Cystathionine Gamma-Lyase Is A Prognostic Biomarker And Related To Immune Infiltrates In Hepatocellular Carcinoma

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Abstract

Purpose

The role of cystathionine γ -lyase (CTH) in the prognosis and immune invasion of hepatocellular carcinoma (HCC) has not been reported.

Methods

Combined with the clinical characteristics of HCC, CTH was analyzed via R package and various databases in this study.

Results

Compared with normal tissues, the level of CTH in HCC was significantly decreased, the extent of which was related to tumor clinicopathologic stage, gender, tumor status, residual tumor, histologic stage, race, alpha-fetoprotein (AFP), albumin, drinking and smoking. The results suggested that CTH might be a protective factor for the survival of HCC patients. Further functional analysis showed that the high expression of CTH was enriched in the reactome signaling by interleukins and the reactome neutrophil degranulation. Moreover, the expression of CTH was negatively correlated with the CD56 (bright) NK cells and Follicular helper T cell (TFH), positively correlated with Th17 cells and central memory T cell (Tcm), and closely correlated with the gene markers of a variety of immune cells. Also, high expression of CTH based on immune cells predicted the better prognosis of HCC. Pyridoxal phosphate, L-cysteine, Carboxymethylthio-3-(3-chlorophenyl)-1,2,4-oxadiazol, 2-[(3-Hydroxy-2-Methyl-5-Phosphonooxymethyl-Pyridin-4-Ylmethyl)-Imino]-5-phosphono-pent-3-enoic acid and L-2-amino-3-butynoic acid may be the targeted candidate medications for the treatment of CTH in HCC.

Conclusion

In brief, CTH can be used as a biomarker to predict the prognosis and immune infiltration of HCC.

Introduction

Hepatocellular carcinoma (HCC), the most common primary liver malignant tumor, approximately accounts for 75% to 90% of liver cancers (1) and is prone to metastasis and rapid invasive growth with high degree of malignancy and poor prognosis (2-4). At present, with the wide application of high-throughput sequencing, many genes and signal pathways relevant to the occurrence and development of HCC have been disclosed, though, the effective early diagnosis and key driver genes, especially those ones that may affect the formation of the immune micro-environment for HCC are still in need of investigation (5-6).

Cystathionine gamma-lyase (CTH) is located on the short arm of chromosome 1, and consists of 13 exons and 12 introns (7). Previous studies have shown that the expression of CTH will affect progression

and metastasis of different tumors, and is related to the immune micro-environment, however, the role of which remains controversy. For instance, H₂S produced by CTH passes interleukin (IL)-1 β /NF- κ B signaling pathways promotes the occurrence and development of prostate cancer (8). In addition, CTH has higher expression and activity in the neuroblastoma cells (9). Conversely, the CTH substrate inhibits the progression of melanoma (10). Also, the prognosis of HCC patients with positive forkhead box C1 (FOXC1) and negative CTH was poor (11). Moreover, oxidized LDL (ox-LDL) probably down-regulate the CTH/H₂S pathway, which plays an anti-inflammatory role in ox-LDL-stimulated macrophage via inhibiting c-jun n-terminal kinase (JNK)/NF- κ B signal (12). However, the potential molecular mechanism and immunological progress of CTH in HCC remains unknown.

To clarify the effect of CTH in HCC prognosis, RNA-sequencing (RNA-seq) datum were retrieved from the cancer genome atlas (TCGA) database, and the significance of CTH in HCC was systematically analyzed with bioinformatics and statistical methods, including differential expression genes (DEGs) analysis, gene ontology (GO) term analysis, kyoto encyclopedia of genes and genomes (KEGG) pathway analysis, gene set enrichment analysis (GSEA), Kaplan-Meier survival analysis, logistic & Cox regression analysis, nomogram analysis and time-dependent receiver operating characteristic (ROC) curve analysis. In addition, we analyzed the correlations between CTH and tumor-infiltrating lymphocytes (TILs) in tumor micro-environment based on tumor immune estimation resource (TIMER) database, and the prognoses of different TIL expression in HCC was investigated based on Kaplan-Meier plotter. Moreover, the molecular mechanisms of CTH dysregulation were further explored via integrative molecular database of hepatocellular carcinoma (HCCDB), such as different expressions of CTH in HCC and adjacent tissues. Finally, TISIDB database was used to seek for targeted drug candidates for CTH. These results clarify the important role of CTH in the prognosis of HCC and provide a potential mechanism by which CTH expression may regulate tumor immunity by regulating TILs of HCC.

Materials & Methods

Dataset and analytic protocols

In this study, high-throughput sequencing data and clinical information of 33 tumor types, containing HCC were retrieved from TCGA and genotype-tissue expression (GTEx) databases. A total of 9379 tumor tissue samples and 6397 normal tissue samples were included in 15776 pan-cancer samples, of which 531 HCC samples included 371 tumor tissue samples and 50 normal tissue samples from TCGA database and 110 normal tissue samples from GTEx database. In this study, differential expressions of CTH in pan-cancer and adjacent tissues, and in HCC and adjacent tissues were conducted with R software (Version 3.6.3, AT&T BellLaboratories, New Zealand). The clinical correlation analysis of CTH in HCC, gene heat maps related to CTH, GO, KEGG and GSEA enrichment analysis, Cox regression analysis, nomogram analysis, time-dependent ROC curve analysis and correlations between CTH and TILs in HCC were investigated. P value less than 0.05 was considered to be statistically significant.

UCSC Xena Database Analysis

UCSC Xena database (<https://xenabrowser.net/datapages/>), providing about 200 public databases, including TCGA, international cancer genome consortium (ICGC), therapeutically applicable research to generate effective treatments (TARGET), GTEx, etc was used to detect somatic mutation, gene expression, protein expression and copy number and methylation (13). In addition, the RNA-seq data in TPM format of TCGA and GTEx was uniformly processed through the Toil process was used in this study (14). Furthermore, the correlation between CTH and DNA methylation, copy number variant (CNV) and somatic mutations in HCC was shown via UCSC Xena.

TIMER Database Analysis

TIMER2.0 (<http://timer.cistrome.org/>), in which six state-of-the-art algorithms were used to provide more reliable estimates of immune infiltration levels for TCGA or user provided tumor profiles (15). It was also used to analyze the expressions of CTH in diverse tumors. TIMER web server (<https://cistrome.shinyapps.io/timer/>) pre-calculated the levels of six TIL subsets (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) for 10897 tumors of 32 cancer types (16).

HCCDB Database Analysis

HCCDB (<http://lifeome.net/database/hccdb>) contained a 4D metric to comprehensively describe the expression pattern of each gene, linking with third-party drugs, proteomics and literature databases, and graphically display the results of computational analysis, including differential expression analysis, tissue-specific and tumor specific expression analysis, survival analysis and co expression analysis, which was employed to compare the different expressions of CTH in HCC, adjacent, cirrhotic and normal tissues (17).

HPA Database Analysis

The human protein atlas (HPA) database (<https://www.proteinatlas.org/>), which is a Swedish-based program launched in 2003 to map all human proteins in cells, tissues and organs by integrating various omics technologies (18). It was used to verify the different expression of CTH between HCC and normal liver tissues.

Kaplan-Meier Plotter Database Analysis

Kaplan-Meier plotter (<http://kmplot.com/analysis/>), which contains 54675 gene survival data from 10461 cancer samples was applied to evaluate the relationship between various factors and clinical prognosis (19). In addition, the expression levels of CTH in immune cell subsets were also analyzed.

TISIDB Database Analysis

In TISIDB (<http://cis.hku.hk/TISIDB>), the role of interested genes in tumor-immune interactions could be cross-checked through literature mining and high-throughput data analysis, and testable hypotheses and

high-quality data can be generated (20). In this study, it was employed to display the correlation heat map of CTH in 28 tumors and the prediction of CTH targeted drug candidates.

Results

Abnormally Low Expression of CTH in HCC

The expressions of CTH in pan-cancer were analyzed by R package and TIMER database, respectively. The outcomes of R packet analysis indicated that the expressions of CTH in 16 tumors were lower, including adrenocortical carcinoma (ACC), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA) and uterine carcinosarcoma (UCS). On the contrary, the CTH is highly expressed in colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), brain lower grade glioma (LGG), lung adenocarcinoma (LUAD), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), skin cutaneous melanoma (SKCM), testicular germ cell tumors (TGCT) and thymoma (THYM) (Fig. 1A; $p < 0.05$). In addition, analysis of TIMER database showed that CTH was lower expressed in BRCA, CHOL, COAD, KICH, KIRC, KIRP, LIHC, PEPG, READ and THCA, but higher expressed in HNSC, LUAD and uterine corpus endometrial carcinoma (UCEC) (Fig. 1B; $p < 0.05$). Taken two results into consideration, it is suggested that CTH was remarkably under-expressed in HCC. To verify this finding, HCCDB database was used to show that CTH expression decreased in HCC compared with adjacent tissues (Fig. 1C; Supplement 1). Moreover, the expressions of CTH in 160 adjacent tissues and 371 HCC tissues were compared. Expressions of CTH were significantly lower in HCC samples ($p < 0.001$) (Fig. 1D). Furthermore, in 50 HCC samples compared with matched paracancerous samples, CTH also decreased significantly in HCC ($p = 0.001$) (Fig. 1E). ROC was employed to analyze the differential effect of CTH between HCC and normal liver tissues. The area under the curve (AUC) of CTH was 0.728, indicating that CTH might be a potential biomarker of HCC (Fig. 1F).

Clinical Correlations and Pathological Validations of CTH in HCC

We classified and compared the data according to the clinical relationship. Notably, the expression of CTH in HCC was related to tumor (T) stage, pathologic stage, gender, histologic grade, race, AFP and albumin (Fig. 2A-G; Supplement Table 2). In addition, the low expression of CTH in HCC was confirmed by immunohistochemistry images (Fig. 3A-E).

Table 2
Correlations between CTH and TILs in HCC

Molecule	Cells	Pearson	P value	Spearman	P value
CTH	aDC	-0.104	0.044	-0.101	0.051
CTH	B cells	-0.166	0.001	-0.155	0.003
CTH	CD8 T cells	0.028	0.593	0.041	0.424
CTH	Cytotoxic cells	0.008	0.874	0.019	0.719
CTH	DC	0.042	0.414	0.083	0.109
CTH	Eosinophils	0.004	0.945	0.021	0.692
CTH	iDC	-0.217	< 0.001	-0.192	< 0.001
CTH	Macrophages	-0.204	< 0.001	-0.198	< 0.001
CTH	Mast cells	0.009	0.857	0.057	0.268
CTH	Neutrophils	0.183	< 0.001	0.197	< 0.001
CTH	NK CD56bright cells	-0.478	< 0.001	-0.470	< 0.001
CTH	NK CD56dim cells	-0.050	0.333	-0.040	0.445
CTH	NK cells	-0.118	0.023	-0.053	0.302
CTH	pDC	-0.058	0.259	-0.067	0.198
CTH	T cells	-0.163	0.002	-0.162	0.002
CTH	T helper cells	0.004	0.932	-0.008	0.885
CTH	Tcm	0.263	< 0.001	0.231	< 0.001
CTH	Tem	-0.152	0.003	-0.140	0.007
CTH	TFH	-0.392	< 0.001	-0.400	< 0.001
CTH	Tgd	0.124	0.016	0.170	< 0.001
CTH	Th1 cells	-0.199	< 0.001	-0.164	0.002
CTH	Th17 cells	0.232	< 0.001	0.253	< 0.001
CTH	Th2 cells	-0.219	< 0.001	-0.241	< 0.001
CTH	TReg	0.068	0.191	0.039	0.448

Correlation and Enrichment Analyses

As the functions and related pathways of CTH indicated by GSEA, the correlations between CTH and other genes in HCC were significantly different ($p < 0.001$) (Fig. 3G, H). Then, the top five genes with the highest and lowest coefficients of correlation with CTH were selected to verify that these genes have lower and higher expressions in HCC and normal liver tissues, respectively (Fig. 3I, J; Supplement Table 3). Moreover, the top 300 CTH-related genes were selected for enrichment analysis. The potential functional pathways were further investigated using the clusterprofiler package (version 3.14.3) (21). GO analysis showed that CTH was mainly associated with small molecule catabolic process, mitochondrial matrix and coenzyme binding (Fig. 4A-C). In addition, KEGG pathway analysis indicated that enrichment and crosstalk of these genes in Phenylalanine metabolism, Fc gamma R – mediated phagocytosis and peroxisome proliferator-activated receptor (PPAR) signaling pathway (Fig. 4D). Furthermore, GSEA results suggested reactome pathways, which revealed that the reactome signaling by interleukins (NES = - 1.446, $p = 0.009$, FDR = 0.005) and the reactome neutrophil degranulation (NES = - 1.585, $p = 0.009$, FDR = 0.005) (Fig. 4E, F) might be malignant tumor related pathways in HCC, especially immune related pathways.

Table 3
Correlation analysis between CTH and TILs related gene markers

Description	Gene markers	HCC			
		None		Purity	
		Core	P	Core	P
CD8 + T cell	CD8A	-0.143	**	-0.150	**
	CD8B	-0.187	***	-0.191	***
T cell (general)	CD2	-0.279	***	-0.265	***
	CD3D	-0.346	***	-0.331	***
	CD3E	-0.259	***	-0.242	***
B cell	CD19	-0.214	***	-0.218	***
	CD79A	-0.256	***	-0.248	***
Monocyte	CD86	-0.220	***	-0.214	***
	CSF1R	-0.173	**	-0.173	**
TAM	CCL2	-0.103	0.056	-0.114	*
	CD68	-0.230	***	-0.228	***
	IL10	-0.158	**	-0.163	**
M1 Macrophage	PTGS2	-0.047	0.379	-0.066	0.220
	NOS2	0.151	**	0.145	**
	IRF5	-0.291	***	-0.291	***
M2 Macrophage	MS4A4A	0.043	0.421	0.012	0.829
	VSIG4	0.001	0.986	-0.024	0.661
	CD163	0.092	0.089	0.056	0.298
Natural killer cell	KIR2DL1	0.063	0.246	0.060	0.263
	KIR2DL3	-0.054	0.315	-0.062	0.246
	KIR2DL4	-0.026	0.636	-0.034	0.523
	KIR2DS4	0.035	0.517	0.033	0.538
	KIR3DL1	0.078	0.150	0.072	0.183
	KIR3DL2	-0.058	0.282	-0.068	0.209
*, p < 0.05; **, p < 0.01; ***, p < 0.001.					

Description	Gene markers	HCC			
		None		Purity	
		Core	P	Core	P
	KIR3DL3	-0.050	0.353	-0.052	0.330
Neutrophils	CCR7	-0.082	0.127	-0.096	0.074
	ITGAM	-0.189	***	-0.195	***
	CEACAM3	-0.156	**	-0.164	**
T cell exhaustion	CTLA4	-0.292	***	-0.287	***
	GZMB	-0.107	*	-0.118	*
	HAVCR2	-0.252	***	-0.242	***
	LAG3	-0.234	***	-0.239	***
	PDCD1	-0.317	***	-0.307	***
Tfh	BCL6	0.048	0.370	0.048	0.375
	IL21	-0.001	0.987	-0.009	0.869
Th1	TBX21	-0.100	0.063	-0.112	*
	STAT4	-0.173	**	-0.179	***
	IFNG	-0.154	**	-0.161	**
	STAT1	-0.133	*	-0.140	**
Th2	GATA3	-0.253	***	-0.244	***
	IL13	-0.097	0.071	-0.098	0.069
	STAT5A	-0.136	*	-0.144	**
	STAT6	0.137	*	0.137	*
Th17	IL17A	0.016	0.768	0.014	0.797
	STAT3	0.164	**	0.148	**
Treg	CCR8	-0.065	0.232	-0.077	0.152
	FOXP3	0.082	0.128	0.069	0.203
	STAT5B	0.116	*	0.122	*
Dendritic cell	CD1C	-0.187	***	-0.190	***
*, p < 0.05; **, p < 0.01; ***, p < 0.001.					

Description	Gene markers	HCC			
		None		Purity	
		Core	P	Core	P
	HLA-DPA1	-0.100	0.064	-0.111	*
	HLA-DPB1	-0.182	***	-0.183	***
	HLA-DQB1	-0.166	**	-0.170	**
	HLA-DRA	-0.094	0.082	-0.106	*
	ITGAX	-0.262	**	-0.255	***
	NRP1	-0.084	0.121	-0.092	0.088
*, p < 0.05; **, p < 0.01; ***, p < 0.001.					

Prognostic Significance of CTH Expression in HCC

The prognostic value of CTH expressions in HCC were analyzed based on Kaplan-Meier plotter database. The low expression of CTH in HCC indicated poor prognosis, that is, overall survival (OS): HR = 0.55, 95% CI = 0.39–0.78, p = 0.00068; disease specific survival (DSS): HR = 0.49, 95%CI = 0.29–0.81, p = 0.0046; progression free survival (PFS): HR = 0.64, 95%CI = 0.47–0.87, p = 0.0045 (Fig. 5A-C). In addition, low expression of CTH in HCC was significantly associated with poor prognosis in stage T1&T2 and T3&T4 of OS and DSS (Fig. 5D-G). Through logistic regression analysis, univariate and multivariate Cox regression analysis, the forest plot showed that CTH was a protective factor for HCC (Table 1; Fig. 5H; Supplement Table 4). Moreover, both drinking and hepatitis could reduce the protective effect of CTH in HCC (Fig. 6A-L). Furthermore, nomogram and time dependent ROC showed the 1-year (AUC = 0.419), 3-year (AUC = 0.443) and 5-year (AUC = 0.389) survival rates of HCC patients (Fig. 7A,B), indicating that CTH was the protective factor.

Table 1
The logistic regression analysis results

Characteristics	Total(N)	Odds Ratio(95%CI)	P value
T stage (T2&T3&T4 vs. T1)	371	0.628 (0.416–0.945)	0.026
N stage (N1 vs. N0)	258	0.355 (0.017–2.814)	0.373
M stage (M1 vs. M0)	272	0.381 (0.019–3.021)	0.406
Residual tumor (R1&R2 vs. R0)	345	0.766 (0.286–1.991)	0.585
Pathologic stage (Stage II&Stage III&Stage IV vs. Stage I)	350	0.632 (0.414–0.962)	0.033
Tumor status (With tumor vs. Tumor free)	355	0.860 (0.564–1.309)	0.482
Histologic grade (G3&G4 vs. G1&G2)	369	0.522 (0.338–0.800)	0.003
Vascular invasion (Yes vs. No)	318	0.713 (0.447–1.132)	0.152
Child-Pugh grade (B&C vs. A)	241	1.066 (0.441–2.623)	0.888
Adjacent hepatic tissue inflammation (Mild&Severe vs. None)	237	0.856 (0.512–1.429)	0.553

Table 4
Cox proportional risk model of CTH and TILs in HCC patients

	coef	HR	95%CI_l	95%CI_u	P value
B_cell	-8.185	0.000	0.000	0.318	0.023
CD8_Tcell	-7.345	0.001	0.000	0.066	0.002
CD4_Tcell	-6.135	0.002	0.000	1.049	0.052
Macrophage	4.538	93.504	0.646	13527.347	0.074
Neutrophil	3.926	50.715	0.001	2548571.888	0.477
Dendritic	5.652	284.944	9.858	8236.484	0.001
CTH	-0.118	0.888	0.802	0.984	0.024

Relationship between CTH expression and Immune Infiltration

The correlation between the expression standard of CTH and TILs standard quantified via ssGSEA was analyzed using spearman correlation. The expression of CTH was positively related to Th17 cells, Tcm, neutrophils, T gamma delta (Tgd), etc. And negatively related to CD56 (bright) NK cells, TFH, Th2 cells, macrophages, immature DC (iDC), Th1 cells, T cells, B cells, T effector memory (Tem), etc (Fig. 8A; Fig. 8C-O; Fig. 9A-M; Table 2). In addition, the correlations of CTH in 28 tumors were analyzed using TIMER 2.0 database (Fig. 8B). Moreover, the correlation between CTH and TIL related gene markers was analyzed using TIMER database. Owing to the tumor purity of clinical samples would affect the immune infiltration analysis, the purity of correlation analysis was adjusted (Table 3).

Prognostic Potential of CTH Expressions in HCC Based on TILs

Kaplan-Meier plotter were employed, following B cells, CD4 + memory T cells, CD8 + T cells, macrophages, NK T cells, Treg T cells, Th1 cells and Th2 cells to analyze the CTH expression in HCC. The results showed that CTH and any of the above TILs were highly enriched, and the prognosis was well in HCC (Fig. 10). Moreover, Cox proportional risk model of CTH and TILs in HCC was analyzed based on TIMER dataset, and the results stated that B cell ($p = 0.023$), CD8 + T cells ($p = 0.002$), dendritic cells ($p = 0.001$) and CTH expression ($p = 0.024$) were correlated with the clinical prognosis of HCC remarkably (Table 4). To sum up, immune infiltration may, partly, influence high CTH expression prognosis of HCC patients.

Mutation, CNV, Methylation and Potential Drug Candidates on CTH

According to the UCSC Xena database, the heat map showed that the expression of CTH was related to CNV, somatic mutation and DNA methylation in HCC (Fig. 11A). Therefore, we speculated that CNV, somatic mutation and DNA methylation may lead to the increase of CTH level in HCC. Now that the target CTH has been found, we further detected five CTH targeted drug candidates via TISIDB database, including Pyridoxal phosphate (DB00114), L-cysteine (DB00151), 2 - [(3 - Hydroxy - 2-Methyl - 5 - Phosphonooxymethyl - Pyridin - 4 - Ylmethyl) - Imino] - 5 - phosphono - pent - 3 - enoic acid (DB00151), Carboxymethylthio - 3 - (3 - Chlorophenyl) - 1, 2, 4 - Oxadiazol (DB02328) and L-2-amino-3-butynoic acid (DB04217) (Fig. 11; Table 5). These drugs may have a great effect on the treatment of HCC and worth further validation.

Table 5
Details on candidate drugs targeting CTH

ID	Name	Drug Type	Targets	Targets
DB00114	Pyridoxal Phosphate	Small Molecule	AADAT, ABAT, AGXT, AGXT2, ALAS1, ALAS2, AZIN2, BCAT1, BCAT2, CBS, CCBL1, CCBL2, CSAD, CTH, DDC, FTCD, GAD1, GAD1, GADL1, GCAT, GLDC, GOT1, GOT2, GPT, GPT2, HDC, IGSF10, KYNU, MOCOS, NFS1, OAT, ODC1, PDXDC1, PDXP, PHYKPL, PNPO, PROSC, PSAT1, PYGB, PYGL, PYGM, SCLY, SDS, SDSL, SEPSECS, SGPL1, SHMT1, NSHMT2, SPTLC1, SPTLC2, SPTLC3, SRR, TAT, THNSL1	54
DB00151	L-Cysteine	Small Molecule	CARS, CARS2, CBS, CDO1, CSAD, CTH, GCLC, GCLM, GOT1, GSS, MGMT, NFS1, SLC19A3	13
DB02328	2-[(3-Hydroxy-2-Methyl-5-Phosphonooxymethyl-Pyridin-4-Ylmethyl)-Imino]-5-Phosphono-Pent-3-Enoic Acid	Small Molecule	CTH	1
DB03928	Carboxymethylthio-3-(3-Chlorophenyl)-1,2,4-Oxadiazol	Small Molecule	CTH	1
DB04217	L-2-amino-3-butynoic acid	Small Molecule	CTH	1

Discussion

In this study, the expression, prognostic value and correlation with TILs of CTH in HCC were analyzed for the first time. Compared with normal tissues, the expression of CTH in HCC decreased significantly. In addition, CTH expression was related to T stage (T1&T2), pathologic stage (stage 1&stage 2), gender (male), histologic stage (grade 1), race (black or African American), AFP (≤ 400 ng/ml) and albumin (< 3.5 g/dl). These results suggested that CTH played an important role in the progression and metastasis of HCC, which were consistent with previous studies (8–12; 22–24). The decrease of CTH activity will lead to the consumption of glutathione (GSH) in cancer patients (22), and GSH is extremely significant for the removal and detoxification of carcinogens (23). In addition, CTH was down-regulated in HCC patients, and individual cancer stage and T grade were negatively correlated with the expression of CTH (24).

As immunohistochemical images from HPA database verified, the CTH was highly expressed in normal liver tissues and low expressed in HCC tissues. Then, the top 50 up and down-regulated genes with the highest correlation with CTH were selected, and the five genes with the highest correlation between them were selected for verification in HCC, respectively. In addition, GO and KEGG analysis showed that CTH

was mainly related to small molecule catabolism, mitochondrial matrix and coenzyme binding, enrichment and crosstalk of these genes in Phenylalanine metabolism, Fc gamma R – mediated phagocytosis and PPAR signaling pathway. As the GSEA results of reactome signaling by interleukins (NES = - 1.446, p = 0.009, FDR = 0.005) and the reactome neutrophil degranulation (NES = - 1.585, p = 0.009, FDR = 0.005) indicated, CTH was related to many pathways related to malignant tumors in HCC, especially immune related pathways, which was the same as previous studies (25–26). CTH silencing induced the expression of the natural-killer group 2, member D (NKG2D) ligands, major histocompatibility complex class I related chain A (MICA) and UL16 binding protein 2 (ULBP2), in the human breast cancer cell line (MDA-MB-231) triple-negative breast cancers (TNBC) cell line, which was associated with improved cytotoxicity of NK cell against H2S-depleted tumor targets, and CTH siRNAs caused the decrease of the production of tumor necrosis factor – α (TNF- α), which is a cytokine that may promote immune cell apoptosis in tumor microenvironment (25). H2S plays a role in regulating several pro-inflammatory cytokines levels in diabetic rat models including TNF - α tumor (26).

Survival analysis showed that low expression of CTH was correlated with poor OS (HR = 0.55, p = 0.00068), DSS (HR = 0.49 p = 0.0046) and PFS (HR = 0.64, p = 0.0045) in HCC. In addition, T stage (T3&T4), pathologic stage (stage 3&stage 4), tumor status (with tumor), alcohol consumption and hepatitis factors will make the prognosis of low expression group of CTH worse, which were consistent with previous reports (27–28). The low expression of a polymorphism of the alanine-glyoxylate aminotransferase 2 (AGXT2), D-amino acid oxidase (DAO) and CTH and the high expression of bisphosphoglycerate mutase (BPGM), cystathionine-beta-synthase (CBS), phosphoserine phosphatase (PSPH) and Acyl-CoA thioesterase 7 (ACOT7) are associated with the poor prognosis of HCC (24). In addition, knockout of CTH in mice will develop acute hepatitis in the case of intaking excessive methionine (27).

CTH expression was reported to be related to several TILs in HCC, especially CD56 (bright) NK cells, TFH, Th17 cells and Tcm. These results suggested that CTH was involved in the regulation of TILs. Han et al (28) revealed that pretreatment with D,L-propargylglycine, an inhibitor of CTH, attenuated plasma levels of TNF- α , IL-6, as well as urea and reduced H2S concentration in the kidney following hemorrhagic shock. According to Cui et al (29), the constitutive sulfurization of liver kinase B1 by H2S derived from CTH activates its target kinase adenosine monophosphate (AMP) activated protein kinase and promotes Treg differentiation and proliferation, so as to reduce the immune inflammation of blood vessels and kidneys and prevent hypertension. The results of studies above have the effect of mutual authentication.

The immune markers in HCC were further analyzed. After cell purity correction, CTH was negatively correlated with many immune cell markers in HCC, including that CTH was related to the immune infiltration of HCC. There is a significant correlation between CTH levels and several T lymphocyte markers (CD8 + T cell, general T cell, Th1, Th2, Th17 and Treg), monocyte and dendritic cell. These connections may indicate a potential mechanism by which CTH regulates T cell function in HCC. Kaplan-Meier and Cox proportional risk analysis showed that the high expression level of CTH enriched in various immune cell cohorts of HCC had a good prognosis. As Wu et al (30) reported, the effect of CTH/H2S on

IL-1 β ese connections might indicate a potential mechanism by which CTH regulates T cell function in HCC. Kaplan-Meier and Cox proportional risk analysis showed that the high expression monocytes and dendritic cells, which is down-regulated in transplantation tolerance and may be involved in maintaining tolerance (31). Moreover, CTH / H₂S pathway over inhibits JNK/NF- κ B signaling plays an anti-inflammatory role in ox-LDL-stimulated macrophage (12). These results may explain that the high expression of CTH partially affects the prognosis of HCC patients via immune infiltration. Moreover, CTH trans-sulfur pathway converts cystathionine to cysteine and is responsible for the increase of total homocysteine (tHcy), which plays an important role in oxidative stress, resulting in the decrease of S-adenosyl-L-methionine (SAM) level and DNA demethylation (32–34). Therefore, we believe that CNV, somatic mutation and DNA methylation may result in the increase of CTH level, which may lead to HCC.

Five candidate drugs were found through TISIBD database (Pyridoxal phosphate, L-cysteinephosphate, 2-[(3-Hydroxy-2-Methyl-5-Phosphonooxymethyl-Pyridin-4-ylmethyl)-imino]-5-phosphono-pent-3-enoic acid, Carboxymethylthio-3- (3-Chlorophenyl)-1,2,4-Oxadiazol and L-2-amino-3-butynoic acid), which have targeted therapeutic effects on CTH. Pyridoxal phosphate is co-enzyme of cystathionine yssynthase (CBS) and CTH, and CTH shows greater loss of activity than CBS during pyridoxal phosphate deficiency (35). According to Yadav et al (36), L-cysteine inhibited the thcystathionine and H₂S synthesis from cysteine by human CTH, which are consistent with our conjecture.

Conclusion

To sum up, the up-regulated CTH is closely related to clinicopathological features, well prognosis and immune infiltration in HCC patients. Moreover, this study provides a novel speculation that CTH may affect the prognosis of HCC via tumor immune infiltration. Furthermore, five candidate drugs are in need of validation for the further study of HCC tumor immunotherapy.

Declarations

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Contributions

XW conducted experiments, analyzed the data, prepared figures and/or tables, authored drafts of the paper, and approved the final draft.

FL analyzed the data, prepared figures and/or tables, and approved the final draft.

SL analyzed the data, prepared figures and/or tables, and approved the final draft.

CS analyzed the data and approved the final draft.

HW conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

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Ethics approval statement

Ethical approval is not required for this study.

Competing Interests

The authors report no conflicts of interest in this work.

Data Availability

The data sets provided in this study could be found in the online repository. The names and login number(s) of the repositories can be found in the article

Patient and public involvement

No patients or the public were involved in the research.

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Figures

Figure 1

Expression levels of CTH in diverse malignant tumors. (A) Expression of CTH in different cancers compared with normal tissues in TCGA and GTEX databases. (B) Expression of CTH among different cancers in the TIMER database. (C) Differential expression levels of CTH in HCC in HCCDB database (D, E) Differential expression levels of CTH in HCC analyzed by R software (F) A ROC curve was established to test the value of CTH to identify HCC tissue. ns, $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 2

Clinical relevance of CTH in HCC. The level of CTH were significantly correlated with the tumor stage (A), pathologic stage (B), gender (C), histologic grade (D), race (E), alpha-fetoprotein (F) and albumin (G) of HCC patients. ns, pnts. ns*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 3

Expression of CTH and CTH related DEGs in HCC tissues. Immunohistochemical images showed high expression of CTH in normal liver tissues (A-C) and low expression of CTH in HCC tissues (D-F). The heat map of the top 50 CTH related up and down-regulated DEGs (G,H). Validations of the expression of the top 5 CTH related up and down-regulated DEGs in HCC (I,J). ns, p pions *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 4

Function and pathway enrichment analysis of CTH in HCC. Considerable GO terms of the top 300 genes most positively related to CTH, including biological processes (A), cell component (B) and molecular function (C). (D) Considerable KEGG pathways of the top 300 genes most positively related to CTH. Enrichment plots from the GSEA, including reactome signaling by interleukins and reactome neutrophil degranulation. NES, normalized enrichment score; p.adj, adjusted p value; FDR, false discovery rate.

Figure 5

Survival analysis of CTH in HCC patients. Kaplan-Meier survival curve analysis showed the overall survival (OS; n=364), disease free survival (DSS; n=362) and progression specific survival (PFS; n=370) of different CTH in HCC using the Kaplan-Meier plotter database (A-C). OS and DSS analysis of tumor stages 1&2 (D, E; n=257). OS and DSS analysis of tumor stages 3&4 (F, G; n=90). Forest plot of univariate analysis (H).

Figure 6

Effects of alcohol consumption and hepatitis virus on the prognosis of CTH in HCC patients. (A, B) none alcohol consumption groups; (C, D) alcohol consumption groups; (E, F) none hepatitis virus groups; (G, H) hepatitis virus groups; (I, J) none alcohol and none hepatitis groups; (K, L) both alcohol consumption and hepatitis group.

A

B

Figure 7

Quantitative methods for predicting HCC patients' probabilities of 1-, 3-, and 5-year OS. (A) A Nomogram for predicting 1-, 3-, and 5-year OS in patients with HCC. (B) Time-dependent ROC plot for predicting 1-, 3-, and 5-year OS probabilities. OS, overall survival; AUC, area under ROC curve.

Figure 8

The correlations between the levels of TILs in human cancers analyzed by R package and TISIDB database. (A) The correlations between 24 TILs and CTH in HCC. (B) Heat map of CTH expression and 28 types of TILs across human heterogeneous cancers. Statistically significant TILs were selected for differential expression in HCC tissues containing CTH, in which iDC (C), B cells (D), macrophages (E), T cells (H), Tem (J), TFH (K), Th1 cells (M), Th2 cells (N) and NK CD56 bright cell (O) decreased, while neutrophils (F), Th17 cells (G), Tcm (I) and Tgd (L) increased. iDC, immature DC; Tem, T effector memory;

TFH, T follicular helper; Tgd, T gamma delta; pDC, plasmacytoid DC; aDC, activated DC; Tcm, T central memory. TIL, tumor infiltrating lymphocyte. ns, pumor in*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 9

The correlations between CTH expression and 13 types of TILs with statistical significance in HCC patients. Among them, CTH level was negatively correlated with iDC (A), B cells (B), macrophages (C), T cells (F), Tem (H), TFH (I), Th1 cells (K), Th2 cells (L) and NK CD56 bright cell (M), and positively correlated with neutrophils (D), Th17 cells (E), Tcm (G) and Tgd (J). iDC, immature DC; Tem, T effector memory; TFH, T follicular helper; Tgd, T gamma delta; pDC, plasmacytoid DC; aDC, activated DC; Tcm, T central memory. TIL, tumor infiltrating lymphocyte.

Figure 10

Comparison of Kaplan-Meier survival curves of the high and low expression of CTH in HCC based on TIL subgroups. (A-H) High CTH level enriched in B cells, CD4+ memory T cells, CD8+ T cells, macrophages, NK T cells, Treg T cells, Th1 cells and Th2 cells had better OS in HCC.

Figure 11

Mutation, CNV, Methylation and Potential Drug Candidates on CTH The CTH multiomics analysis (A) and screening of CTH targeted drug candidates(B). Pyridoxal phosphate (DB00114), L-cysteine (DB00151), 2-[(3-Hydroxy-2-Methyl-5-Phosphonooxymethyl-Pyridin-4-Ylmethyl)-Imino]-5-phosphono-pent-3-enoic acid (DB00151), Carboxymethylthio-3-(3-Chlorophenyl)-1, 2, 4-Oxadiazol (DB02328) and L-2-amino-3-butynoic acid (DB04217) were targets of CTH.

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