Integrated Analysis of the Expression, Prognostic Value, and Relationship with Immune Infiltration of CMTMs in Human Hepatocellular Carcinoma

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Abstract

The chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing (CMTM) family refers to an updated gene family involved in diverse pathophysiological processes. However, the effect exerted by CMTM family members (CMTMs) in hepatocellular carcinoma (HCC) is still elusive. Herein, to elucidate the expression, prognostic value, and immune roles of CMTMs in HCC, a comprehensive bioinformatic analysis was carried out. Our findings showed that CKLF, CMTM1, CMTM3-4, and CMTM7-8 displayed higher mRNA expression levels in HCC tissues than in normal tissues, yet CMTM2, CMTM5, and CMTM6 were lower in HCC tissues. The levels of CMTM1, CMTM2, and CMTM4 were related to tumor stage. Survival analysis showed that high levels of CKLF, CMTM1, CMTM4, and CMTM6-7 were associated with shorter overall survival (OS). Conversely, a high level of CMTM5 in HCC patients was associated with improved OS. We also found that CKLF, CMTM1-4, and CMTM6-7 were closely correlated with immune infiltration in the HCC microenvironment. In conclusion, this study indicates that CMTMs exert a crucial effect on HCC microenvironment remodeling. CKLF, CMTM1, CMTM4, and CMTM6-7 are potential targets of precision therapy, and CMTM5 is a tumor-suppressive gene that was associated with improved survival in HCC patients.

Introduction

Hepatocellular carcinoma (HCC) refers to the predominant histologic type of liver cancer with a high recurrence rate and a poor prognosis. However, the molecular mechanism underlying HCC formation and progression is still highly ambiguous, which hinders the efficient treatment of this disease. Over the past years, alpha-fetoprotein (AFP), which is the major serum biomarker of HCC, has manifested relatively low specificity and sensitivity [1, 2]. Due to tumor heterogeneity and the lack of efficient molecular therapeutic targets, the efficiency of molecular targeted therapy and immunotherapy in HCC is limited. Therefore, it is urgent to explore novel biomarkers to detect early-onset HCC to improve clinical outcomes.

Chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing (CMTM) family, functioning as a novel gene family, is comprised of nine genes in total: CKLF and CMTM1 to CMTM8 [3]. The genes of the CMTM family are located on different chromosomes. CKLF and CMTM1–4 comprise a gene cluster on chromosome 16q, CMTM5 is located at chromosome 14q11.2, and CMTM6–8 form another gene cluster on chromosome 3p22.3. These proteins by linking the chemokine and transmembrane-4 superfamily (TM4SF) exert latent biological roles in tumorigenesis, development, and metastasis [4]. Some researchers found that the abnormal expression of CMTMs is related to various cancers [5, 6]. In addition, previous studies have found aberrant expressions and biological function in some members of CMTM family in HCC. For instance, CKLF was overexpressed in HCC tissues. High expression of CKLF was associated with tumor size, vascular invasion and poorer survival of patients [7]. However, there is a lack of systematic studies to clarify the role and prognostic value of CMTMs in HCC.

Herein, based upon analyses of gene expression and variations in copy numbers published online, we investigated the expression and mutations of different CMTMs in HCC patients to determine the

expression patterns, potential functions, and distinct prognostic values of CMTMs.

Materials And Methods

Oncomine

Oncomine (<https://www.oncomine.org/resource/login.html>) is an integrated online oncogene microarray database and data-mining platform [8]. We analyzed the mRNA expression levels of 9 different CMTMs in HCC tissues and adjacent normal control tissues from Oncomine. Differences were analyzed with Student's t-test. The criteria for significance were as follows: P value < 0.05; fold change 1.5; and gene rank 10%.

GEPIA

GEPIA (<http://gepia.cancer-pku.cn/>) is a recently developed interactive web server used to analyze the RNA sequencing expression data of 9,736 tumor samples along with 8,587 normal samples from The Cancer Genome Atlas (TCGA) and Genotype Tissue Expression (GTEx) databases. This method was established with a standard processing pipeline [9]. Student's t-test was used to generate the P value from the analyses of presentation and pathological stage. A P value < 0.05 and fold change > 1.5 were considered to indicate statistical significance.

Kaplan-Meier Plotter

The prognostic value of different CMTMs was estimated with Kaplan-Meier Plotter (www.kmplot.com), which comprises gene expression data and survival information from HCC patients. Information regarding the number-at-risk cases, median values of mRNA expression levels, hazard ratios (HRs) based on 95% confidence intervals (CIs) and log-rank P values was traced and obtained from the Kaplan-Meier Plotter webpage. A P value < 0.05 was considered to indicate statistical significance.

HPA

HPA (www.proteinatlas.org) functions as a user-friendly website that is composed of expression profiles on the basis of immunohistochemistry of the top 20 cancers [10]. Aiming to prevent bias resulting from a single database, HPA was employed to identify tumor-specific protein expression patterns of CMTMs. Immunohistochemistry images of CMTMs in HCC and normal tissues were directly visualized.

cBio Cancer Genomics Portal (cBioPortal)

cBioPortal (<https://www.cbioportal.org/>) is an integrated web resource that can be used to visualize and analyze multidimensional cancer genomic data. The data used to analyze the relationship between genetic mutations and HCC prognosis in cBioPortal were based on the TCGA database [11]. A P value < 0.05 was considered statistically significant. mRNA expression z-scores (RNA Seq V2 RSEM) were obtained with a z-score threshold of ± 2.0 .

STRING

STRING (<https://string-db.org/>) is a database that can be applied to construct a PPI network [12]. In this research, STRING was employed to analyze the relevance of CMTMs and their coexpressed genes. We set *Homo sapiens* as the analytic species. A synthesized score > 0.9 was regarded as statistically significant. The nodes represent proteins, and the edges represent the interactions between proteins. STRING also serves as a web tool for functional enrichment analysis. Functional enrichment analyses were implemented on CMTMs and their 50 coexpressed genes in our study. GO functional enrichment was performed for the biological process (BP), cellular component (CC), and molecular function (MF) categories. In addition, pathway analysis was implemented with the KEGG pathway tool.

Immune Infiltration Analysis

TIMER

TIMER (<https://cistrome.shinyapps.io/timer/>) refers to a web tool that can be used to systematically evaluate the infiltration of different immune cell types and their clinical impacts [13]. We assessed the mRNA expression levels of CMTMs in HCC in addition to their relevance to tumor purity and infiltrating immune cells, including B cells, macrophages, neutrophils, CD4 + T cells, CD8 + T cells, and DCs. Further analysis of CMTM associations with immune markers of different immune cells was conducted with TIMER [14]. Since the tumor purity of clinical samples may influence the analysis of immune infiltration, samples were adjusted for purity before the correlation analyses.

ESTIMATE

Estimation of stromal and immune cells in malignant tumor tissues using expression data (ESTIMATE) is an algorithm used to calculate immune, stromal and ESTIMATE scores which could reflect the infiltration patterns of immune cells and stromal cells in the tumor microenvironment [15].

ssGSEA

Single-sample GSEA (ssGSEA), an extension of Gene Set Enrichment Analysis (GSEA), calculates separate enrichment scores for each pairing of a sample and gene set. Each ssGSEA enrichment score represents the degree to which the genes in a particular gene set are coordinately up- or down-regulated within a sample [16].

Results

mRNA Expression Levels of CMTMs in HCC Patients

Aiming to investigate the mRNA expression levels of different CMTMs, we used the Oncomine database to analyze 20 different kinds of cancers and compared the expression levels of CMTMs in cancerous tissues with those in normal tissues (Fig. 1a). As shown in Table 1, the mRNA expression levels of CKLF,

CMTM4 and CMTM7 were notably higher in HCC tissues. By contrast, in the Wurmbach dataset, the mRNA expression levels of CMTM2 and CMTM6 were lower in HCC tissues than in normal tissues, with fold changes of -1.743 and - 1.636 ($P = 2.8E-2, 8.95E-4$), respectively. Additionally, the mRNA expression levels of CMTMs in HCC and control tissues were compared with Gene Expression Profiling Interactive Analysis (GEPIA). The results showed elevated mRNA expression levels of CKLF, CMTM1, CMTM3, CMTM4, CMTM7 and CMTM8 in HCC tissues, although there was no significant difference in CMTM1 and CMTM8 between HCC and normal tissues (Fig. 1b). HCC tissues had much lower mRNA expression levels of CMTM2, CMTM5, and CMTM6. These results nearly conform to the findings from Oncomine.

Table 1
Significant Changes in mRNA Expression Levels of CMTMs Between Hepatocellular Carcinoma and Normal Liver Tissues (Oncomine)

Gene ID	Type of HCC Tissue versus Normal Liver Tissue	Fold Change	P value	t Test	Source
CKLF	HCC	1.581	1.93E-12	7.481	Chen Liver
	HCC	2.427	5.10E-48	16.661	Roessler Liver 2
	HCC	1.535	2.82E-06	5.028	Mas Liver
	Cirrhosis	1.571	4.63E-09	7.748	Mas Liver
	HCC	1.891	1.44E-05	4.978	Roessler Liver
CMTM1	NA	NA	NA	NA	NA
CMTM2	HCC	-1.743	2.8E-02	-2.169	Wurmbach Liver
CMTM3	Cirrhosis	1.765	6.38E-05	4.868	Wurmbach Liver
CMTM4	HCC	1.509	8.53E-04	3.359	Wurmbach Liver
	Liver Cell Dysplasia	-1.511	6.16E-06	-5.451	Wurmbach Liver
CMTM5	NA	NA	NA	NA	NA
CMTM6	HCC	-1.636	8.95E-04	-3.557	Wurmbach Liver
CMTM7	HCC	1.654	9.58E-04	3.507	Wurmbach Liver
	Cirrhosis	2.329	6.95E-06	5.744	Wurmbach Liver
CMTM8	Cirrhosis	-2.246	6.96E-07	-6.867	Wurmbach Liver

HCC hepatocellular carcinoma. *NA* not available.

Protein Levels of CMTMs in HCC Patients

After determination of the mRNA expression levels of the nine CMTMs, the protein levels of CMTM1, CMTM3, CMTM4, CMTM5, CMTM6 and CMTM8 in HCC patients were analyzed using the Human Protein Atlas (HPA) database (Fig. 1c). There were 11 cases in CMTM1 and CMTM3 groups, 12 cases in CMTM4-

6 groups, and 10 cases in CMTM8 group. The results showed that CMTM1/3/4/6/8 were a medium or high expression in HCC. However, CMTM5 had no detected expression in HCC. This result was basically consistent with our previous findings on mRNA levels of expression.

The Correlation of CMTMs and the Pathological Stage

Then, we estimated the correlation of CMTMs expression and the pathological stage of patients with HCC. The expression levels of CMTM1, CMTM2, and CMTM4 were notably variable in different stages of HCC, whereas the expression levels of CKLF, CMTM3, CMTM5, CMTM6, CMTM7 and CMTM8 did not present remarkable differences (Fig. 2a). These data revealed that CMTM1, CMTM2, and CMTM4 might play an indispensable role in the progression of HCC.

Prognostic and Diagnostic Value of CMTMs in HCC Patients

Our in-depth exploration focused on the prognostic and diagnostic value of CMTMs in HCC patients. Firstly, Kaplan-Meier Plotter was employed to analyze the relevance of CMTM mRNA expression levels and the survival of HCC patients. According to the Kaplan-Meier curves and log-rank tests, it was determined that HCC patients with low mRNA expression levels of CKLF, CMTM4 and CMTM7 or high mRNA levels of CMTM5 had improved overall survival (OS) (Fig. 2b) and disease-free survival (DFS) (Fig. 2c). In addition, high mRNA levels of CMTM1 and CMTM6 were closely correlated with short OS. In contrast, HCC patients with high mRNA levels of CMTM2 were predicted to have improved DFS.

Genetic Alterations in CMTMs Affect OS and DFS in HCC Patients

The frequencies of genetic alterations in the nine CMTMs in HCC patients were determined by virtue of the cBioPortal database. As displayed in Fig. 3a, 102 (29.57%) HCC patients harbored genetic alterations in CMTMs, including amplifications, missense mutations, and transcriptional upregulation and downregulation. In particular, the percentage changes of genetic alterations in CKLF and CMTM1-8 among HCC patients were as follows: 5, 5, 3, 6, 6, 1.1, 5, 5 and 6%, respectively (Fig. 3b). Moreover, our group examined the relevance of CMTM genetic alterations and HCC prognosis using the cBioPortal database. Kaplan–Meier curves were utilized to determine the OS and DFS of HCC patients who had altered or unaltered CKLF and CMTM1–8 genes. As the results revealed, altered CMTM genes in HCC patients were closely correlated with OS and DFS (Fig. 3c-d). HCC patients with CMTM genetic alterations exhibited markedly worse OS ($P = 0.0393$) and DFS ($P = 0.0192$) than those without CMTM genetic alterations.

Functional Enrichment Analysis of CMTMs and their 50 Coexpressed Genes in Patients with HCC

Having completed the analysis of genetic alterations in CMTMs and their prognostic value in HCC patients, our research group conducted analyses on CMTMs and their 50 coexpressed genes. As shown

in Fig. 4a, we used STRING to construct a protein-protein interaction (PPI) network of 59 genes (group 1). For comparison, as shown in Fig. 4b, we built a PPI network of the 9 CMTMs (group 2). Then, we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses on the genes in the two groups.

As shown in Fig. 4c, we found that in group 1, the CMTMs as well as their 50 coexpressed genes were enriched in the following cellular component terms: GO:0030667 (secretory granule membrane), GO:0035579 (specific granule membrane), GO:0098805 (whole membrane), GO:0035577 (azurophil granule membrane), and GO:0005765 (lysosomal membrane). Meanwhile, in group 2, we found that the CMTMs were enriched in only GO:0005615 (extracellular space) and GO:0016021 (integral component of membrane) (Fig. 4c). Moreover, the genes in group 1 were enriched in the following molecular function terms GO:0005125 (cytokine activity) and GO:0005484 (SNAP receptor activity). In group 2, we found that CKLF and CMTM1-8 were similarly enriched in the term GO:0005125 (cytokine activity) (Fig. d). Furthermore, the biological process terms GO:0006935 (chemotaxis) and GO:0050896 (response to stimulus) were found in two different groups. In addition, the CMTMs and their coexpressed genes in HCC were enriched in the terms GO:0043312 (neutrophil degranulation), GO:0045321 (leukocyte activation), GO:0032940 (secretion by cell), GO:0002376 (immune system process), and GO:0051179 (localization) (Fig. 4e). In the KEGG analysis, the CMTMs and their coexpressed genes in HCC were enriched in the following pathways: hsa04145 (phagosome), hsa04142 (lysosome), hsa04130 (SNARE interactions in vesicular transport), hsa04140 (autophagy) and hsa05120 (epithelial cell signaling in *Helicobacter pylori* infection) (Fig. 4f).

Immune Infiltration Analysis in HCC Patients

Taking into consideration that the inflammatory response and infiltrating immune cells were able to affect the prognosis of HCC, our group evaluated the correlation between distinctively expressed CMTMs and immune cell infiltration through the TIMER database. As displayed in Fig. 5, there were positive correlations between CKLF (Fig. 5a), CMTM1-4 (Fig. 5b-r) and CMT6-7 (Fig. 5g-h) expression with infiltrating B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells. However, there were positive correlations between CMTM5 expression and only infiltrating CD8 + T cells and macrophages (Fig. 5f). There was no correlation between CMTM8 expression and infiltrating immune cells (Fig. 5i). In order to verify the above research results and further explore the relationship between CMTMs and immune infiltration, we firstly investigated the correlations between CMTMs. Then we used the ESTIMATE algorithm and the ssGSEA algorithm to explore the tumor microenvironment score and the relationship between 24 immune infiltrating cells and CMTMs. The results showed that the CKLF and CMTM1-3 were positively correlated with each other. The same results could be found in CMTM5-7. In addition, CMTM6 and CMTM1-8 showed a significant positive correlation (Fig. 6a).

The results also indicated that immune score was significantly correlated with the expression of CKLF and CMTM2-8, the stromal score was significantly correlated with the expression of CMTM1-3 and CMTM5-8. And the ESTIMATE score was associated with the expression of CKLF and CMTM1-8 (Fig. 6b). In addition, through ssGSEA analysis, we found that CMTMs were significantly correlated with a

variety of infiltrating immune cells (Fig. 6c). This confirmed the results of TIMER. All these results indicated that CMTMs were closely related to immune infiltration in HCC.

To further investigate the mechanism of CMTMs affecting immune infiltration, we investigated the correlations of CMTMs and immune markers of tumor-infiltrating immune cells (Table S1). In particular, the expression levels of chemokine (C-C motif) ligand 2 (CCL2), CD68, and IL10 in TAMs and PTGS2 and IRF5 in M1 macrophages were significantly correlated with the expression levels of CKLF, CMTM1-4 and CMTM6-7 expression in HCC. In addition, the expression levels of CD163, VSIG4 and MS4A4A in M2 macrophages were significantly correlated with the expression levels of CKLF, CMTM1-3 and CMTM6-7 in HCC (Table S1). These discoveries showed that CMTMs might work as regulators of macrophage polarization in HCC. Furthermore, DC markers such as HLA-DPB1, BDCA-1, BDCA-4 and CD11c displayed significant correlations with CKLF, CMTM1-4 and CMTM6-7 expression (Table S1). These results suggested that the association between CMTMs and DC infiltration.

In recent years, multiple studies had confirmed that immune checkpoint blockade (ICB) could remove the immune escape effect of HCC by removing the immunosuppressive function of immune checkpoint, which has become the latest method of anti-tumor treatment in clinical application [17, 18]. In this study, we explored the relationship between CMTMs and ICBs. Our findings revealed significant correlations between CKLF, CMTM1-4, and CMTM6-7 and marker genes of exhausted T cells, such as PD-1, PD-L1, CTLA4 and TIM-3 (Fig. 6d and Table S1). Therefore, in addition to being associated with immune infiltration, CMTMs may also play a key role in regulating immune evasion.

Discussion

According to previous reports, CMTMs exert a crucial role in regulating tumor development in multiple cancer types [19, 20]. They are closely related to tumor growth, metastasis, and antitumor immunity [21, 22]. In this study, we systematically explored the relationship between CMTMs and HCC occurrence and prognosis. Analysis of the potential signaling pathways in which CMTMs are involved further revealed the significant biological roles of CMTMs in HCC development.

CKLF is a member of the CMTM family, and there are four alternative RNA splicing forms for CKLF: CKLF1, CKLF2, CKLF3 and CKLF4. Liu et al. concluded that CKLF1, which is highly expressed in HCC tissues, was associated with vascular invasion. Moreover, CKLF1 works as an activator of the IL-6/STAT3 signaling pathway to induce HCC development and metastasis [7]. Our findings were consistent with theirs, showing the overexpression of CKLF at the mRNA level in HCC. Furthermore, we also found that a high mRNA level of CKLF in predicted a poor prognosis in HCC patients, which confirms the prognostic value of CKLF in HCC.

Previous studies reported that CMTM1 was highly expressed in various tumors, including HCC [23]. Our results also followed the same trend. However, there was no statistically significant difference in the CMTM1 mRNA expression levels between HCC tissues and normal tissues. We considered that this might be a result of the inclusion of FC > 1.5 in the GEPIA database. We also found that patients with high

mRNA levels of CMTM1 experienced short OS. This was consistent with the results of Song et al [24]. Their research suggested that the survival of HCC patients with CMTM1 positive expression was significantly shorter than those with CMTM1 negative expression. Taken together, these results suggest that CMTM1 is closely associated with HCC progression and patient prognosis. Further study on the mechanism of CMTM1 promoting the progression of HCC is one of the important research directions in the future.

CMTM2 has shown clinical significance in different tumors, such as diffuse-type gastric cancer [25]. In our study, we found that the mRNA level of CMTM2 was lower in HCC tissues than in normal tissues. CMTM2 expression levels were significantly correlated with different stages of HCC, which is consistent with Guo et al.'s findings. Similarly, they found that CMTM2 was correlated with tumor grade in HCC [26]. Furthermore, our findings revealed that patients with high mRNA expression levels of CMTM2 tended to have better DFS. Guo et al showed a similar tendency. They applied immunohistochemistry to confirm that HCC patients with negative CMTM2 expression had a significantly shorter OS time. Zhang et al. conducted an study on the mechanism by which CMTM2 down-regulation promotes the progression of HCC. They determined the epithelial-mesenchymal transition (EMT) process following down-regulation of CMTM2 expression by examining the expression of EMT-related factors. Western blot results showed that epithelial markers were decreased, while mesenchymal markers were increased after CMTM2 was knocked-down. These results showed down-regulated CMTM2 could induce the EMT process in HCC cells. They also found a significant positive correlation between CMTM2 and E-cadherin expression in HCC tissues. These results suggested that down-regulation of CMTM2 expression in HCC tissues can promote HCC metastasis by inducing EMT process [27]. Combined with our results, CMTM2 may be a potential tumor suppressor gene, and high levels of CMTM2 expression may be related to a good prognosis. However, in-depth research is needed to determine the regulatory mechanism of CMTM2 in HCC.

CMTM3 acts as a tumor suppressor in a variety of tumors, and DNA methylation of the CMTM3 promoter can inhibit CMTM3 expression in tumor tissues [28, 29]. However, there were few studies on the role of CMTM3 in HCC. Li et al. found that CMTM3 was weakly expressed in multiple HCC cell lines included HepG2, 97H, Hep3B, and HCCLM3. Moreover, in their study, the results of Western blot analysis showed that compared with the vector group, overexpression of CMTM3 significantly down-regulated the phosphorylation level of JAK2 and the downstream JAK substrate STAT3 in HepG2 cells. Based on these results, they suggested that CMTM3 may play an important role in HCC metastasis by inhibiting the JAK2/STAT3 signaling pathway [30]. Zhao et al. found that CMTM3 might be involved in the regulation of the tumor microenvironment in HCC [31]. Our research showed that the overexpression of CMTM3 in HCC patients occurred at both the mRNA and protein levels, which is inconsistent with previous findings. Moreover, the relationship of CMTM3 with the prognosis of HCC could not be elucidated. However, CMTM3 expression was closely correlated with immune infiltration in HCC in our research. Based on the above results, we hypothesized that the role of CMTM3 in HCC may be affected by the cell or matrix components in the tumor microenvironment, and thus displayed different expression patterns from cell

lines. Until now, the prognostic value of CMTM3 in HCC has not been clear. Further investigation is needed to explain the function and prognostic value of CMTM3 in HCC.

Previous studies have explored the function of CMTM4 in various types of cancer, including renal cell carcinoma [32], colorectal cancer [33], and pancreatic cancer [34]. Several studies have demonstrated the clinical importance of CMTM4 in HCC. Bei et al. found a low expression level of CMTM4 in HCC tissues and suggested that low CMTM4 expression was a risk factor for a poor prognosis [35]. In our report, we found that CMTM4 was overexpressed in HCC patients at both the mRNA and protein levels. A high expression level of CMTM4 was correlated with shorter OS and DFS, which is inconsistent with Bei's perspectives. Furthermore, we found that CMTM4 might exert an essential role in the tumorigenesis and progression of HCC. Zhou's findings were in line with ours. The expression of CMTM4 was significantly upregulated in HCC tissues and correlated with a poor prognosis in patients with HCC [36]. One study demonstrated that CMTM4 can participate in immune escape through the synergistic protective effect of PD-L1 and is extensively involved in the process of tumor proliferation [37]. Recently, Chui et al. reported new findings in their study on CMTM4 in HCC. They demonstrated that CMTM4 acts as a positive regulator of PD-L1 in a variety of HCC cell lines, and CMTM4 stabilizes PD-L1 expression through posttranslational mechanisms. They also found that suppression of CMTM4 can inhibit HCC growth and increased CD8 + T-cell infiltration in immunocompetent mice [38]. These findings might explain the crucial role of CMTM4 in HCC occurrence and progression, which can shorten a patient's survival time. In our TIMER database analysis, CMTM4 was found to be positively correlated with the immune markers of exhaustion T cells included PD-1, PD-L1, CTLA4 and TIM-3. This was basically consistent with the research results of Chui et al. These results suggested that CMTM4 may promote HCC progression by inducing the immunosuppressive tumor microenvironment. Considering the existing positive research results, the role of CMTM4 in HCC is worthy of further study.

Similar to CMTM3, CMTM5 has a tumor-suppressive function, and the promoter methylation of CMTM5 can cause a tumor to evade anticancer effects [39]. Our study demonstrated that patients with high mRNA expression levels of CMTM5 experienced better OS and DFS, which was consistent with previous findings. According to the report by Guan et al., miR-10b-3p is notably upregulated in HCC cell lines based on the significant suppression of CMTM5 expression, which further promotes HCC metastasis and progression. In addition, other studies have shown that CMTM5 expression can be restored in HCC by treatment with the histone deacetylase inhibitor PXD101 [40]. Therefore, it was reasonable to assume that CMTM5 may be a valuable therapeutic target for HCC. However, the expression pattern of CMTM5 in HCC and its effect on prognosis still need to be verified by a larger sample study.

CMTM6 is regarded as a critical regulator in terms of the maintenance of PD-L1 expression in multiple cancers [41]. The researchers found that PD-L1 relied on CMTM6/4 to effectively perform its inhibitory function [37]. In this study, we found that the expression of CMTM6 and CMTM4 showed significant positive correlation. In addition, according to our research, the mRNA expression levels of CMTM6 were decreased in HCC patients, and high mRNA expression levels of CMTM6 were associated with short OS. These findings were in line with those described by Zhu et al [42]. One study showed that CMTM6

expression was positively correlated with immunosuppressive factors, such as induced T cell tolerance and regulatory T cell differentiation [43]. Yugawa et al. confirmed that CMTM6 and PD-L1 were co-expressed in HCC. They also found that high CMTM6 and PD-L1-positive expression was associated with malignant aggregates and shorter OS of HCC patients [44]. This may be due to its function in stabilizing PD-L1 expression, thereby facilitating escape from T-cell-mediated cytotoxicity. Our correlation analysis further showed that there were positive correlations between CMTM6 expression and the immune markers of exhaustion T cell such as PD-L1, PD-1, CTLA4, TIM-3 and LAG3. This was consistent with Yugawa, K's research results. Further study of the relationship between CMTM6 and PD-L1 will open up a new path for the immunotherapy of HCC.

CMTM7 is broadly expressed in normal human tissues, particularly in immune cells. Huang et al. revealed that CMTM7 was notably downregulated in HCC tissues along with cell lines and exhibited tumor-suppressor activities [45]. In addition, they concluded that CMTM7 overexpression led to cell cycle arrest at G0/G1 phase and then inhibited HCC cell growth and migration. Our results were contrary to existing findings, which showed that CMTM7 was overexpressed at the mRNA level, and low CMTM7 expression was closely correlated with a good prognosis in HCC. In addition, through immune infiltration analysis, we found that CMTM7 was significantly positively correlated with PD-1, CTLA4, LAG3, TIM-3, GZMB and PD-L1. This results suggested that CMTM7 may play an important role in immune escape and induce of immunosuppressive microenvironment. Therefore, it is necessary to focus on the function and systematic performance of CMTM7 in the HCC microenvironment.

CMTM8, which functions as a tumor suppressor, accelerates the internalization of EGFR to attenuate the EGFR-mediated signaling pathway [46]. In our research, CMTM8 expressed higher in HCC tissues, but did not differ significantly between HCC and normal tissues. In addition, the immune infiltration analysis showed that CMTM8 was almost independent of various tumor infiltrating immune cells. Prior studies revealed the possible silencing of CMTM8 in tumors, which was similar to our findings [47]. At present, the role and prognostic value of CMTM8 in HCC need to be further studied.

In this study, we carried out a systematic analysis of the expression, prognostic value and relationship of immune infiltration and CMTMs in HCC. We concluded that high expression levels of CKLF, CMTM1, CMTM4 and CMTM6-7 expression could also serve as molecular markers to identify high-risk subgroups of HCC patients. The genetic alterations in CMTMs were correlated with a poor prognosis in HCC. CKLF, CMTM1-4 and CMTM6-7 demonstrated close associations with immune infiltration in HCC.

Conclusions

In conclusion, CMTMs might greatly affect the prognosis of HCC patients by remodeling the tumor microenvironment. CKLF, CMTM1, CMTM4 and CMTM6-7 may serve as therapeutic targets for HCC, and CMTM5 works as a suppressor associated with improved survival in HCC patients.

Declarations

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Figures

Figure 1

The expression profiles of CMTM family members in HCC patients. (a) mRNA expression levels of CMTM family members in different human cancers and corresponding normal tissues (Oncomine). The number in the colored cell is the number of datasets meeting the thresholds. The cell color is determined by the gene rank. The color intensity (red or blue) is directly proportional to the significance level of upregulation or downregulation, respectively. (b) mRNA expression levels of CMTM family members in HCC and normal liver samples (GEPIA). Red, HCC tissues; Grey, normal tissues. (c) Immunohistochemical expression of CMTM family members in normal liver tissues and HCC tissues (Human Protein Atlas).

Figure 2

Prognostic value of CMTM family members and relationship between CMTM family members and clinical stage in HCC patients. (a) Kaplan-Meier curve analysis of OS comparing the high and low levels of CMTM members (Kaplan-Meier plotter). (b) Kaplan-Meier curve analysis of DFS comparing the high and low levels of CMTM members (Kaplan-Meier plotter).

and low expression levels of CMTM members (Kaplan-Meier plotter). Red, high expression; Black, low expression. (c) The violin pot shows the correlations with the clinical stage (GEPIA).

Figure 3

Genetic Mutations in CMTMs and their Associations with the Survival of Hepatocellular Carcinoma Patients (cBioPortal). (a) Types of alterations in CMTMs. (b) Distribution and proportion of samples with alterations in CMTMs. (c-d) Genetic alterations in CMTMs were related to short survival.

Figure 5

Correlations between mRNA Expression Levels of CMTMs and Immune Infiltrates in HCC. (a–i) The effect of CKLF and CMTM1–8 on the immune cell infiltration (TIMER).

Figure 6

Immune infiltration analysis of CMTMs in HCC patients. (a) Correlation between each CMTMs. (b) The relationship between immune, stromal, ESTIMATE score and CMTMs. (c) Correlation heatmap between CMTMs and immune infiltration. (d) Correlation heatmap between CMTMs and immune checkpoint genes.

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