CD97 Serves as a Novel Biomarker of Immune Cell Infiltration in Hepatocellular Carcinoma

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Abstract

Background: CD97 is the most widely expressed G protein-coupled receptor in the epidermal growth factor seven-span transmembrane family. It plays a vital role in cell adhesion, migration, and cell connection regulation. We explored the role of CD97 in hepatocellular carcinoma (HCC).

Methods: We evaluated CD97 mRNA expression in HCC using TNMplot and the Gene Expression Omnibus database. The clinical prognostic significance of CD97 in HCC was evaluated by gene expression profiling interactive analysis, the Kaplan–Meier plotter, and the UALCAN database. The Tumor Immune Estimation Resource (TIMER) and CIBERSORT databases were used to analyze the relationships among CD97, CD97 co-expression genes, and tumor-infiltrating immune cells.

Results: CD97 was highly expressed in HCC and was associated with a poor prognosis. CD97 and its co-expressed genes were positively correlated with tumor-infiltrating immune cells and strongly correlated with tumor-infiltrating macrophages (all $r \geq 0.513$, $P < 0.0001$). CD97 was closely related to M2 macrophage and tumor-associated macrophage markers (both $r \geq 0.464$, $P < 0.0001$). CD97 was found to be an immune-related gene in HCC and positively correlated with the TOX, PD-L1, PD-L2, CTLA4, and PD-1 immune checkpoint genes. CD97 copy number alterations affect the level of immune cell infiltration and mRNA expression.

Conclusions: CD97 can be used as a potential molecular marker of prognosis in HCC, which is associated with immune cell infiltration.

Background

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. The incidence and mortality rate of HCC rank sixth and third, respectively[1], posing a severe threat to human health. HCC is increasing in incidence in China and is the primary causative factor in chronic hepatitis B virus infection[2]. At present, hepatitis B accounts for the largest HCC burden globally[3]. Recently, with in-depth tumor research and rapid developments in science and technology, the early detection rate and treatment efficacy of HCC have improved, but HCC patients still have an extremely poor prognosis[4]. Gene mutations and abnormal gene expression are key factors in the occurrence and progression of HCC[5]. However, the molecular mechanism of hepatocellular carcinogenesis remains unclear.

Adhesion G protein-coupled receptors (GPCRs) are seven-span transmembrane receptors widely expressed in various cells that play vital roles in cell adhesion and signal transduction[6]. The epidermal growth factor seven-span transmembrane family comprises adherent GPCRs with multiple functions, constituting the largest family of cell surface proteins[7]. CD97/ADGRE5 belongs to class B GPCRs, consisting of an extracellular α -subunit and a transmembrane β -subunit[8]. CD97 is the most widely expressed member of the epidermal growth factor seven-span transmembrane family and is found on the surface of lymphocytes, monocytes, macrophages, dendritic cells, granulocytes, and smooth muscle[9]. It plays a role in cell adhesion, migration, and regulation of intercellular junctions[9]. Previous studies have

investigated the relationship between CD97 expression and tumors. Ward *et al.* found that CD97 and lysophosphatidic acid receptor 1 heterodimerize and function synergistically, mediate Gα12/13 to increase RHO-GTP levels in thyroid and prostate cancers, and promote tumor invasion[10, 11]. Aust *et al.* found high expression of CD97 in gastric, pancreatic, and esophageal carcinomas[12]. Staining of CD97 was stronger in aggressive tumor cells in > 50% of gastric cancer (GC) patients, and a similar expression pattern was also observed in colorectal cancer. The high expression of CD97 was associated with a worse clinical stage and lymphatic invasion[13]. In a study on rectal adenocarcinoma, the expression of CD97 was significantly higher in patients with recurrence or metastasis than in patients without recurrence or metastasis[14]. Other studies found that CD97 increased the migration ability of tumor cells in glioblastoma, but with no effect on proliferation, and the survival of patients with CD97 overexpression was significantly shorter than that in patients with lower expression[15]. Liu *et al.* found that CD97 was related to the invasion depth and TNM stage of human GC, and that CD97 promoted GC cell proliferation and invasion via the MAPK signaling pathway, mediated by exosomes[16, 17]. Yin *et al.* found that the synergy between CD97 and GPCR kinase 6 (GRK6) promoted epithelial–mesenchymal transition by regulating the expression of downstream MMP2/MMP9, leading to metastasis of HCC[18]. The function of CD97 in tumors remains unknown. Although many studies have shown that CD97 plays a key role in tumor invasion, metastasis, and angiogenesis, the specific regulatory mechanisms remain unclear. With this background, it is especially appealing to explore the role of CD97 in the pathogenesis and development of tumors.

Here, we found that CD97 was correlated with immune cell infiltration and can be used as a prognostic marker in HCC. First, using multiple databases, we revealed that CD97 and genes co-expressed with CD97 were abnormally expressed and related to prognosis in HCC. At the same time, we analyzed the signaling pathways that CD97 is involved in using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. In addition, we analyzed the relationships among CD97, tumor-infiltrating immune cells (TIICs), and immune-related genes using multiple databases. This study aims to provide a new perspective on the underlying mechanisms of hepatocellular carcinogenesis and to help identify new potential targets for diagnosing and treating HCC.

Materials And Methods

CD97 gene expression analysis

We used the pan-cancer analysis in the TNMplot database to evaluate the mRNA levels of CD97 in various tumors. The TNMplot database[34] is a collection of 56,938 high-quality tissue samples, including RNA-seq data from the Genotype-Tissue Expression (GTEx), The Cancer Genome Atlas (TCGA), and the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) databases, as well as GeneChip data from the Gene Expression Omnibus (GEO) database. Next, we selected two datasets (GSE14323[35] and GSE64041[36]) containing HCC and normal hepatic tissue gene expression data from the GEO database[37]. Differences in CD97 mRNA expression between HCC and normal hepatic tissues were analyzed, and plots were created using GraphPad Prism 8 software. We also used

the Human Protein Atlas (HPA) database to analyze differences in CD97 protein expression between HCC and normal hepatic tissues.

Correlation analysis between CD97 and clinicopathological features

We downloaded the clinicopathological data of primary HCC patients from the UCSC Xena browser[38]. The HCC cohort in TCGA contains 115 clinical parameters. We selected age, sex, tumor grade, invasion depth, lymph node metastasis, distant metastasis, TNM stage, and survival status as the clinicopathological parameters. After excluding the HCC patients with missing clinicopathological data, we divided the remaining 365 patients into two groups according to the median expression level of CD97: high and low CD97 expression groups. The chi-square test was used to analyze the correlations between CD97 expression and clinicopathological parameters.

Prognostic analysis of CD97 expression in HCC

The Kaplan–Meier plotter (KM plotter) online database[39] was used to evaluate the prognostic value of specific genes in HCC patients (n = 364). The Gene Expression Profiling Interactive Analysis (GEPIA) database[40] contains TCGA and GTEx data, which are widely used to determine the prognostic value of a gene. UALCAN is a web analysis tool[41] of TCGA that is also used to analyze the prognostic impact of individual genes. We used the KM plotter, GEPIA, and UALCAN databases to analyze the relationship between CD97 expression and prognosis in HCC. Furthermore, we used a KM plotter to analyze the clinicopathological features of patients with HCC, and these were mapped using the R package “forest plot”.

Significant prognostic marker analysis

We downloaded prognostic data of patients with primary HCC from the UCSC Xena browser. We used SPSS software to determine the independent prognostic factors by univariate and multivariate Cox regression analyses. The results are presented as hazard ratios (HRs), 95% confidence intervals (CIs), and P-values.

Gene set enrichment analysis

We downloaded processed data of patients with HCC from the UCSC Xena browser and divided the patients into high and low CD97 expression groups according to the median expression of CD97. Gene set enrichment analysis (GSEA) software was used to identify the enriched KEGG pathways in both groups. In the GSEA, the number of genes parameters was 1000, and the nominal P-value, false discovery rate, and normalized enrichment score were used to evaluate the enriched pathways.

Profiling of genes co-expressed with CD97

To investigate the molecular mechanism of CD97, we used the UALCAN web analysis tool to screen CD97 co-expressed genes and select CD97-associated proteins. We selected the top six genes most closely

related to CD97 for further in-depth research. The relationship between these genes was verified using the Tumor Immune Estimation Resource (TIMER) database[42]. Moreover, we used TNMplot and UALCAN web analysis tools to explore expression differences among CD97 co-expressed genes. Finally, we used the KM plotter and UALCAN to analyze the prognostic value of the CD97 co-expressed genes.

Correlation analysis between CD97 or CD97 co-expressed gene expression and immune infiltrating cells

We used the TIMER database to analyze TIICs, including CD8+ T cells, CD4+ T cells, B cells, macrophages, neutrophils, and dendritic cells. We also explored the relationships of the expression of CD97 and its co-expressed genes (IQGAP1, GMIP, MOBKL2A, ARPC2, CD68, and FAM102B) with TIICs. Furthermore, we used CIBERSORT software[43] to identify the LM22 gene in the HCC samples (including 22 immune cell types) to explore the proportion of immune cells in the HCC high and low CD97 expression groups further. Next, we used “vioplot” in the R package to create the Fig.s. The TIMER database was used to analyze the relationships between CD97 and gene markers of M1 macrophages, M2 macrophages, and tumor-associated macrophages (TAMs) in HCC.

Correlation analysis between CD97 and immune parameters

We evaluated changes in the tumor microenvironment (TME) in HCC based on the CIBERSORT analysis results and used “ggplot2”, “heatmap”, and “corrplot” in R to generate the corresponding images. Next, we used the TIMER database to analyze the relationship between the expression of CD97 and that of five immune checkpoints (TOX, PD-L1, PD-L2, CTLA4, and PD-1). We also analyzed the relationship between CD97 copy number changes and the level of immune cell infiltration in HCC. Finally, we used the cBioportal database[44] to analyze the relationship between CD97 copy number alternations and mRNA level changes.

Data analysis

We used RStudio (version 3.5.2) and SPSS (version 25) for the statistical analyses, and the values are presented as means $\pm$ standard deviation. We used independent and paired sample *t*-tests to analyze differences in CD97 mRNA expression between HCC and normal hepatic tissues based on the TNMplot and GEO databases. The Pearson chi-squared test was used to analyze the association between CD97 expression and characteristic clinical variables. We then used the KM plotter and UALCAN database to generate survival curves. Univariate and multivariate analyses were performed using Cox proportional hazards regression. Statistically significant differences were defined by $P < 0.05$.

Results

Elevated expression of CD97 in HCC

We evaluated CD97 mRNA levels in different tumors by analyzing RNA-seq and GeneChip data in the TNMplot database (Fig. 1a). We showed that CD97 mRNA expression was significantly higher in HCC tissue than in normal hepatic tissue (Fig. 1b). Comparison of the corresponding normal tissues revealed

high CD97 expression in colon adenocarcinoma, esophageal carcinoma, ovarian cancer, pancreatic adenocarcinoma, rectum adenocarcinoma, kidney renal papillary cell carcinoma, skin cancer, GC, and thyroid cancer but low expression in acute myelogenous leukemia, breast cancer, lung adenocarcinoma, lung squamous cell carcinoma, kidney chromophobe, uterine corpus endometrial carcinoma, and uterine carcinosarcoma. These results indicate that CD97 is abnormally expressed in various tumor tissues. Next, we evaluated CD97 mRNA expression in HCC and normal hepatic tissues using the GEO database and found higher expression in HCC tissue than in normal hepatic tissue (Fig. 1c-1d). Moreover, immunohistochemical data from the HPA database showed a significantly higher CD97 protein level in HCC than normal hepatic tissues (Fig. 2). These results suggest higher CD97 expression in HCC than normal hepatic tissues.

Relationship between CD97 expression and clinicopathological features in HCC patients

We downloaded HCC data from TCGA via the UCSC Xena tool to assess the relationship between CD97 mRNA expression and clinicopathological features of HCC patients, including age, sex, tumor grade, invasion depth, lymph node metastasis, distant metastasis, TNM stage, and survival status. Based on the median CD97 mRNA level, we assigned 183 HCC patients to the CD97 high expression group and 182 patients to the CD97 low expression group. As shown in Table 1, there were no significant differences in clinicopathological characteristics, including survival status ($P = 0.052$), invasion depth ($P = 0.099$), and TNM stage ($P = 0.099$), between the two groups.

Table 1
Relationship between CD97 expression level and clinicopathological variables in HCC patients.

Classification	Total	CD97 expression		χ^2	<i>P</i>
		High	Low		
Age				1.732	0.188
< 60	169	91	78		
≥ 60	196	92	104		
Sex				1.902	0.168
Male	247	130	117		
Female	118	53	65		
Grade				1.172	0.760
G1	55	25	30		
G2	177	87	90		
G3	121	65	56		
G4	12	6	6		
Invasion depth				6.269	0.099
T1	181	79	102		
T2	91	52	39		
T3	80	44	36		
T4	13	8	5		
Lymph node metastasis				0.000	1
N0-Nx	361	181	180		
N1	4	2	2		
Distant metastasis				0.247	0.619
M0-Mx	361	180	181		
M1	4	3	1		
TNM stage				6.283	0.099
I	179	78	101		
II	89	51	38		

Classification	Total	CD97 expression		χ^2	<i>P</i>
		High	Low		
III	89	49	40		
IV	8	5	3		
Status				3.778	0.052
Alive	239	111	128		
Dead	126	72	54		
<i>P</i> < 0.05, statistically significant.					

The prognostic value of CD97 expression in HCC

First, we analyzed the association between CD97 expression and the prognosis of HCC patients. The prognosis of HCC patients with high CD97 expression was significantly worse in the GEPIA (HR = 1.6, P = 0.012; Fig. 3a), KM plotter (HR = 1.8, P = 0.00069; Fig. 3b), and UALCAN (P = 0.00052; Fig. 3c) databases compared with the patients with low expression. Kaplan–Meier survival analysis showed that male sex (HR = 2.37, P = 0.0002), Asian ethnicity (HR = 3, P = 0.0002), alcohol consumption (HR = 1.96, P = 0.036), no alcohol consumption (HR = 1.89, P = 0.0068), and no hepatitis viral infection (HR = 1.86, P = 0.0068) were associated with a poor prognosis in the high CD97 expression group (Fig. 3d). The prognosis of HCC patients with high CD97 expression was significantly worse at TNM stages III and IV, pathological grades II and III, and American Joint Committee on Cancer stage III (Fig. 3d). These results indicate that the prognosis of HCC patients with high CD97 expression is correlated with clinicopathological features, especially for those with advanced HCC. Furthermore, Cox univariate survival analysis showed that grade (P < 0.001), invasion depth (P < 0.001), distant metastasis (P = 0.026), TNM stage (P < 0.001), and CD97 expression (P = 0.048) were significantly associated with the prognosis of HCC patients (Table 2). Cox multivariate survival analysis revealed that TNM stage and CD97 expression were independent risk factors for poor prognosis in HCC (all P < 0.05; Table 3). In summary, our results suggest that CD97 expression predicts a poor prognosis and acts as an independent prognostic marker in HCC.

Table 2
Univariate analysis of the prognostic factors in HCC patients using a Cox regression model.

Parameters	Univariate analysis		
	Hazard ratio	95% CI	P value
Ages, year (≥ 60 vs. < 60)	1.195	0.839–1.702	0.323
Sex (male vs. female)	1.198	0.834–1.720	0.328
Grade (G3-G4 vs. G1-G2)	2.594	1.819–3.701	< 0.001
Invasion depth (T3/T4 vs. T1/T2)	2.539	1.767–3.647	< 0.001
Lymph node metastasis (N1 vs. N0-Nx)	1.869	0.458–7.543	0.368
Distant metastasis (M1 vs. M0-Mx)	3.681	1.166–11.615	0.026
TNM stage (III-IV vs. I-II)	2.566	1.801–3.657	< 0.001
CD97 expression (High vs. Low)	1.428	1.003–2.034	0.048
CI, confidence interval. P-value < 0.05, statistically significant.			

Table 3
Multivariate analysis of the prognostic factors in HCC patients using a Cox regression model.

Parameters	Multivariate analysis		
	Hazard ratio	95% CI	P-value
Ages, year (≥ 60 vs. < 60)	1.189	0.828–1.708	0.349
Sex (male vs. female)	1.124	0.773–1.636	0.540
Grade (G3-G4 vs. G1-G2)	1.183	0.820–1.708	0.369
Lymph node metastasis (N1 vs. N0-Nx)	1.019	0.242–4.296	0.980
Distant metastasis (M1 vs. M0-Mx)	1.709	0.517–5.655	0.380
TNM stage (III-IV vs. I-II)	2.498	1.735–3.597	< 0.001
CD97 expression (High vs. Low)	1.445	1.010–2.068	0.044
CI, confidence interval. P-value < 0.05, statistically significant.			

CD97-related signaling pathways in HCC identified by GSEA

We explored the molecular mechanism of CD97 in the development of HCC using GSEA and found 131 KEGG signaling pathways associated with the high CD97 expression group, of which 19 were significantly enriched in the high CD97 expression group (nominal $P < 0.05$, false discovery rate < 0.1 , and normalized enrichment score > 1.6 ; Table 4). These signaling pathways were closely related to immunity

and inflammation, including “cytokine receptor interaction”, “T cell receptor signaling pathway”, “B cell receptor signaling pathway”, “graft versus host disease”, “primary immunodeficiency”, “NOD-like receptor signaling pathway”, “intestinal immune network for IgA production”, “allograft”, “chemokine signaling pathway”, “toll-like receptor signaling pathway”, and “FC gamma R mediated phagocytosis”, and to cell adhesion, including “cell adhesion molecules CAMs” (Fig. 4).

Table 4
GSEA pathways upregulated due to high expression of CD97.

GS < br > follow link to MSigDB	SIZE	NES	NOM p-val	FDR q-val
KEGG_LEISHMANIA_INFECTION	69	1.90	0.000	0
KEGG_GRAFT_VERSUS_HOST_DISEASE	37	1.83	0.000	0
KEGG_PRIMARY_IMMUNODEFICIENCY	35	1.82	0.000	0
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	60	1.80	0.000	1.86E-04
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	46	1.79	0.000	1.49E-04
KEGG_TYPE_I_DIABETES_MELLITUS	41	1.78	0.000	2.50E-04
KEGG_HEMATOPOIETIC_CELL_LINEAGE	84	1.78	0.000	2.15E-04
KEGG_ALLOGRAFT_REJECTION	35	1.76	0.000	1.88E-04
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	258	1.73	0.000	4.26E-04
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	74	1.72	0.000	4.60E-04
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	106	1.71	0.000	6.26E-04
KEGG_ASTHMA	28	1.70	0.000	5.74E-04
KEGG_CELL_ADHESION_MOLECULES_CAMS	128	1.69	0.000	6.47E-04
KEGG_CHEMOKINE_SIGNALING_PATHWAY	185	1.69	0.000	6.56E-04
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	54	1.69	0.000	7.14E-04
KEGG_AUTOIMMUNE_THYROID_DISEASE	50	1.66	0.000	1.24E-03
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	100	1.64	0.000	1.70E-03
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	92	1.63	0.000	1.86E-03

GS < br > follow link to MSigDB	SIZE	NES	NOM p-val	FDR q-val
KEGG_DILATED_CARDIOMYOPATHY	90	1.61	0.000	2.77E-03
NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate. Gene sets with NOM p-value < 0.05 and FDR q-value < 0.1 are considered as significant.				

Co-expression analysis of CD97 in HCC

We used the UALCAN analysis tool to mine for genes co-expressed with CD97 to further evaluate the effect of CD97 on HCC development. We selected the top six genes co-expressed with CD97 ($r \geq 0.66$) (Fig. 5a-5f). Next, we used the TIMER database to verify the co-expression relationships between CD97 and these genes. The results showed that CD97 expression was positively related with IQGAP1 ($r = 0.679$, $P = 2.06e-51$), GMIP ($r = 0.673$, $P = 2.33e-50$), MOBKL2A ($r = 0.642$, $P = 1.45e-44$), ARPC2 ($r = 0.607$, $P = 1.2e-38$), CD68 ($r = 0.568$, $P = 4.92e-33$), and FAM102B ($r = 0.558$, $P = 8.3e-32$) (Fig. 5G-5L). UALCAN analysis showed that only CD68 expression was decreased in HCC, while the other five genes had high expression (Fig. 6a-6e). The TNMplot database (Fig. 7a-7e) mirrored these findings. We also explored the correlations of the six genes with the prognosis of HCC patients using the UALCAN database and found that HCC patients with high GMIP, MOBKL2A, ARPC2, CD68, or FAM102B expression had a significantly worse prognosis (Fig. 8a-8e). The KM plotter database showed the same results: patients with high GMIP (HR = 1.46, $P = 0.031$), MOBKL2A (HR = 1.49, $P = 0.022$), ARPC2 (HR = 2.03, $P = 0.002$), CD68 (HR = 1.44, $P = 0.046$), or FAM102B (HR = 1.57, $P = 0.011$) expression had a poor prognosis (Fig. 9a-9e). These results suggested that CD97 and CD97 co-expressed genes are associated with the development and poor prognosis of HCC.

Significant correlations of CD97 and its co-expressed genes with TIICs in HCC

We used the TIMER database to explore the relationship between CD97 expression and TIICs in the HCC microenvironment. Expression of CD97 was positively correlated with different TIICs, including B cells ($r = 0.406$, $P = 4.30e-15$), CD8 + T cells ($r = 0.482$, $P = 2.88e-21$), CD4 + T cells ($r = 0.405$, $P = 5.34e-15$), neutrophils ($r = 0.543$, $P = 8.39e-28$), and dendritic cells ($r = 0.531$, $P = 4.23e-26$) and was significantly related to macrophages ($r = 0.605$, $P = 1.91e-35$) (Fig. 10a). Further analysis of the relationships between CD97-co-expressed genes and TIICs in HCC showed that IQGAP1, GMIP, MOBKL2A, ARPC2, CD68, and FAM102B were significantly positively correlated with TIICs, especially macrophages (all $r \geq 0.281$, $P < 0.0001$). The expression of IQGAP1 was positively correlated with B cells ($r = 0.47$, $P = 2.92e-20$), CD8 + T cells ($r = 0.551$, $P = 1.36e-28$), CD4 + T cells ($r = 0.583$, $P = 1.12e-32$), macrophages ($r = 0.725$, $P = 7.59e-57$), neutrophils ($r = 0.654$, $P = 1.94e-43$), and myeloid dendritic cells ($r = 0.686$, $P = 1.27e-48$) (Fig. 10b). The expression of GMIP was positively related to B cells ($r = 0.562$, $P = 4.85e-30$), CD8 + T cells ($r = 0.508$, $P = 7.41e-24$), CD4 + T cells ($r = 0.533$, $P = 1.23e-26$), macrophages ($r = 0.646$, $P = 1.38e-41$), neutrophils ($r = 0.591$, $P = 7.31e-34$), and myeloid dendritic cells ($r = 0.617$, $P = 5.28e-37$) (Fig. 10c). The expression of MOBKL2A was positively correlated with B cells ($r = 0.445$, $P = 4.03e-18$), CD8 + T cells ($r = 0.401$, $P = 1.27e-14$), CD4 + T cells ($r = 0.547$, $P = 3.49e-28$), macrophages ($r = 0.611$, $P = 2.97e-36$), neutrophils ($r =$

0.502, $P = 2.23 \times 10^{-23}$), and myeloid dendritic cells ($r = 0.575$, $P = 2.88 \times 10^{-31}$) (Fig. 10d). The expression of ARPC2 was positively correlated with B cells ($r = 0.479$, $P = 3.55 \times 10^{-21}$), CD8 + T cells ($r = 0.445$, $P = 4.57 \times 10^{-18}$), CD4 + T cells ($r = 0.469$, $P = 3.21 \times 10^{-20}$), macrophages ($r = 0.623$, $P = 5.03 \times 10^{-38}$), neutrophils ($r = 0.504$, $P = 1.19 \times 10^{-23}$), and myeloid dendritic cells ($r = 0.625$, $P = 2.94 \times 10^{-38}$) (Fig. 10e). The expression of CD68 was positively correlated with B cells ($r = 0.389$, $P = 7.46 \times 10^{-14}$), CD8 + T cells ($r = 0.392$, $P = 4.84 \times 10^{-14}$), CD4 + T cells ($r = 0.281$, $P = 1.11 \times 10^{-7}$), macrophages ($r = 0.513$, $P = 2.54 \times 10^{-24}$), neutrophils ($r = 0.444$, $P = 3.91 \times 10^{-18}$), and myeloid dendritic cells ($r = 0.559$, $P = 2.49 \times 10^{-29}$) (Fig. 10f). The expression of FAM102B was positively correlated with B cells ($r = 0.358$, $P = 7.58 \times 10^{-12}$), CD8 + T cells ($r = 0.344$, $P = 6.31 \times 10^{-11}$), CD4 + T cells ($r = 0.545$, $P = 5.03 \times 10^{-28}$), macrophages ($r = 0.623$, $P = 4.88 \times 10^{-38}$), neutrophils ($r = 0.562$, $P = 4.56 \times 10^{-30}$), and myeloid dendritic cells ($r = 0.485$, $P = 1.87 \times 10^{-21}$) (Fig. 10g). These results suggest that CD97 and its co-expressed genes are involved in the immune response in the TME by affecting TIICs, especially macrophages.

CIBERSORT analysis showed that CD97 expression was significantly associated with TIICs, including CD8 + T cells ($P < 0.0001$), naive CD4 + T cells ($P = 0.0097$), regulatory T cells ($P = 0.0074$), resting NK cells ($P = 0.0048$), monocytes ($P = 0.0067$), M0 macrophages ($P = 0.001$), activated dendritic cells ($P = 0.0426$), and resting mast cells ($P < 0.0001$) (Fig. 11). In short, CD97 and its co-expressed genes showed close correlations with TIICs in HCC.

CD97 is associated with M2 macrophage polarization

There was a significant positive correlation between CD97 and the infiltration level of macrophages (Fig. 11a). We explored the associations between CD97 expression and expression of markers of M1 macrophages, M2 macrophages, and TAMs in HCC using the TIMER database to determine the relationships between CD97 and the different macrophage subtypes. We found that expression of the M1 macrophage gene markers NOS2 ($r = 0.054$, $P = 2.99 \times 10^{-1}$), IL1A ($r = 0.278$, $P = 5.31 \times 10^{-8}$), and ARG2 ($r = 0.001$, $P = 9.81 \times 10^{-1}$) was significantly but weakly correlated with the expression of CD97 (Fig. 12a), while that of the M2 macrophage gene markers CD163 ($r = 0.464$, $P = 3.25 \times 10^{-21}$), MS4A4A ($r = 0.536$, $P = 0 \times 10^0$), and VSIG4 ($r = 0.525$, $P = 0 \times 10^0$) and the TAM markers CD86 ($r = 0.661$, $P = 0 \times 10^0$), CCL2 ($r = 0.479$, $P = 0 \times 10^0$), and IL10 ($r = 0.508$, $P = 9.07 \times 10^{-26}$) showed stronger positive correlations with CD97 expression (Fig. 12b, 12c). These findings suggest that high CD97 expression promotes M2 macrophage polarization and differentiation into TAMs, contributing to hepatocellular carcinogenesis.

CD97 is associated with M2 macrophage polarization

We used the CIBERSORT algorithm to evaluate immune cell infiltration in the TME of HCC. We obtained data from 373 primary HCC and 50 normal hepatic samples from TCGA and found significant differences in the proportions of TIICs between HCC and normal hepatic tissues (Fig. 13a, 13b). There was an apparent discrepancy in the proportion of TIICs between the CD97 high and low expression groups in HCC (Fig. 13c). A heatmap showed that the proportions of different TIICs in HCC were strongly correlated with each other. There were significant negative correlations between M0 and M2 macrophages ($r = -0.44$), between CD8 + T cells and resting memory CD4 + T cells ($r = -0.42$), and between resting and

activated NK cells ($r = -0.42$) and a significant positive correlation between CD8 + T cells and activated memory CD4 + T cells ($r = 0.38$) (Fig. 13d). We used the TIMER database to evaluate the relationships between the proportions of diverse types of TIICs and prognosis in HCC but found no significant associations (Fig. 14a). We also analyzed the relationship between CD97 and the immune checkpoint genes TOX, PD-L1 (or CD274), PD-L2 (or PDCD1LG2), CTLA4, and PD-1 (or PDCD1), which are critical targets of immunotherapy. The results showed that CD97 expression was positively correlated with expression of the immune checkpoint genes and negatively correlated with tumor purity (Fig. 14b). In addition, we explored whether CD97 copy number alterations had a significant impact on the infiltration level of immune cells and CD97 mRNA expression (Fig. 15). Our results indicate that the copy number of CD97 may affect immune cell infiltration and thus the prognosis of HCC. Therefore, CD97 has a potential application in HCC immunotherapy.

Discussion

HCC is a common malignant tumor of the digestive system[1]. Although there have been substantial advancements in the diagnosis and treatment of HCC over recent decades, patient prognosis remains poor[4]. Abnormal gene expression may be involved in tumor progression and is closely related to patient prognosis[5]. However, the molecular mechanisms of HCC remain unknown. This study showed that CD97 is abnormally highly expressed in HCC tissues and is related to the TNM stage and patient stage at initial presentation. Furthermore, CD97 overexpression is significantly associated with the prognosis and overall survival (OS) of HCC patients, suggesting that CD97 is an oncogene that participates in HCC development. However, our knowledge of the role of CD97 molecules in HCC is limited. We aimed to comprehensively analyze the biological functions of CD97 in HCC using public databases and to reveal its related regulatory pathways.

Transcriptomic analysis of multiple databases revealed significantly higher CD97 expression in HCC tissues than in normal hepatic tissues. Analysis of the HCC data in TCGA showed that expression of CD97 was related to tumor invasion depth, TNM stage, and survival of patients with HCC. In addition, the prognosis of patients with high CD97 expression was significantly worse in multiple databases, especially patients with advanced HCC. Univariate and multivariate Cox survival analyses of HCC data in TCGA showed that CD97 was an independent prognostic risk factor in HCC. We speculate that CD97 is highly expressed in HCC and can be used as a molecular marker for diagnosis and prognosis, but more clinical cohorts are needed for verification.

We used GSEA to explore the signaling pathways of HCC samples with high CD97 expression to investigate the molecular function and potential mechanism of CD97 in HCC. We identified 19 upregulated signaling pathways predominantly related to immune and inflammatory responses, including “cytokine receptor interaction”, “T cell receptor signaling pathway”, “B cell receptor signaling pathway”, “graft versus host disease”, “primary immunodeficiency”, “NOD-like receptor signaling pathway”, “intestinal immune network for IgA production”, “allograft”, “chemokine signaling pathway”, “toll-like receptor signaling pathway”, and “FC gamma R mediated phagocytosis”. CD97 is widely expressed in

various immune cells, including lymphocytes, monocytes, all types of macrophages, and antigen-presenting cells[19]. CD97 is an important mediator of the host immune defense and plays an essential role in T cell activation[19]. The expression of CD97 is rapidly upregulated during activation of lymphocytes and myeloid cells, which may promote adhesion and migration of these cells to inflammatory sites[8]. In addition to playing a role in host immunity, CD97 is also associated with several autoimmune diseases. Kop *et al.* used a mouse model of collagen-induced arthritis to demonstrate that CD97 inhibitors reduce arthritis-related joint injury and inflammation by neutralizing antibodies[20], demonstrating that CD97 is closely related to immune cell infiltration. Moreover, the significantly upregulated “cell adhesion molecules CAMs” signaling pathway participates in cell adhesion. CD97 has been proven to play a vital role in intercellular connections. Cell adhesion based on E-cadherin is important for maintaining epithelial integrity[21]. E-cadherin is inactivated in HCC, resulting in loss of contact between HCC cells, and promotes strong motility that spreads to the surrounding and distant tissues, promoting HCC progression[21]. CD97 plays a key role in the adhesion and migration of immune cells and mediates the invasion of human tumors. Together, these results indicate that CD97 participates in tumorigenesis and immune-related KEGG signaling pathways. We showed that high CD97 expression in HCC predicted adverse outcomes, including poor prognosis. Therefore, CD97 may play a role in the development of HCC by affecting these signaling pathways.

Co-expression and TIMER analyses showed that CD97 expression was positively correlated with IQGAP1, GMIP, MOBKL2A, ARPC2, CD68, and FAM102B expression. These genes are involved in inflammation, the immune response, or tumorigenesis[22–26]. CD97 co-expressed genes were highly expressed in HCC tissues, except for CD68, and were associated with a poor prognosis in HCC patients, except for IQGAP1. TIICs are closely related to the progression and prognosis of HCC[27]. The mRNA expression levels of CD97 and its co-expressed genes were significantly and positively correlated with B cells, CD8 + T cells, CD4 + T cells, neutrophils, dendritic cells, and macrophages. At the same time, we evaluated the proportion of TIICs in HCC based on the differential expression of CD97 using CIBERSORT analysis and found significant differences in the proportions of eight types of immune cells: CD8 + T cells, naive CD4 + T cells, regulatory T cells, resting NK cells, monocytes, M0 macrophages, activated dendritic cells, and resting mast cells. The results further suggest that CD97 is strongly associated with TIICs in HCC, and that CD97 and CD97 co-expressed genes may be involved in the immune response to HCC development, leading to a poor prognosis.

We identified a strong positive correlation between CD97 mRNA expression and macrophages in HCC and speculated that CD97 expression is related to macrophage polarization. Therefore, we explored the relationship between CD97 expression and various macrophages and showed that CD97 expression in HCC was strongly associated with marker genes of M2 macrophages and TAMs, but there were only weak associations with the marker genes of M1 macrophages. These results indicate that high CD97 expression in HCC can promote the polarization of macrophages to M2 macrophages that eventually differentiate into TAMs, contributing to HCC development. The development and progression of HCC are complex and are associated with cumulative gene mutations in tumor cells but are regulated by the TME[28]. Scientific evidence shows that the TME plays a key role in tumor stimulation and progression.

TAMs are vital components of the TME in HCC[28] and are composed of M1 and M2 macrophages. M1 macrophages, known as anti-tumor macrophages, activate host defenses and kill tumor cells by producing reactive oxygen/nitrogen species and pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α . M2 macrophages, known as tumor-promoting macrophages, can induce anti-inflammatory cytokines such as IL-10, IL-13, and TGF- β to promote tumor progression[29]. Previous studies have shown that tumor-promoting factors affect the polarization of macrophages to M2 macrophages in the TME. TAMs are similar to M2 macrophages and have similar characteristics[30]. TAMs play important roles in tumor growth by enhancing tumor cell resistance to chemotherapy and radiotherapy and promoting tumor angiogenesis (including invasion, infiltration, and metastasis) and immunosuppression[30]. Therefore, we speculate that high CD97 expression promotes the differentiation of macrophages into TAMs, accelerating their progression and leading to a poor prognosis in HCC patients.

There was a significant positive correlation between the expression of CD97 and immune checkpoint genes such as TOX, PD-L1, PD-L2, CTLA4, and PD-1. Immune checkpoint inhibitors have made significant breakthroughs in cancer treatment as new tumor therapies[31]. In HCC, the influence of immune checkpoints on the prognosis of patients is extensive and complex; increased expression of PD-1, PD-L1, and tumor-infiltrating lymphocytes in HCC induces immunosuppression[32]. Furthermore, high expression of CTLA-4 in HCC patients inhibits activation of T cell antigen presentation and proliferation and induces apoptosis, leading to immunosuppression and HCC development and progression[33]. Therefore, we believe that CD97 may affect the progression of HCC and the prognosis of patients by altering the expression of immune checkpoint genes such as TOX, PD-L1, PD-L2, CTLA4, and PD-1.

This study has certain limitations. First, our research data were obtained from the TCGA, GTEx, GEO, and TARGET databases, and our results must be verified using larger sample sizes. Moreover, the results obtained by the algorithm based on RNA sequences may not be accurate. Additional *in vivo* and *in vitro* experiments are required to explore the potential physiological mechanism of CD97 in HCC, including its role in tumor–immune interactions.

Conclusion

In summary, our results suggested that CD97 has prognostic value in HCC and affects tumor immunity. Patients with high CD97 expression had poorer clinicopathological features and worse prognoses compared with patients with low expression. High CD97 expression is associated with TIICs and can promote the conversion of macrophages to TAMs. Therefore, CD97 can be used as a prognostic marker and therapeutic target in HCC.

Abbreviations

GPCRs: G protein-coupled receptors; HCC: Hepatocellular carcinoma; GEO: Gene Expression Omnibus; TCGA: The Cancer Genome Atlas; GEPIA: Gene Expression Profiling Interactive Analysis; KM: Kaplan-Meier; GSEA: Gene Set Enrichment Analysis; TIMER: Tumor Immune Estimation Resource; GC: gastric

cancer; GRK6: G protein-coupled receptor kinase 6; EMT: Epithelial-mesenchymal Transition; KEGG: Kyoto Encyclopedia of Genes and Genomes; GTEx: Genotype-Tissue Expression; TARGET: Therapeutically Applicable Research to Generate Effective Treatments; HPA: the Human Protein Atlas; HR: hazard ratio; NES: normalized enrichment score; TME: tumor microenvironment; OS: Overall survival; TIICs: tumor infiltrating immune cells; TAMs: tumor associated macrophages.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The raw data of this study are derived from the TCGA database (<https://portal.gdc.cancer.gov/>), GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) and UCSC Xena database (<http://xena.ucsc.edu>), which are publicly available databases.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

QS and JR conceived and designed the study. QS, LL and XL performed the data curation and analysis. QS, JR, WL and XZ analyzed and interpreted the results. QS, JR, YD, LH and DW drafted and reviewed the manuscript. All authors reviewed and approved the final manuscript.

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Figures

Figure 1

CD97 mRNA expression in HCC. **a** CD97 mRNA levels in different tumors analyzed in TNMplot. **b** CD97 mRNA levels in HCC tissues and normal hepatic tissues in TNMplot. CD97 mRNA levels in HCC tissues and normal hepatic tissues in the GSE14323 (**c**) and GSE64041 (**d**) datasets. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 2

CD97 protein levels in HCC in the HPA database.

Figure 3

Prognostic value of CD97 expression in HCC. **a** Correlation between CD97 expression and OS in HCC patients using the KM plotter. **b** Correlation between CD97 expression and OS in HCC patients using the GEPIA database. **c** Correlation between CD97 expression and OS in HCC patients using the UALCAN database. **d** OS analysis of HCC patients using the KM plotter database. OS, overall survival.

Figure 4

GSEA pathways enriched in HCC samples with high CD97 expression. The GSEA results showed that the terms “cytokine receptor interaction” (**a**), “T cell receptor signaling pathway” (**b**), “B cell receptor signaling pathway” (**c**), “graft versus host disease” (**d**), “primary immunodeficiency” (**e**), “NOD-like receptor signaling pathway” (**f**), “intestinal immune network for IgA production” (**g**), “allograft” (**h**), “chemokine signaling pathway” (**i**), “toll-like receptor signaling pathway” (**j**), “FC gamma R mediated phagocytosis” (**k**), and “cell adhesion molecules CAMs” (**l**) were enriched in HCC samples with high CD97 expression. NES, normalized enrichment score.

Figure 5

The top six genes co-expressed with CD97 in HCC. **a-f** the genes co-expressed with CD97 in HCC ($r \geq 0.66$) were assessed using the UALCAN database. **g-l** CD97 was positively correlated with IQGAP1 ($r = 0.679$, $P = 2.06e-51$), GMIP ($r = 0.673$, $P = 2.33e-50$), MOBKL2A ($r = 0.642$, $P = 1.45e-44$), ARPC2 ($r = 0.607$, $P = 1.2e-38$), CD68 ($r = 0.568$, $P = 4.92e-33$), and FAM102B ($r = 0.558$, $P = 8.3e-32$) in HCC in the TIMER database.

Figure 6

The mRNA expression of CD97 co-expressed genes in HCC using the UALCAN database. a-f the mRNA expression levels of IQGAP1, GMIP, MOBKL2A, ARPC2, CD68, and FAM102B in HCC in the UALCAN database.

Figure 7

The mRNA expression of CD97 co-expressed genes in HCC using the TNMplot database. a-f the mRNA expression levels of IQGAP1, GMIP, MOBKL2A, ARPC2, CD68, and FAM102B in HCC in the TNMplot database.

Figure 8

The prognostic value of CD97 co-expressed genes in HCC using the UALCAN database. a-f Correlations between OS and the mRNA levels of IQGAP1, GMIP, MOBKL2A, ARPC2, CD68, and FAM102B in HCC in the UALCAN database.

Figure 9

The prognostic value of CD97 co-expressed genes in HCC using the KM plotter database. a-f Correlations between OS and the mRNA levels of IQGAP1, GMIP, MOBKL2A, ARPC2, CD68, and FAM102B in HCC in the KM plotter database.

Figure 10

Correlations of CD97 and its co-expressed genes with immune cell filtration levels in HCC. Correlations of CD97 (a), IQGAP1 (b), GMIP (c), CDH11 (d), MOBKL2A (e), ARPC2 (f), and FAM102B (g) expression with TIICs in HCC.

Figure 11

Violin plots visualizing the distributions of TIICs according to CD97 expression using the CIBERSORT database. Blue and red represent the CD97 low and high expression groups, respectively. There were

significant differences in the numbers of CD8+ T cells ($P < 0.0001$), naive CD4 + T cells ($P = 0.0097$), regulatory T cells ($P = 0.0074$), resting NK cells ($P = 0.0048$), monocytes ($P = 0.0067$), M0 macrophages ($P = 0.001$), activated dendritic cells ($P = 0.0426$), and resting mast cells ($P < 0.0001$).

Figure 12

Correlation of CD97 expression with macrophage polarization in HCC. **a** Correlations between CD97 expression and gene markers of M1 macrophages (NOS2, IL1A, and ARG2). **b** Correlations between CD97 expression and gene markers of M2 macrophages (CD163, MS4A4A, and VSIG4). **c** Correlations between CD97 expression and gene markers of TAM (CD86, CCL2, and IL10).

Figure 13

Landscape of immune cell infiltration in HCC by CIBERSORT. **a-b** the composition of 22 types of immune cells in HCC and normal hepatic tissues. **c** Heatmap showing the differences in the infiltration levels of 22 types of immune cells between the CD97 high and low expression groups in HCC. **d** Correlation heatmap visualizing the correlations among 22 types of immune cells in HCC.

Figure 14

Correlation analysis between CD97 expression and immune parameters. The associations between immune cells and prognosis in the TIMER database (**a**). The associations between CD97 and immune checkpoint expression (**b**).

Figure 15

The levels of CD97 and immune cell infiltration were associated with CD97 copy number alternations. **a-c** the relationship between CD97 copy number and its mRNA level. **d** the relationships between CD97 copy number and immune cell infiltration levels, * $P < 0.05$.