), 57 (20.58%) were between − 786.8 and − 427.1 (intermediate immune scores subcohorts), and 192 (69.31%) patients were greater than − 427.1 (high immune scores subcohorts). The median OS time was 20.81 months (range 0 -120.82 months) and the median DFS time was 13.08 months (range 0-120.82 months). A summary of differentially expressed CDC7 gene and clinical characteristics for immune scores subcohorts are shown in Table 1.

Table 1
Differentially expressed CDC7 gene and clinical characteristics for immune scores subcohorts in 277 patients with HCC.

	Immune scores					
Characteristics	**Total**	**≤ -786.8**	**-786.8 to -427.1**	**> -427.1**	**χ2 value**	**P**
Sample sizes	277	28	57	192	-	-
Age (y)					**3.971**	**0.868**
≤ 40	24	2	6	16		
40–50	34	3	7	24		
50–60	85	12	16	57		
60–70	85	8	15	62		
> 70	49	3	13	33		
Sex					**1.311**	**0.519**
Male	183	17	35	131		
Female	94	11	22	61		
Race					**0.718**	**0.698**
White	134	12	26	96		
Asian	143	16	31	96		
Stage					**7.049**	**0.133**
Stage I	139	10	26	103		
Stage II	70	8	12	50		
Stage III - IV	68	10	19	39		
CDC7					**2.972**	**0.226**
Negative	179	14	37	128		
Positive	98	14	20	64		

Univariate and Multivariate Analyses for DFS and OS.

As shown in Fig. 2 to Fig. 5, there were statistically significant differences in univariate analysis/multivariate analysis for DFS and OS among patients with CDC7 positive (hazard ratios [HR]:1.6, 95% confidence interval [CI]: 1.1–2.2, P = 0.013; HR:2.1, CI:1.4–3.3, P < 0.001; HR:1.62, CI:1.12–2.36, P = 0.011; HR: 2.18, CI:1.40–3.39, P < 0.001. Meantime, the analyses in Fig. 2 to Fig. 5 indicating the

lower immune scores are, the worse the prognosis is for the patient with HCC. The results in Fig. S2 also confirmed this tendency.

High CDC7 Expression Impacts the Prognosis of Patients with HCC.

Kaplan–Meier survival curves in Fig. 1e and 1f demonstrated that high expression level of CDC7 in patients with HCC was significantly correlated with shorter DFS and OS. This was consistent with previously published results [22].

The prognostic nomogram that integrated all considered independent factors for DFS and OS were shown in Fig. 6. The C-index for DFS and OS predictions were 0.662 (95%CI, 0.688-0.636) and 0.692(95% CI,0.66-0.724), respectively. Figure 7 showed that ROC for nomograms for DFS (AUC of 3-year = 0.686, AUC of 5-year = 0.67) and nomograms for OS (AUC of 3-year = 0.76, AUC of 5-year = 0.728).

Correlation Analysis between CDC7 Expression and Immune Cells.

The scatterplots in Fig. 8 revealed the high CDC7 expression was significantly correlated with CD4 + T cells, macrophages and DCs in HCC. Macrophages are divided into M1/pro-inflammatory macrophages and M2/anti-inflammatory macrophages, which possess different cytokine profiles. M2 macrophages can promote HCC cells invasion and migration via miR-149-5p/MMP9 signaling in previous research [23]. And tumor-associated macrophages (TAMs) are also important components of the tumor microenvironment, which have been shown to make malignant tumor cells acquired cell migration and invasion capabilities [24, 25]. For each type of above cells in Fig. 9 we determined the number of three types biomarkers according to previous studies [26]. The partial correlation coefficient between CDC7 expression and biomarkers of TAMs was 0.227 ($p < 0.01$), 0.269 ($p < 0.01$) and 0.333 ($p < 0.01$), respectively. The partial correlation coefficient between CDC7 expression and biomarkers of M1 macrophages was 0.382 ($p < 0.01$), 0.02 ($p > 0.05$) and 0.201($p < 0.01$), respectively. The partial correlation coefficient between CDC7 expression and biomarkers of M2 macrophages was 0.383 ($p < 0.01$), 0.539 ($p < 0.01$) and 0.459 ($p < 0.01$), respectively.

Correlation Analysis between CDC7 Expression and HCC Metastasis.

Some biomarkers, such as NUF2, ENO1 and FOXM1, have been verified to be associated with HCC cell invasion and metastatic ability [27–32]. The partial correlation coefficient between CDC7 expression and above biomarkers was 0.777 ($p < 0.01$), 0.438 ($p < 0.01$) and 0.796 ($p < 0.01$) in Fig. 10, respectively.

Discussion

CDC7 is a protein coding gene. This gene encodes a cell division cycle protein with kinase activity that is critical for the G1/S transition. GSEA indicated that cell cycle enrichment occurred when CDC7 was highly expressed, while fatty acid metabolism was enriched when CDC7 was lowly expressed in the TCGA datasets. A study used XL413, a potent inhibitor of the DNA-replication kinase CDC7, and sertraline to kill liver hepatocellular carcinoma cells [5]. Another study shows that PHA-767491 as a dual inhibitor of

CDC7 and Cdk9 has synergistic antitumor effect with 5-FU to suppress human HCC cells [33]. Therefore, inhibition of CDC7 expression has been validated as a target for anticancer therapy.

Like other genetically cancers, HCC is the results of combinational effect of multiple genetic and environmental factors. In addition to genetic factors, clinical factors, such as age, sex, race and TNM stage, were reported to be associated with patients' survival [34–36]. Therefore, we took, for the first time, CDC7 expression, clinical characteristics and immune scores together in our study. As a result, for univariate and multivariate analyses in Fig. 2 to 5, there was an inverse correlation between both high CDC7 expression and low immune scores, respectively, and DFS. The results in OS followed the same trend. ROC in Fig. 7 for nomograms performed a satisfactory level of area under curve values. Moreover, trends in the survival curves also indicated worse outcomes for those patients with high CDC7 expression. HCC microenvironment with low immune scores means fewer effector immune cells to suppress or restrict tumor growth. Furthermore, the mechanisms for a high level expression of CDC7 leads to short survival are still largely unknown. The reason for this phenomenon may be due to changes in the tumor microenvironment that suppress anti-immunity with high CDC7 expression.

To test our hypothesis, we next to analyze the correlation between the expression levels of CDC7 and immune cells. The results in Fig. 8 revealed high CDC7 expression was positively correlated with CD4 + T cells, macrophages and DCs in HCC. In a previous study by Chen et al confirms that inhibition of CDC7 expression by XL413 can suppress activation of T cell [37]. Moreover, M2 macrophage was strongly correlated with the level of CDC7 expression and makers' partial correlation coefficient were 0.383, 0.539 and 0.459 ($n = 371$, $p < 0.001$). Results in Fig. 9 indicated that M2 macrophages were increased with high expression of CDC7. Such information in the present study may provide new ideas into the inhibition of CDC7 for HCC treatment.

Meanwhile, the shorter DFS and OS curves may be associated with patients who has unfavorable-phenotype of HCC cells. The high expression of NUF2, ENO1, or FOXM1 is considered a key step promoting liver tumor invasion and metastasis [27–32]. Importantly, high expression level of the CDC7 in analyses had been shown to correlate with the above-mentioned genes in Fig. 10. The close correlation in their expression suggests that mechanisms that expression of CDC7 may impact expression of the other. Thus, our study provides insights in understanding the potential role of CDC7 in tumor immunology and the metastatic mechanisms of HCC by bioinformatic analysis.

Conclusions

The significance of our study is multifaceted, CDC7 is a promising prognostic indicator, provides insight into hepatic carcinogenesis and may facilitate the development for cancer diagnosis and targeted therapy. High expression of CDC7 is correlated with TIME and HCC metastasis, which implies that CDC7 might promote HCC cell invasion and metastatic ability by inducing macrophage differentiation and remodeling the tumor microenvironment. Cellular and molecular studies are required to elucidate the role of this gene in future works. Furthermore, we developed a nomogram that is clinically simple to use,

integrating data on CDC7 expression, clinical factors and immune signature to improve the management of the patients.

Declarations

Supplementary Information

The detailed results are discussed in the supplementary information with Fig.s S1 to S2.

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We acknowledge TCGA and Oncomine database for providing their platforms and contributors for uploading their meaningful datasets.

Author’ contributions

R.L. and L.G. conceived the study, R.L., L.G., Z.Z., X.L., L.Z., Y.X. and H.Y. analyzed the data, R.L. and L.G. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Publicly available datasets were analyzed in this study. This data can be found here: Liver Hepatocellular Carcinoma (TCGA, PanCancer Atlas) in cBioPortal, http://www.cbioportal.org/.

Ethics approval and consent to participate

Ethical review and approval were waived for this study, due to this research is based on public database data.

Ethics Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

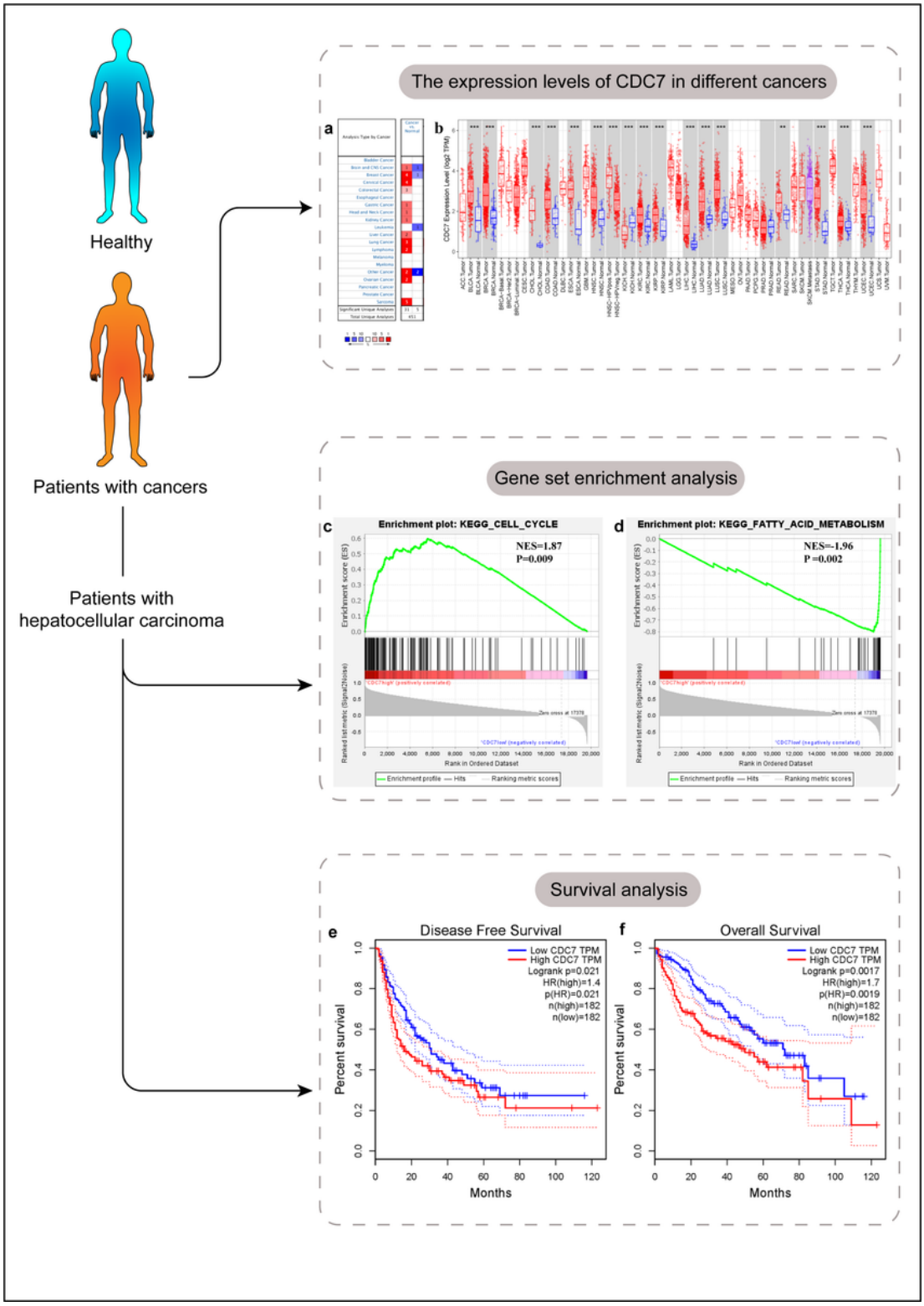

Figure 1

The analyses for differentially expressed CDC7 online. (a) Increased or decreased CDC7 in data sets of different cancers compared with normal tissues in the Oncomine database. (b) CDC7 expression levels in different tumor types from TCGA database were determined by TIMER (*P < 0.05, **P < 0.01, ***P <

0.001). (c) GSEA indicated that cell cycle enrichment occurred when CDC7 was highly expressed. (d) Fatty acid metabolism was enriched when CDC7 is lowly expressed. Kaplan-Meier curves online depicting associations of different levels of CDC7 expression with (e) DFS and (f) OS for patients with HCC.

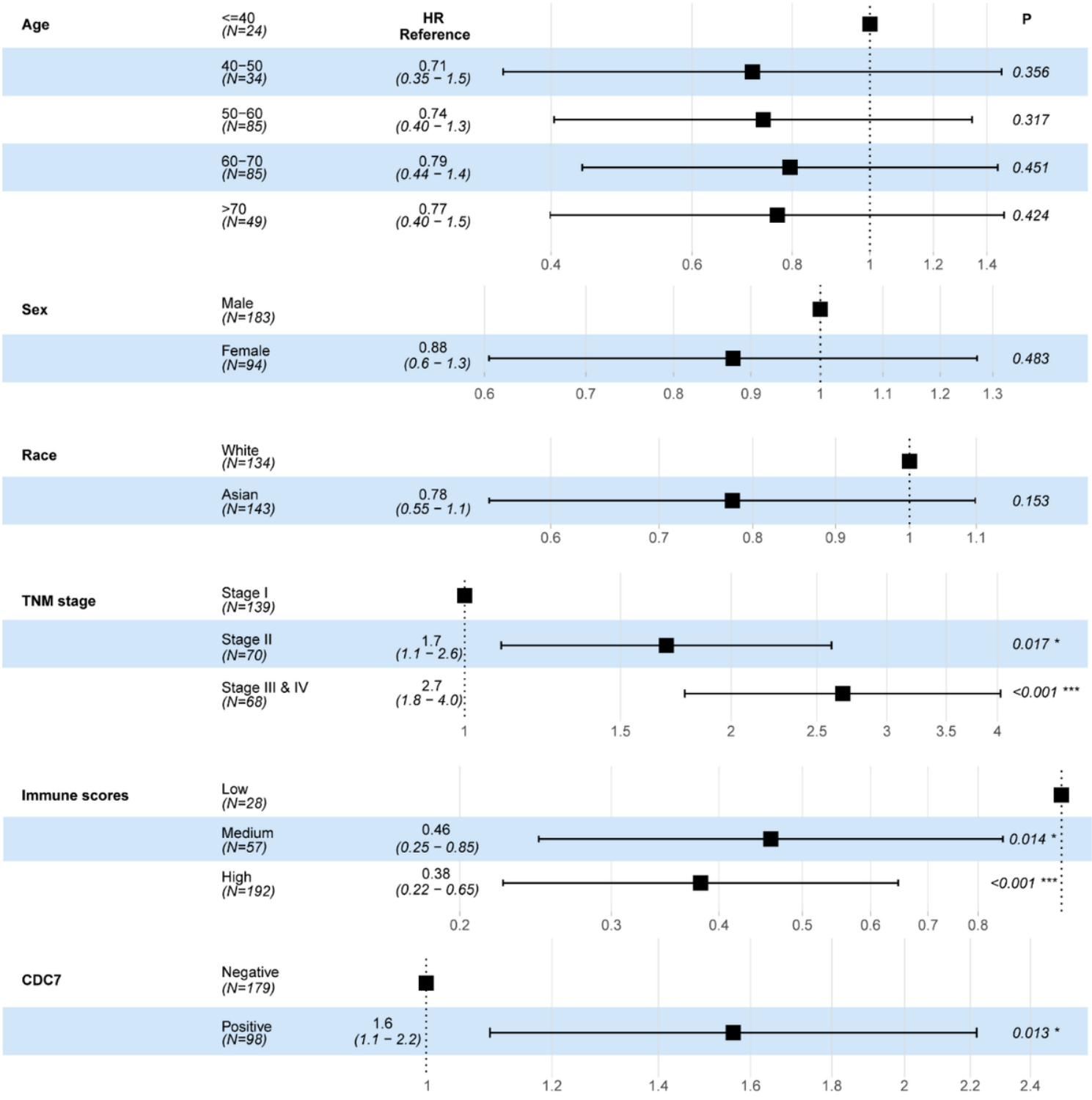

Figure 2

Univariate analyses of DFS among patients with HCC according to clinic pathological characteristics, immune scores and the expression levels of CDC7.

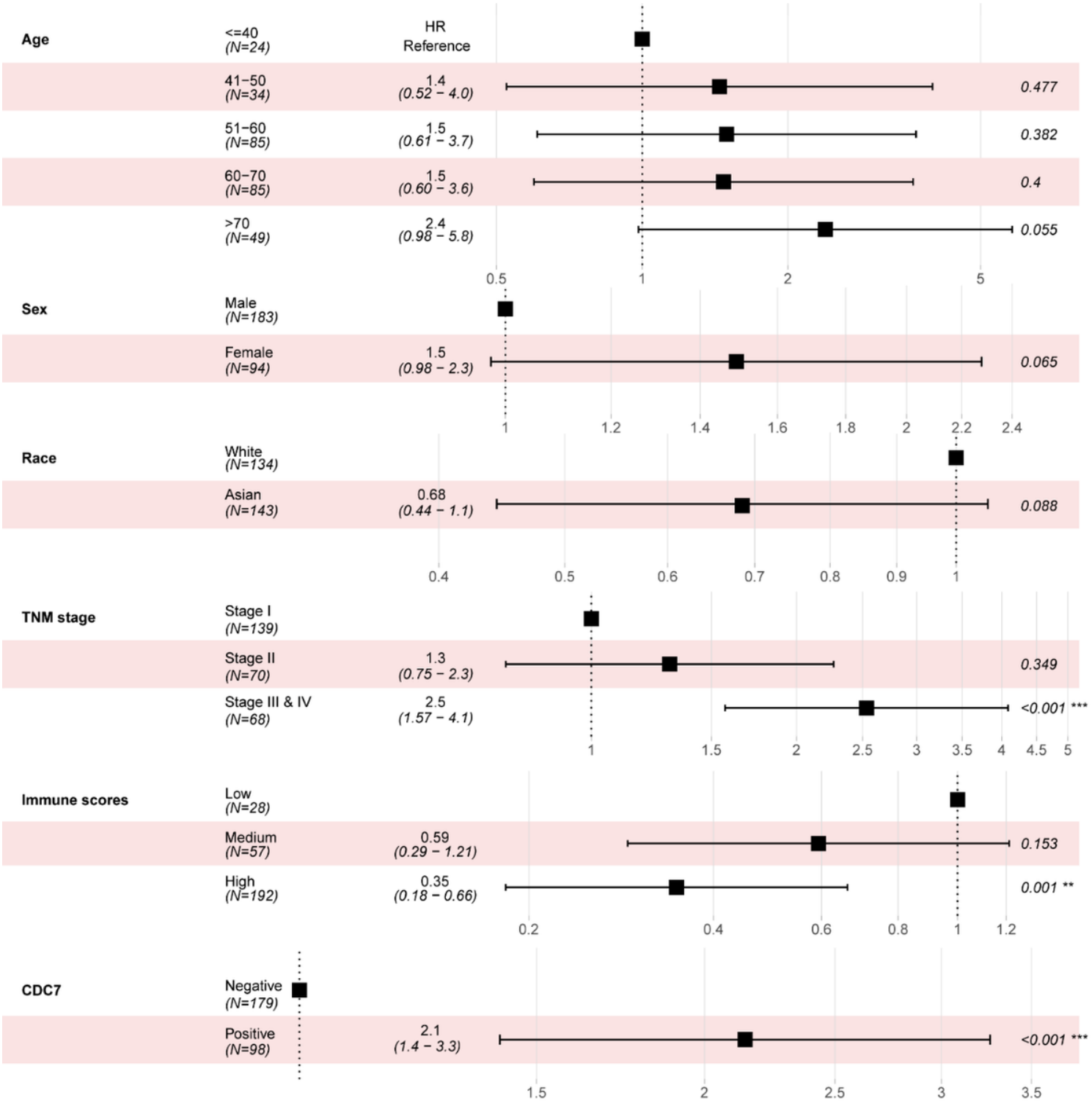

Figure 3

Univariate analyses of OS among patients with HCC according to clinic pathological characteristics, immune scores and the expression levels of CDC7.

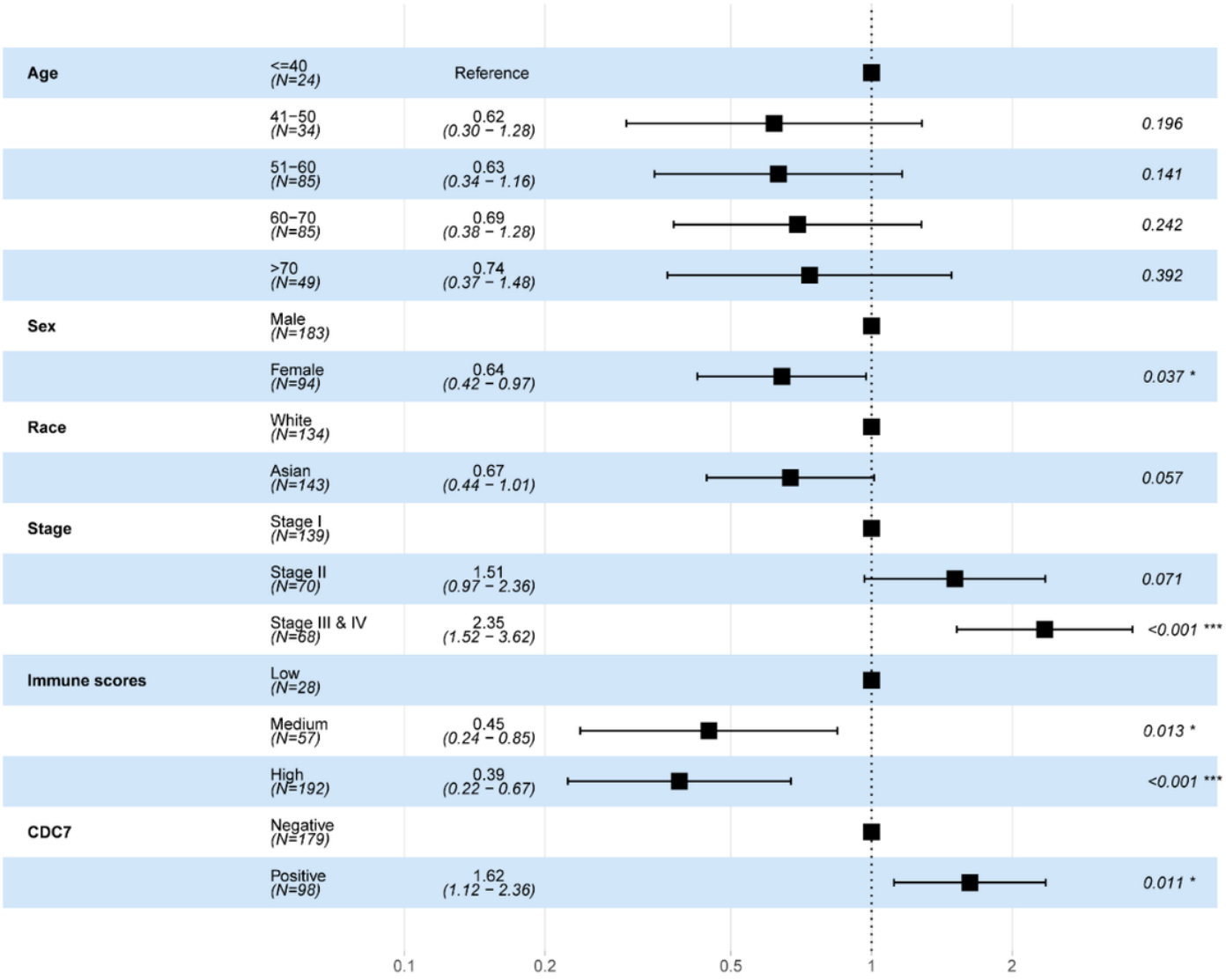

Figure 4

Multivariate analyses of DFS among patients with HCC according to clinical characteristics, immune scores and the expression levels of CDC7.

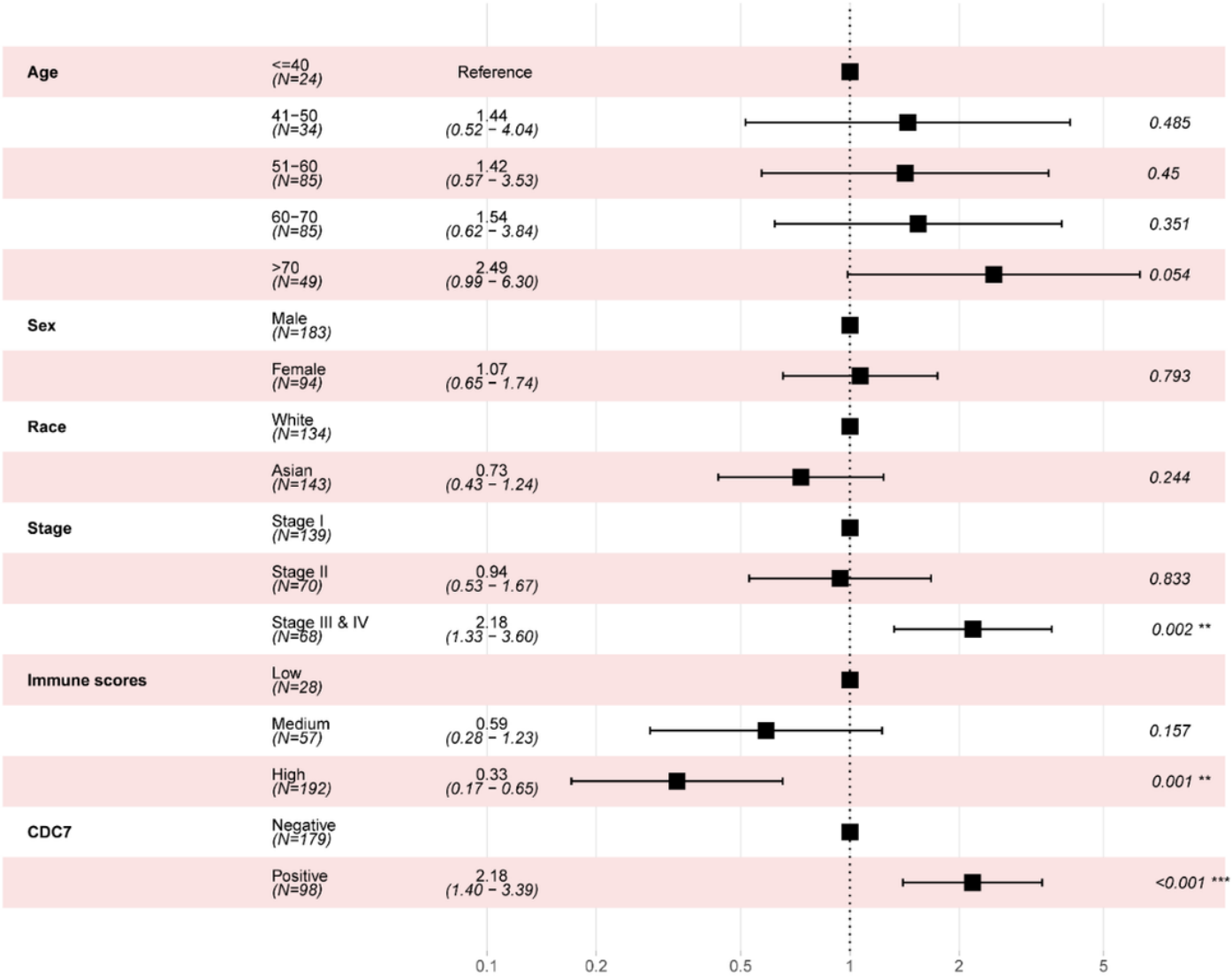

Figure 5

Multivariate analyses of OS among patients with HCC according to clinical characteristics, immune scores and the expression levels of CDC7.

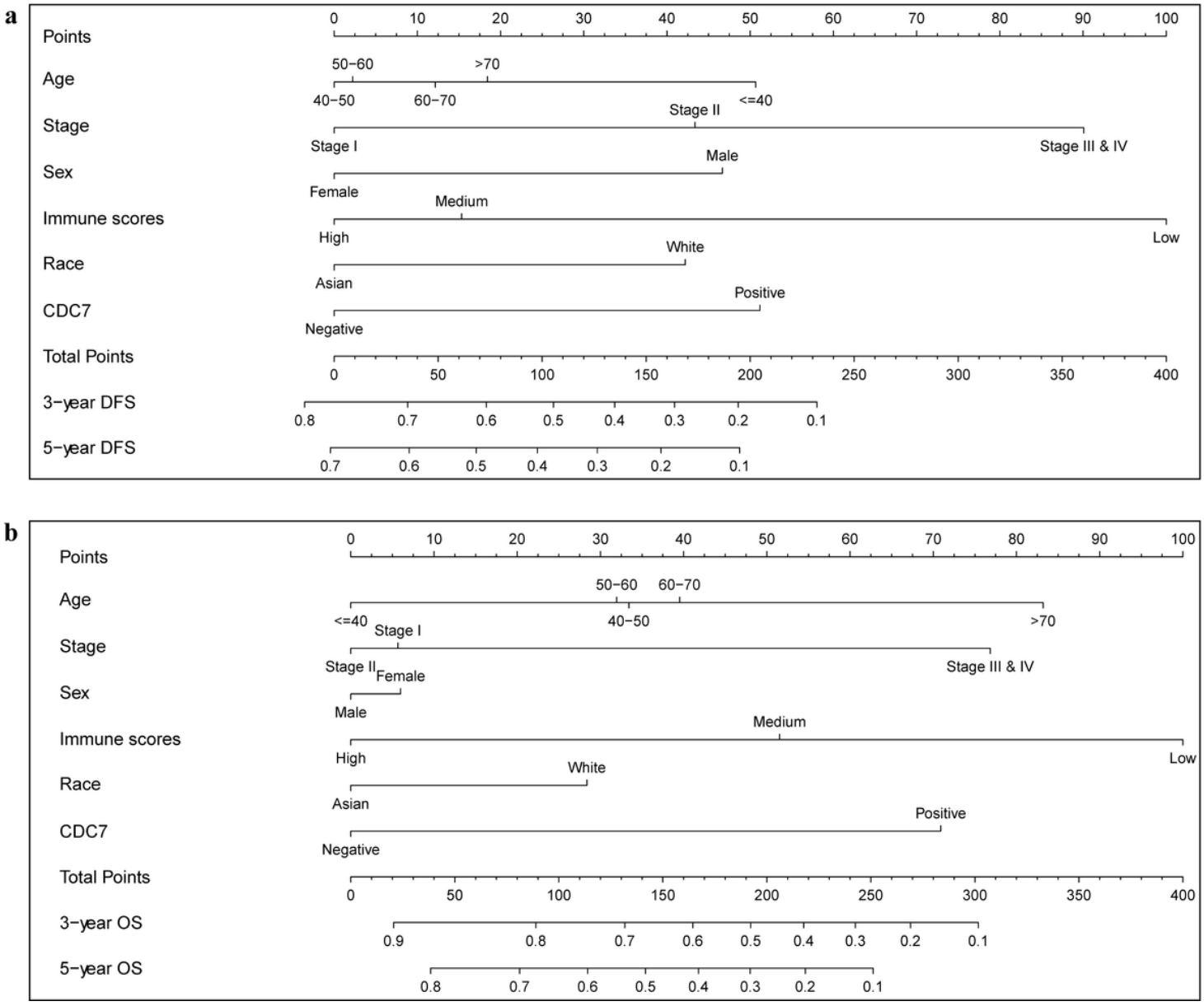

Figure 6

HCC survival nomograms. (a) The nomograms for estimate 3-year and 5-year DFS and (b) the nomograms for estimate 3-year and 5-year OS.

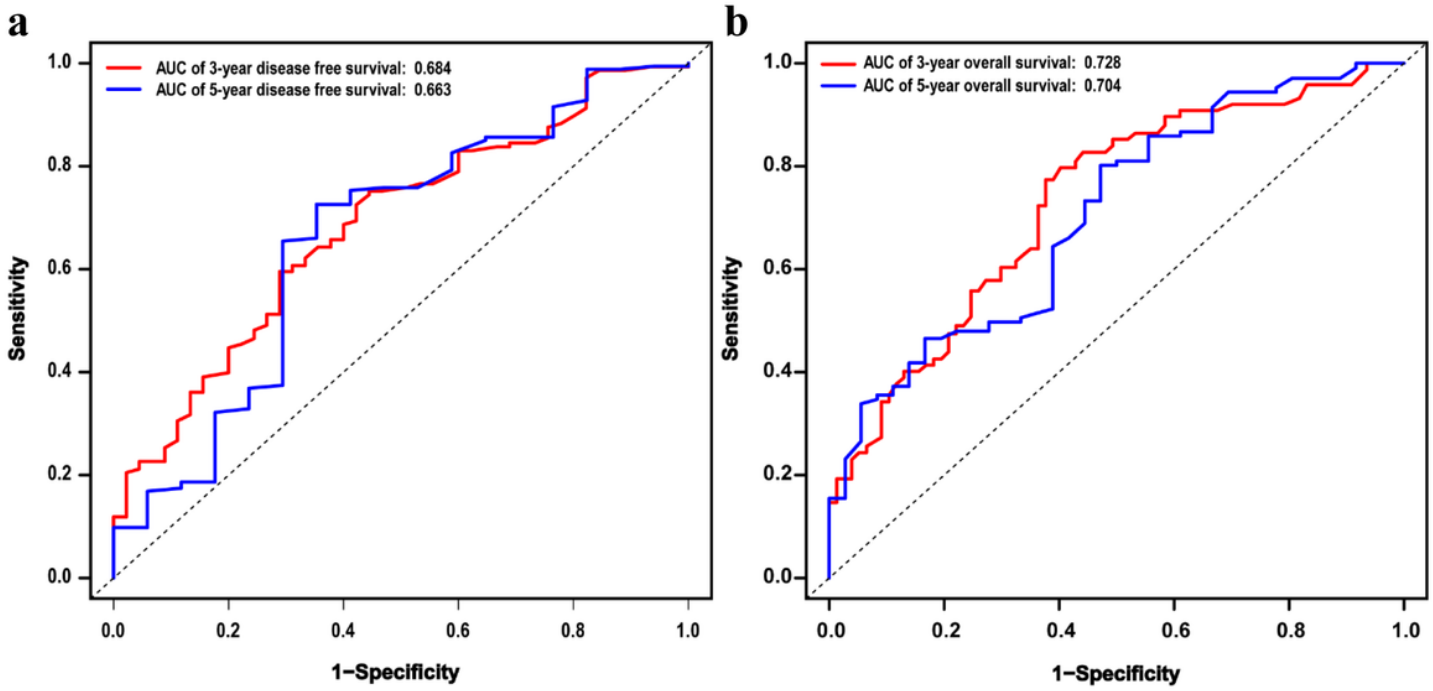

Figure 7

ROC curve analysis for nomograms. (a) The nomograms for estimate 3-year and 5-year DFS and (b) the nomograms for estimate 3-year and 5-year OS.

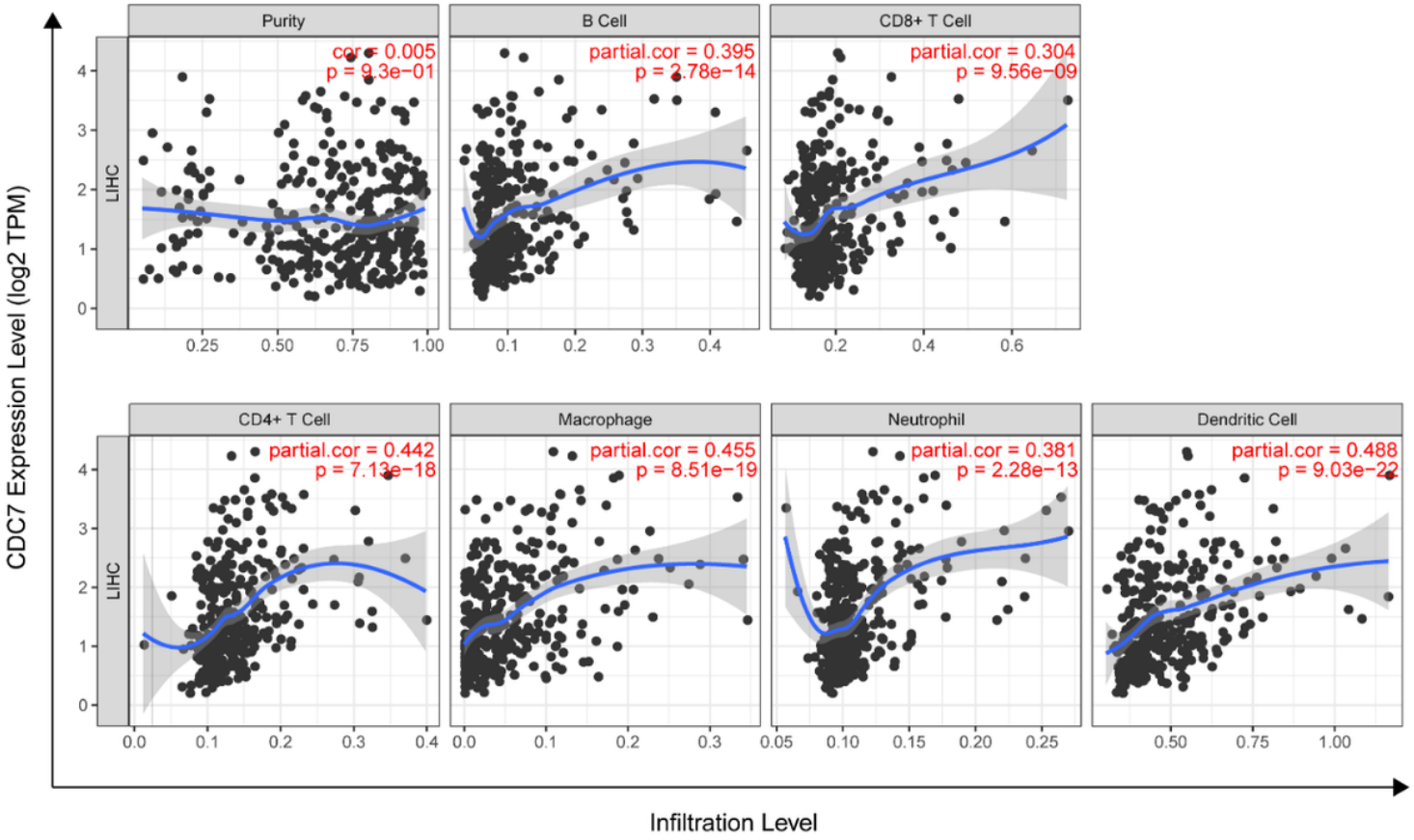

Figure 8

Correlation of CDC7 expression with immune infiltration level in HCC (n = 371).

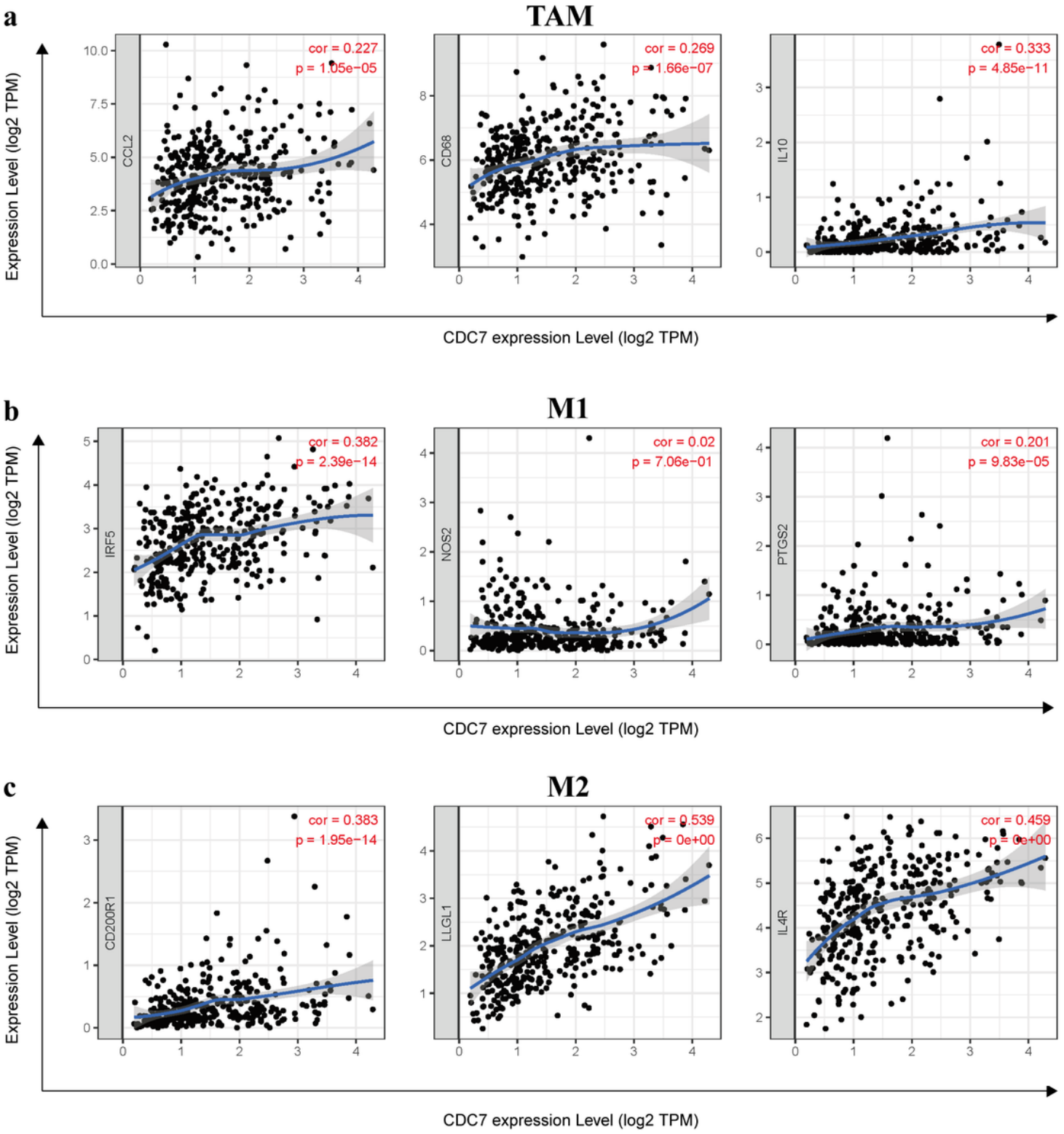

Figure 9

CDC7 expression correlated with macrophage polarization in HCC. (a) Markers include CCL2, CD68, and IL10 of TAMs (tumor-associated macrophages), (b) IRF5, NOS2 and PTGS2 of M1 macrophages, and (c) CD200R1, LLGL1 and IL4R of M2 macrophages. Scatterplots of correlations between CDC7 expression and gene markers of TAMs, and M1 and M2 macrophages in HCC (n = 371).

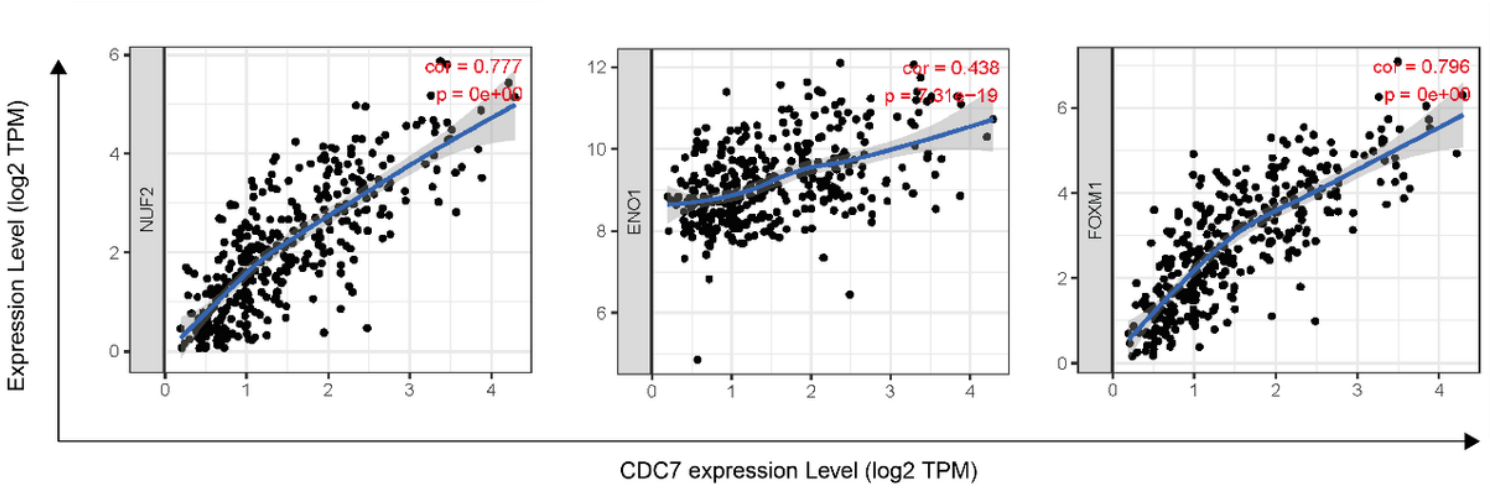

Figure 10

CDC7 expression correlated with NUF2, ENO1 and FOXM1 in HCC (n = 371).

Supplementary Files

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- SupportInformation.docx