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Original Article

# CTHRC1 overexpression in gastric cancer patients contributes to a poor prognosis and is related to immune cell infiltration

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**Abstract**

Gastric cancer is a common solid tumor of the digestive system, This research aimed to investigate the relationships among CTHRC1 expression, prognostic values and tumor-infiltrating immune cells (TIICs) in GC patients.The expression of CTHRC1 in gastric cancer patients was analyzed using the GEPIA database and the TCGA database. The relationship between CTHRC1 expression and survival of gastric cancer patients was then explored using the Kaplan-Meier Plotter database and TCGA database.Subsequently, we explored whether there was an associative effect between the expression of CTHRC1 and TIICs in gastric cancer tissues. Then we constructed a prognostic model using immunomodulatory genes related to CTHRC1 and verified the specificity and accuracy of the model.Compared with normal tissues, the expression of CTHRC1 was significantly upregulated in gastric cancer tissues. And the high expression of CTHRC1 was associated with poor prognosis of gastric cancer patients.It is suggested that CTHRC1 is a reliable prognostic indicator for predicting the prognosis of gastric cancer patients.Then we successively used univariate and multivariate COX methods to obtain three high-risk immunomodulatory genes (TGFB2, CXCR4 and NT5E) and one low-risk immunomodulatory gene (TNFRSF18) associated with CTHRC1. These four immunomodulatory genes were used to construct a prognostic model for gastric cancer.Gastric cancer patients with high CTHRC1 expression have a poor prognosis and are associated with immune cell infiltration. Therefore, CTHRC1 can be considered as a potentially reliable prognostic indicator for gastric cancer patients.

**Keywords:** CTHRC1, Gastric cancer, Immunity, Prognosis, Models.

## 1. Introduction

Gastric cancer (GC) is a common solid malignant tumor of the digestive system with high incidence and mortality. It is the fifth most common cancer, and is one of the causes of cancer deaths worldwide[1].GC is a global public health event that places a heavy burden on the global health and wellness sector. According to epidemiological findings the incidence of gastric cancer has a distinct geographic profile. The incidence is higher in East Asia, Eastern Europe and the Americas, while it is lower in North America, Northern Europe and Australia, perhaps due to differences in dietary structure[1, 2]. Gastric cancer is associated with a number of risk factors, such as *Helicobacter pylori* infection, dietary habits, hereditary factors, lifestyle habits such as smoking and alcohol consumption, additionally gastritis and gastric ulcers may also induce[3-9]. Reduction of gastric cancer mortality can be achieved by early detection such as radiologic examination, serologic examination and endoscopic screening, but the overall prognosis of gastric cancer patients is still poor [9-11]. Therefore, it is necessary to find good prognostic indicators and prognostic models for gastric cancer and to adjust the treatment strategy for patients with high risk of death in a timely manner.

Collagen triple helix repeat 1 (CTHRC1) is a member of the TGF-β family that inhibits the production of collagen type I matrix and exhibits a significant role in promoting cellular metastasis [10]. CTHRC1 is the first glycosylated protein identified in arterial injury, and has been shown to be functionally enriched in both tumors and non-tumors. CTHRC1 is a promising new diagnostic and prognostic indicator in rheumatoid arthritis, and is related to the progression and severity of rheumatoid arthritis[11]. CTHRC1 is a conjugated factor secreted by osteoclasts, which regulates bone remodeling, targeting stromal cells to stimulate osteogenesis and maintain bone integrity[12]. CTHRC1 also plays an essential role in the prevention of osteoporosis. Studies have shown that extracellular vesicles of human urinary stem cells can prevent osteoporosis by transporting CTHRC1 and osteoclastogenesis inhibitory factor (OPG), which provides ideas for the development of new drugs for osteoporosis[13]. Upregulation of CTHRC1 expression during skin repair may promote skin wound healing [14]. Nedd4l inhibits the development and progression of interstitial pulmonary fibrosis (IPF) by modulating the function of CTHRC1/HIF-1α pathway through the induced ubiquitination of β-catenin [15]. The effect of CTHRC1 on tumor progression is associated with the regulation of various key signaling pathways.

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For example, CTHRC1 is involved in tumor progression and metastasis by interacting with Wnt, and can bind to integrin-β to activate downstream signaling pathways related to tumor progression and metastasis [16].

It is noteworthy that in some recent studies, CTHRC1 also plays a crucial part in tumors. CTHRC1 is overexpressed in various tumors, such as pancreatic cancer, colorectal cancer, squamous cell carcinoma, cervical cancer and so on[17]. A previous study confirmed that CTHRC1 expression was related to the progression and prognosis of cancer patients [18]. Hepatocellular carcinoma (HCC) patients with high CTHRC1 expression have a poor prognosis, and CTHRC1 expression can influence tumor size and stage [19]. CTHRC1 affects the liver metastasis of colorectal cancer by regulating the polarization of infiltrating macrophages to M2 via interaction with TGF-β signaling[20].CTHRC1 promotes GC metastasis through the HIF-1 α/CXCR4 axis[21]. CTHRC1 may promote the metastasis of epithelial ovarian carcinoma (EOC) through the induction of epithelial-mesenchymal transition (EMT) process, and can serve as a potential therapeutic and prognostic target in cancer patients [22]. The association between CTHRC1 and the prognosis of gastric carcinoma has been partially reported, but whether CTHRC1 expression is associated with immune cell infiltration in gastric carcinoma is still unclear. Tumor-infiltrating immune cells (TIICs) may influence tumor progression and provide insights into anticancer therapy [23]. Studies have shown that integrating multiple types of TIICs is an effective strategy for revealing the prognosis of cancer patients [24]. The comprehensive analysis of immune cell content in tumors may reveal the mechanism of tumor immune escape, thus affecting tumor progression and prognosis of tumor patients and providing new ideas for tumor treatment [25]. This study aimed to further investigate the relationships among CTHRC1 expression, the prognosis of GC patients and immune cell infiltration, and prognostic outcome in GC patients, which can provide new ideas for GC immunotherapy.

## 2. Materials and methods

### 2.1. Data on GC patients

The gene expression and clinical data of GC patients were downloaded from GDC Data Portal website(https://cancergenome.nih.gov/). GDC integrates and classifies data to provide unified cancer genome data, including 68 primary sites and 33,549 cases. It contains data from several large-scale cancer genome research projects, including TCGA and OCG. The gene expression data were standardized. The analysis used data from 463 patients with stomach cancer. Patients with missing and duplicate clinical information were deleted, and 439 patients' information was analyzed.

### 2.2. GEPIA database analysis

GEPIA is an online interactive website for gene expression analysis, which integrates RNA-Seq data of cancers and their normal tissues from various cohorts in the TCGA database and normal tissues from the GETx database, and is able to perform gene differential expression analysis, survival analysis, gene correlation analysis, and similar gene analysis, which allows us to perform data mining more easily and conveniently, to screening of key genes associated with cancer development, so as to further explore new cancer markers and targets[26]. In this paper, the analysis conditions of CTHRC1 were set as follows: |Log2FC|Cutoff>2, p-value Cutoff<0.05.

### 2.3. UALCAN database analysis

UALCAN (http://ualcan.path.uab.edu/index.html), is based on PERL CGI, javascript and CSS of the on-line analysis and mining sites[27, 28] .On the UALCAN database homepage, there are three main analysis options: TCGA transcript level analysis, CPTAC protein level analysis, and Pediatric Brain Tumor Analysis (CBTTC). Among them, TCGA database has a relatively complete analysis function, including differential, correlation and survival analyses, as well as miRNA and LncRNA analyses. UALCAN database was applied to determine the relationship between CTHRC1 expression and clinicopathological features in GC patients.

### 2.4. TIMER database analysis

The TIMER database is based on the transcriptome profiles of various types of cancers in the TCGA database and algorithmically calculates the level of infiltration of six types of immune cells in tumor tissues, B-cells, CD4 T-cells, CD8 T-cells, Neutrophils, Macrophages, and Dendritic cells [29, 30]. In addition, it allows analysis of the correlation between tumor immune infiltration and changes in the cancer genome and transcriptome, providing insights into tumor immune interactions. We used the TIMER database to analyze CTHRC1 expression in pan-cancer. This site was also used to determine whether CTHRC1 copy number variation (CNV) affects immune cell infiltration.

### 2.5. Kaplan-Meier (KM) plotter database analysis

By matching survival profiles with gene expression data, the Kaplan Meier database enables meta-analysis to explore whether target genes are associated with survival and to screen for prognostically relevant molecular markers.KM plotter was employed to analyze the relationship between CTHRC1 expression and prognostic outcomes in GC patients, including first progression (FP), overall survival (OS) and postoperative survival (PPS).

### 2.6. TISIDB database analysis

TISIDB(http://cis.hku.hk/TISIDB/) is a database that reflects the interaction between tumors and the immune system [31]. It can help explore the immune response in the tumor microenvironment and its impact on cancer progression and treatment.We can use it to explore the association between certain target genes and immunity in tumorigenesis and development. We analyzed the correlation between CTHRC1 and immune cell infiltration and immunostimulant genes using the TISIDB database.

### 2.7. ROC curve drawing and independent prognostic analysis

To test the possibility of CTHRC1 to predict prognosis, we plotted ROC curves to estimate 1-, 3- and 5-year survival rates using the sevivalROC R software package v4.1.0.The higher the AUC value, the higher the specificity and sensitivity. Univariate and multivariate independent prognostic analyses were performed using the survival R package to investigate whether CTHRC1 could be used as a prognostic indicator for gastric cancer patients.

### 2.8. Immune cell infiltration analysis

We used CIBERSORT, an immune cell infiltration algorithm, as well as the preprocessCore and Limma packages in R software to convert the gene expression matrix into an immune cell infiltration matrix. Then, the CorrPlot package was utilized to obtain the amount of immune cells in each sample. The Corrplot package was then employed to visualize immune cell infiltration. Finally, Pheatmap and Vioplot software packages were used to produce heat maps and violin maps of immune cell differences. The correlation between CTHRC1 and TIICs was calculated using the Limma, ggpubr, and ggExtra packages, and the obtained results were visualized.

### 2.9. CCLE database analysis

The CCLE database (Cancer Cell Line Encyclopedia:https://portals.broadinstitute.org/ccle) is a comprehensive database focusing on cancer cell lines and contains thousands of cancer cell lines that can provide a wide range of molecular characterizations to facilitate cancer exploration efforts.We used Perl software to search the data resources of all cancer cell lines in the database and extracted the gene expression data of gastric cancer cell lines from them.It was used in limma, ggplot2, ggpubr and ggExtra packages in R software to obtain genes with co-expression relationship with CTHRC1 through co-expression analysis. The screening criteria for co-expression analysis were set as follows: correlation coefficient threshold > 0.5, and correlation test p-value < 0.001. Subsequently, in the R software, we performed GO and KEGG enrichment analysis on the CRHTC1 co-expression-related genes obtained using relevant R packages.

### 2.10. Establishment of immunoregulatory gene prognostic model

We screened survival-related genes from all immune regulatory genes and constructed an immune regulatory gene risk model based on these genes. Using a specific formula, we calculated the risk score for each patient, defined as: Riskscore = coef(gene1) * expr(gene1) + coef(gene2) * expr(gene2) + coef(gene3) * expr(gene3) + ... + coef(genen) * expr(genen). Patients were then divided into high-risk and low-risk groups based on the median risk score. Subsequently, we combined survival data to plot survival curves and investigated whether there were survival differences between the high-risk and low-risk groups. To further validate the accuracy of the model, we plotted risk curves to explore whether there were differences in mortality rates between the high-risk and low-risk groups. Additionally, to determine whether this model could serve as an independent diagnostic tool for predicting patient prognosis, we performed univariate and multivariate independent prognostic analyses using R software.To verify the prediction accuracy of clinical factors and survival model, survivalROC R software package was employed to draw multi-index receiver operating characteristic curve (ROC). The higher the AUC value, the higher the specificity and sensitivity. In order to further verify the model's accuracy, the calibration line diagram and calibration curve were also constructed.

### 2.11. Statistical analysis

Spearman's correlation coefficient was used for correlation analysis. The hazard ratio (HR) and 95% confidence interval (CI) were applied to evaluate univariate and multivariate Cox proportional hazards regression models. Data processing and statistical analyses were performed using R software v4.1.0, and a p-value < 0.05 was considered statistically significant.

## 3. Results

### 3.1. Expression of CTHRC1 in GC and pan-cancer

The mRNA expression of CTHRC1 was remarkably elevated in most cancer tissues compared to normal tissue, especially in GC, as shown by TIMER online biomarker analysis ( 1A). To assess the differential expression of CTHRC1 in GC patients, we downloaded gene expression data from the TCGA database and performed differential analysis and paired difference analysis of CTHRC1 expression in all GC patients using R software. The data indicated that the mRNA expression of CTHRC1 was markedly higher in GC tissue than in normal tissue (Fig 1B,C). GEPIA database was utilized to further verify the differential expression of CTHRC1 in GC tissue and adjacent tissue (Fig 1D). We then assessed the correlation of CTHRC1 expression with Stomach adenocarcinoma(STAD) clinicopathological characteristics (age, sex, race, clinical stage, histology, TP53 mutation status) using the UALCAN online tumorigenomics database. It was found that CTHRC1 expression in COAD (Colon adenocarcinoma) patients was correlated with clinical stage and grading. The expression levels of CTHRC1 were dramatically higher in stages 2, 3 and 4 than in stage 1. Similarly, those of CTHRC1 were markedly higher in grade 3 than in grade 1 and 2

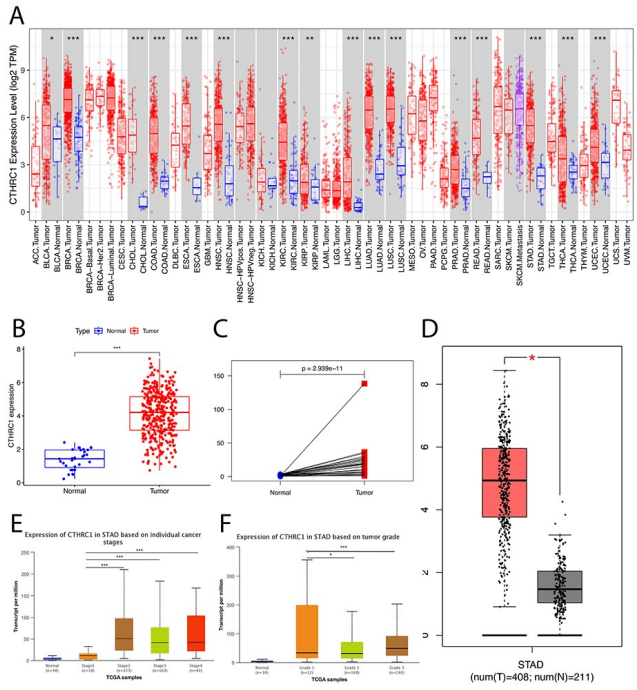


**Fig. 1.** The expression of CTHRC1 was analyzed by using TIMER, TCGA, GEPIA and UALCAN databases. (A) TIMER shows that CTHRC1 is upregulated in most cancers. (B) The expression level of CTHRC1 was significantly higher in GC patients compared with normal tissues in TCGA database. (C) The mRNA expression of CTHRC1 in TCGA database was higher in GC tissues than in paired normal adjacent tissues. (D)GEPIA database showed that CTHRC1 was highly expressed in GC. (E,F) CTHRC1 expression was correlated with clinical stage and grade. * p < 0.05, **p < 0.01, ***p < 0.001. (R software v4.1.0).

(Fig 1E).

### 3.2. Poor prognosis for stomach cancer patients with CTHRC1 overexpression

To analyze the correlation between CTHRC1 expression and GC prognosis, we divided all stomach cancer patients into high- and low-expression groups. We analyzed the relationship of CTHRC1 overexpression with OS, FP and PPS in 875 GC patients using the Kaplan Meier plotter database, and the findings demonstrated that CTHRC1 overexpression was associated with shorter OS, FP and PPS (Fig 2 A-C). We also obtained correlations between CTHRC1 expression levels and patient survival using R software to merge transcriptomic data for performing TCGA with clinical data. Then, survival curves were plotted. The findings demonstrated that the survival rate of GC patients with CTHRC1 upregulation was reduced (Fig 2D). We validated the sensitivity and specificity of CTHRC1 to predict prognosis by plotting ROC curves. The results showed that the AUC values were 0.561, 0.618 and 0.727 for estimating 1-, 3- and 5-year survival, respectively (Fig 2E). To determine whether CTHRC1 could be used as a clinical characteristic to predict patient prognosis, we performed one-way and multi-way independent prognostic analyses. The results showed HR of 1.192 (95%CI 1.048-1.356, P<0.008) and 1.156 (95%CI, 1.012-1.312, P=0.033) for the risk scores in both univariate and multifactor Cox regression analyzes (Fig 2F, 2G). Therefore, CTHRC1 is a fairly reliable prognostic indicator.

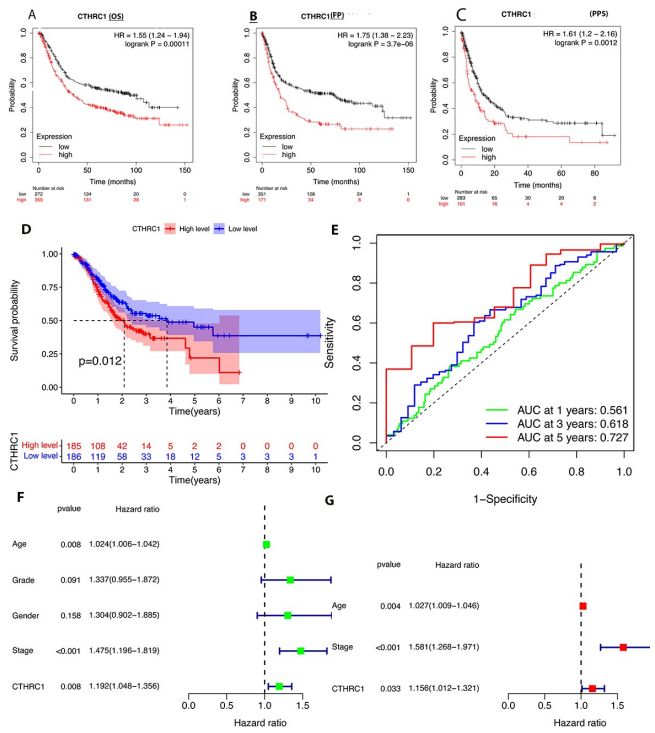


**Fig. 2.** CTHRC1 is a reliable prognostic biomarker in GC. (A-C) The prognosis of gastric cancer patients with high expression of CTHRC1 is poor. (D) Survival curves were plotted using data from the TCGA database. ( E) The results of the ROC curve showed that the AUC values were 0.561, 0.618 and 0.727, respectively, which were used to estimate 1, 3 and 5-year survival rates. (F,G) Univariate and multifactorial independent prognostic analyses were conducted to determine whether CTHRC1 could be used as an independent clinicopathological factor to determine the prognosis of patients. * p < 0.05, **p < 0.01, ***p < 0.001. (R software v4.1.0).

### 3.3. Functional analysis of CTHRC1 co-expressed genes

We used CCLE database to obtain data on GC cell lines. Genes co-expressed with CTHRC1 were obtained by co-expression method. The results showed that 388 genes were co-expressed with CTHRC1(Additional file1 in supplementary section). Figure S1 (Additional file2 in supplementary section) shows some of the co-expressed genes. CTHRC1 co-expressed genes were also selected for functional analysis and GSEA enrichment analysis. The findings showed that CTHRC1 co-expressed genes were enriched in many cancers, including GC, non-small cell lung cancer and colorectal cancer (Figure S2, Additional file3). Additional file 4 (in supplementary section ) revealed 61 pathways in which CTHRC1 co-expressed genes were enriched. To further elucidate the role of CTHRC1 in GC, GSEA was performed on the H-CTHRC1 and L-CTHRC1 groups, which were divided by the median level of CTHRC1 expression. H-CTHRC1 is abundant in TGF BETA SIGNALING PATHWAY, PATHWAYS IN CANCER and WNT SIGNALING PATHWAY (Fig S3A-3C). L-CTHRC1 is mostly enriched in OXIDATIVE PHOSPHORYLATION, GLYCOLYSIS/GLUCONEOGENESIS and CITRATE_CYCLE_TCA_CYCLE (Figure S3D-3F, Additional file5 in supplementary section). Suggesting that CTHRC1 acts as an oncogenic gene.

### 3.4. Correlation analysis of CTHRC1 expression with immune cell infiltration

The tumor microenvironment harbors a variety of cells that can contribute to tumor progression and metastasis, including immune cells. To explore immunotherapeutic approaches, conducting tumor immune analysis is essential. In this study, the immune infiltration algorithm CIBERSORT was employed to quantify the proportion of 22 TIICs in GC tissue and normal tissue (Fig 3A). To further investigate the underlying mechanism of this immune response, we analyzed the correlation of CTHRC1 expression with TIICs in GC (Fig 3B). The red box represents a positive relationship among TIICs, while the blue box indicates a negative relationship among TIICs. The values in the boxes represent correlations. The higher the numerical value, the stronger the pertinency. Activated memory CD4 T cell was positively correlated with CD8 T cell and macrophage M1; neutrophil was positively correlated with activated mast cell; and activated mast cell was positively correlated with activated dendritic cell. Activated memory CD4 T cell was negatively correlated with resting memory CD4 T cell. There were negative correlations between CD8T cell and macrophage M0, resting NK cell and activated NK cell, activated mast cell and resting mast cell. Reveals the composition of immune cells in the tumor microenvironment of gastric cancer tissues and the correlations among them. Subsequently, we analyzed the infiltration levels of 22 immune cells in GC tissue compared to normal stomach tissue (Fig 3C, 3D). The activated naive B cell, macrophage M0 and macrophage M1 were remarkably higher in GC tissue than in normal tissue. Plasma cells, CD8 T cells, resting memory CD4 T cells and resting mast cells were dramatically lower in tumor tissue than in normal tissue. To investigate whether there is an associa-

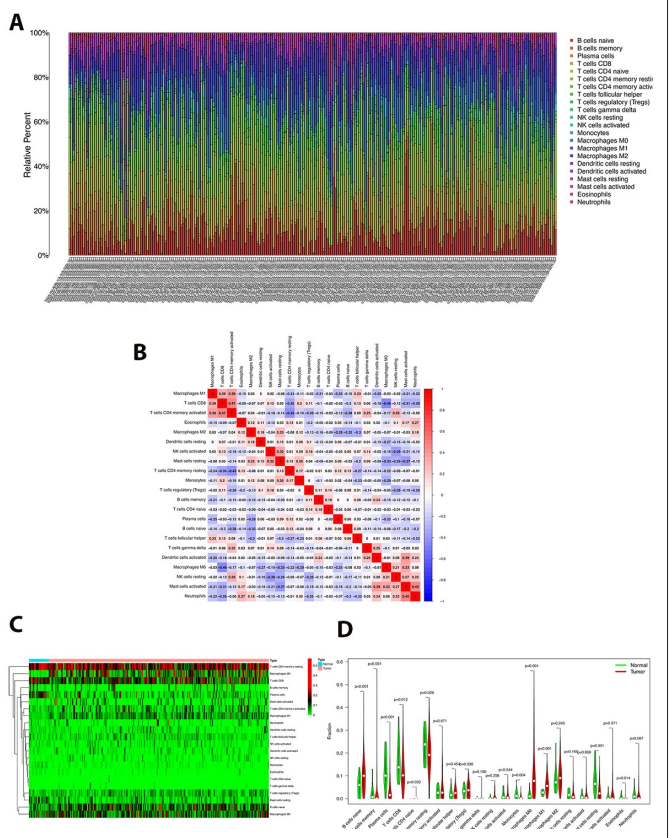

**Fig. 3.** CTHRC1 is a reliable prognostic biomarker in GC. (A-C) The prognosis of gastric cancer patients with high expression of CTHRC1 is poor. (D) Survival curves were plotted using data from the TCGA database. ( E) The results of the ROC curve showed that the AUC values were 0.561, 0.618 and 0.727, respectively, which were used to estimate 1, 3 and 5-year survival rates. (F,G) Univariate and multifactorial independent prognostic analyses were conducted to determine whether CTHRC1 could be used as an independent clinicopathological factor to determine the prognosis of patients. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (R software v4.1.0).

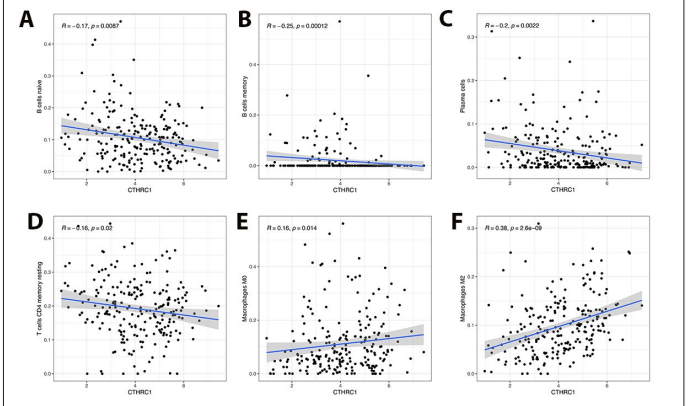

**Fig. 4.** Immune cells co-expressed with CTHRC1 expression level. (A-D) Immune cells negatively correlated with CTHRC1 expression. The R value represents the magnitude of the correlation. (E,F) Immune cells with positive correlation with CTHRC1 expression.

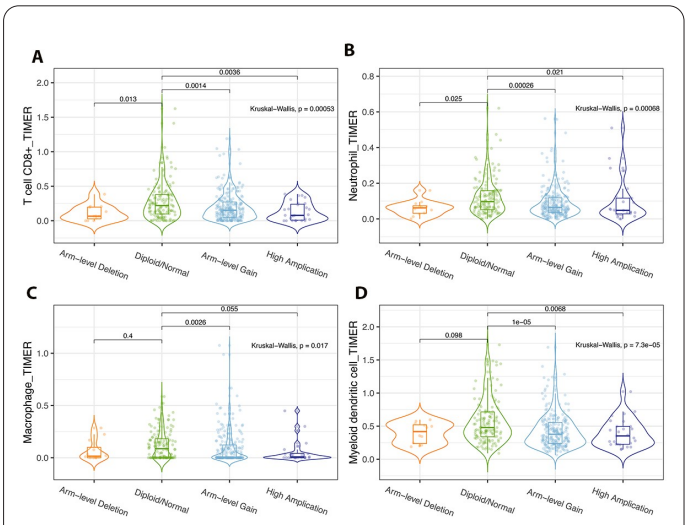

**Fig. 5.** Effect of CTHRC1 copy number variation(CNV) on immune cell infiltration.

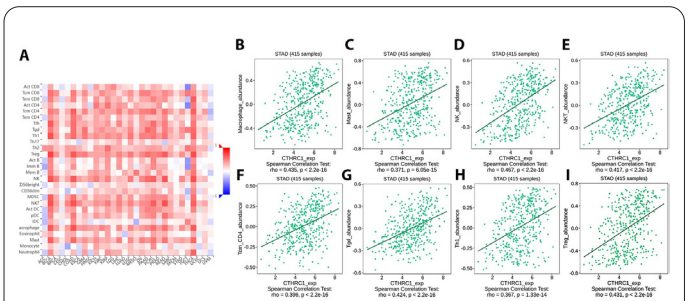

**Fig. 6.** The TISIDB database was used to obtain Spearman correlations of CTHRC1 with various lymphocytes. (A) The effects of CTHRC1 on lymphocyte infiltration in a variety of cancers (B-I) The eight cell types that were most associated with CTHRC1 expression.

tion between the aforementioned characteristics of tumor immune infiltration and CTHRC1.We further explored the association of CTHRC1 expression with immune cells. CTHRC1 expression was negatively correlated with naive B cell, memory B cell, plasma cell and resting memory CD4 T cell. CTHRC1 expression was positively correlated with macrophage M0 and macrophage M2 (Fig 4). The results suggest that CTHRC1 is associated with immune infiltration. Next,to further validate the above conclusions, we utilized TIMER database to determine the relationship between CTHRC1 CNV and TIICs. The findings demonstrated that CD8 T cell and neutrophil infiltration of arm-level Gain, arm-level Deletion and High Amplification were attenuated compared with Diploid/Normal group (Fig 5 A,5B). Macrophage of arm-level Gain group was significantly lower compared with Diploid/Normal group (Fig 5C). Mveloid dendritic cells of arm-level Gain and High Amplification were significantly decreased compared with Diploid/Normal group (Fig 5D). We thus concluded that CTHRC1 is indeed associated with immune infiltration in gastric cancer tissues. We also employed TISIDB database to evaluate the relationship between CTHRC1 expression and tumor-infiltrating lymphocytes (TILs) in 28 samples. Gene expression profiles-based GSVA analysis was conducted to infer the relative abundance of TILs. The results showed that 19 TILs were associated with CTHRC1 expression. Fig 6A shows the relationship between TILs abundance and CTHRC1 expression in different human cancers. Fig 6B-6I shows the correlation between some tumor- infiltrating lymphocytes with high rho values and CTHRC1 expression. These cells can promote immune escape of tumor cells and promote tumor progression.These results suggest that CTHRC1 is associated with tumor immune infiltration and that gastric cancer patients with high CTHRC1 expression have a poor prognosis.

### 3.5. The function of CTHRC1 in regulating immune molecules

To further investigate the mechanism by which CTHRC1 influences immune infiltration.We evaluated the association between two types of immune modulators and CTHRC1 expression utilizing the TISIDB database, which encompasses 24 immunoinhibitors and 45 immunostimulators. Figure 7A illustrates the correlation between immunoinhibitors and CTHRC1 expression across various human cancers, revealing that 11 immunoinhibitors were significantly associated with CTHRC1 expression. Figure 7B highlights six immunoinhibitors that exhibited strong correlations with CTHRC1. Figure 7C demonstrates the relationship between immunostimulators and CTHRC1 expression in different human cancers, with 29 immunostimulators showing significant associations. Figure 7D identifies six immunostimulators that were strongly correlated with CTHRC1. These results suggest that CTHRC1 may play a role in tumor immune infiltration by regulating immune modulators.

### 3.6. Construction of immunoregulatory gene prognostic model

To further predict GC prognosis, a prognostic model was established using immunomodulatory molecules associated with CTHRC1. Seven prognostic immunoregulatory molecules were screened by univariate COX from 40 immunoregulatory molecules, including one low-risk immunoregulatory molecule (HR<1) and 6 high-risk immunomodulatory molecules (HR>1) (Fig 8A). In addition, by multivariate Cox analysis, four immunoregulatory molecules were screened out from the above 7 prognostic immunoregulatory molecules, including one low-risk immunoregulatory molecule (TNFRSF18) and three high-risk immunoregulatory molecules (CXCR4, NT5E and TGFB2) (Fig 8B). These four immunomodulatory molecules were subsequently used to establish optimal prognostic risk models. All GC patients were assigned to low- and high-risk groups according to the model formula. The survival analysis revealed that GC patients in high-risk group had a worse prognosis (Fig 8C). Fig. S4A-C (Additional file 6) shows that patients with high expression of three high-risk immunoregulatory molecules (CXCR4, NT5E and TNFRSF18) have a poor prognosis, while patients with overexpression of low-risk immunoregulatory molecule (TNFRSF18) have a better prognosis, which is consistent with our previous findings. Risk curves and scatter plots were used to demonstrate the risk scores and survival status of patients, suggesting that high-risk patients had higher mortality (Fig 9A, 9B). Heatmap of four immunomodulatory molecules showed that TGFB2, CXCR4 and NT5E expression was upregulated in high-risk group, while TNFRSF18 was overexpressed in low-risk group (Fig 9C). To further validate the model's accuracy, the predictive value was evaluated. The specificity and sensitivity of the prognostic models were assessed by calculating the AOC values. The results showed that the survival model combined with other clinicopathological parameters had a good prediction effect. AUC value was 0.725, suggesting that the prediction model is very reliable (Fig 9D). Univariate and multivariate Cox regression analyses were employed to determine whether the four immunoregulatory molecular prognostic models could serve as independent predictors of GC. In univariate and multivariate Cox regression analyses, the HR values of risk score were 2.278 (95%CI 1.652−3.141, P<0.001) and 2.3991 (95%CI 1.697−3.390, P<0.001) (Fig 9E, 9F). Therefore, it can be seen from the above results that the survival model of four immunomodulatory molecules may serve as an independent predictor. In order to establish a clinically relevant quantitative method for predicting 1-, 2-, and 3-year survival, a nomogram integrating the prognostic model with clinicopathological risk factors was constructed (Fig

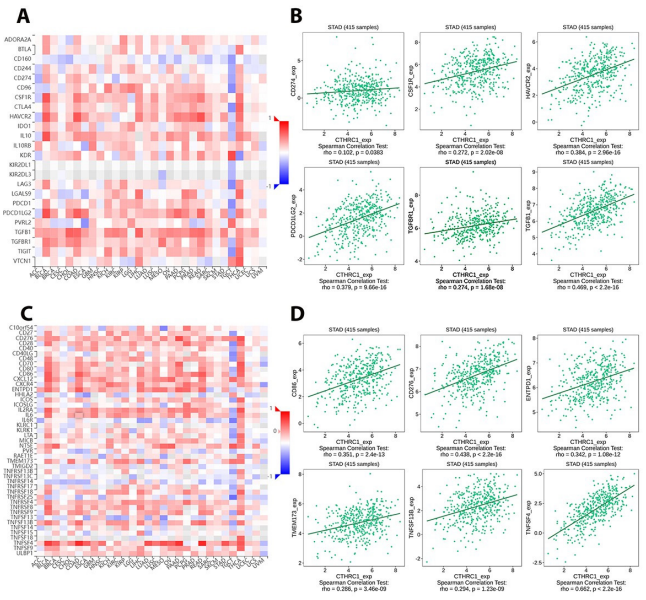


**Fig. 7.** The TISIDB database was used to obtain correlations between CTHRC1 and immunomodulators. (A) Association of CTHRC1 expression with all immunomodulators in different human cancers. (B)Six immunoinhibitors that were most correlated with CTHRC1 expression were shown. (C)Relationship between the abundance of immunostimulators and CTHRC1 expression in different human cancers. (D)Six immunostimulators that were most correlated with CTHRC1 expression were shown.

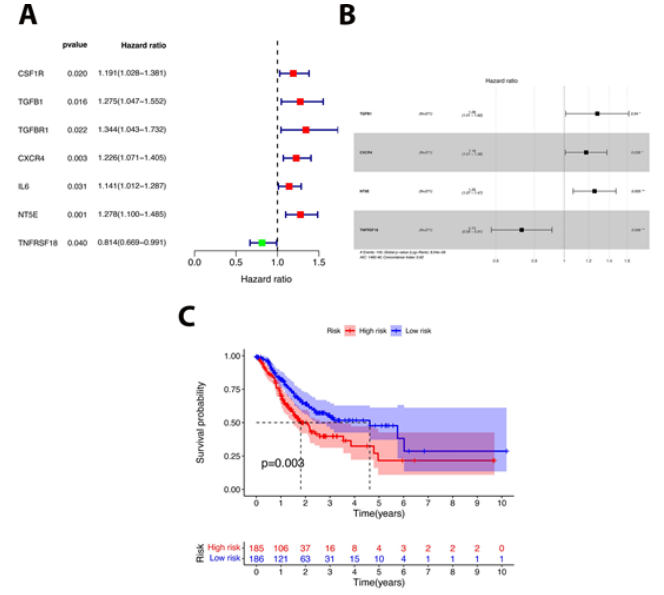


**Fig. 8.** Screening of immunomodulatory genes in prognostic models(A,B). Survival curves of GC patients in different groups(C). (R software v4.1.0)

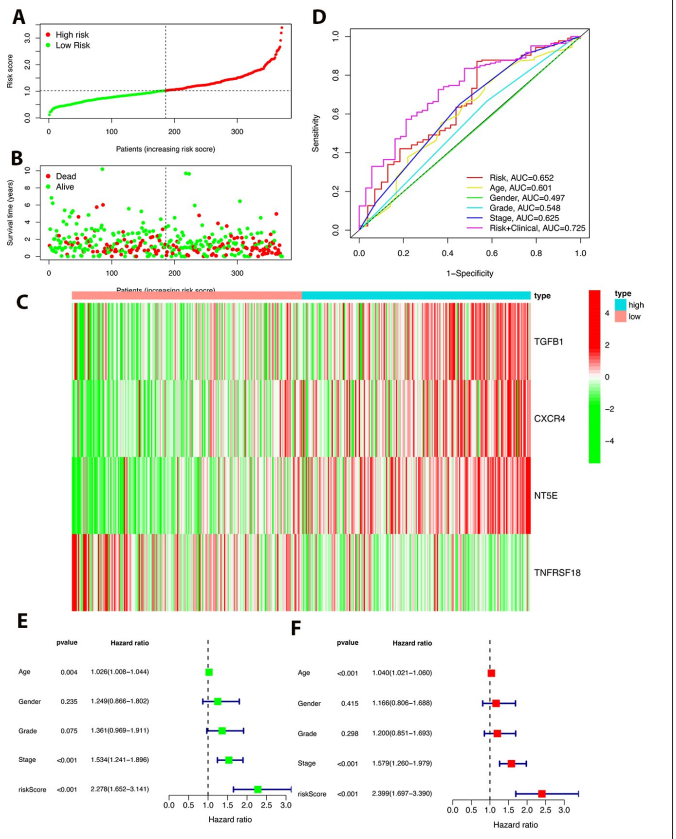


**Fig. 9.** Prognostic value of a survival model consisting of four immunomodulatory genes. (A) Low to high risk curves for all GC patients. (B) The band tabulates the survival status of all GC patients. The blue dots represent survival, and the red dots represent death. The number of deaths was significantly higher in GC patients with higher risk. (C) Heat map showing that TGFB2, CXCR4 and NT5E were upregulated in the high-risk group. TNFRSF18 was upregulated in the low-risk group. (D) AUC values and ROC curves for risk scores and clinical factors. (E) Results of univariate Cox regression analysis of the risk model. (F) Results of multivariate Cox regression analysis of the risk model.

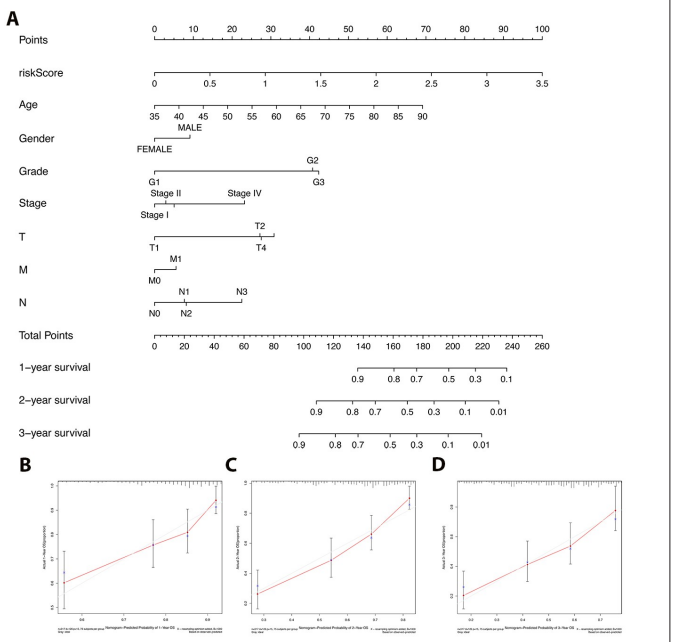


**Fig. 10.** Nomograms and calibration of prognostic models.( R software v4.1.0)

10A). The calibration diagram showed that the estimation of 1-, 2-, and 3-year survival was more accurate than the ideal model (Fig 10B). The results indicate that the constructed immunoregulatory gene prognostic model can serve as an independent predictive factor.

## 4. Discussion

GC is one of the most frequent cancers around the world, with a higher incidence in men than in women, mostly occurring between 60 and 80 years old. It is highly malignant and has a high mortality rate. Although the incidence and mortality of GC have decreased recently owing to the emphasis on early screening and improvement of treatment techniques, GC still poses a serious medical burden, especially in East Asia [32]. TNM staging is the most important clinical indicator for prognostic prediction and treatment. However, some studies have reported that TNM staging cannot be fully used to predict the prognostic outcomes of cancer patients[33-35]. Due to the tumor heterogeneity, individual differentiation is considerably great, and even the prognosis of patients with the same stage may be different. Therefore, it is necessary to discover new molecular markers or models for exploring the molecular mechanisms of GC, so as to more accurately judge the prognosis of GC patients and better develop clinical treatment strategies.

CTHRC1 is a secretory glycoprotein that regulates collagen deposition through TGFβ/BMP and Wnt/PCP pathways and has been found to play an indispensable role in several human cancer types [36]. In recent years, the roles of CTHRC1 in cancers have been increasingly studied. CTHRC1 overexpression promotes tumor invasion by inducing MMP9 expression in CRC, which is related to poor clinical prognosis[37]. Studies have shown that CTHRC1 overexpression is associated with late tumor stage, lymph node metastasis and poor prognosis in kidney renal papillary cell carcinoma patients [38]. CTHRC1 can affect the prognosis of prostate cancer by modulating the tumor microenvironment, and CTHRC1 targeted therapy may inhibit immune function in prostate cancer[39]. Patients with squamous cell carcinoma of the head and neck overexpressing CTHRC1 generally showed poorer disease, and the possible mechanism was through activation of tumor immune pathways leading to altered immune cell infiltration in the tumor microenvironment and macrophage polarization.[40]Studies have reported that CTHRC1 is highly expressed in breast cancer and is associated with macrophage infiltration, making it a promising immune-related biomarker for the treatment of breast cancer. This study also explored and identified that CTHRC1 is highly expressed in gastric cancer and is associated with poor prognosis, while also influencing tumor immune infiltration, which aligns with findings reported in previous studies. Perhaps, clinically, we can use predictive models to forecast patient prognosis and, based on these results, adopt tailored treatment strategies to improve patient survival rates. Additionally, gastric cancer has many biomarkers, including diagnostic, prognostic, and therapeutic markers. Exploring whether there is a certain connection between CTHRC1 and other prognosis-related biomarkers, or combining CTHRC1 with other known prognostic markers to assess the prognosis of gastric cancer patients and modify treatment methods, is a feasible decision. It has been reported that CTHRC1 is a secreted protein involved in pro-

cesses such as cell migration and invasion. Investigating whether there is a regulatory network between CTHRC1 and HER2 as well as E-cadherin, and combining their detection to better predict the prognosis of gastric cancer patients, is of significant interest.

TIICs in the tumor microenvironment have been shown to play a key role in tumor occurrence and influence the clinical outcomes of cancer patients. TIICs are highly heterogeneous and plastic, which can inhibit cancer or support tumor growth[41]. It has been reported that the distribution and morphology of TIICs are varied in different stages of GC, thereby affecting the progression and prognosis of GC patients [42, 43]. Many studies have shown that tumor-related genes can be used as molecular markers to reflect the level of immune cell infiltration in tumors. For example, CALD1 expression may influence the prognosis of GC patients by regulating GC immune cells[44]. In GC patients, LCP1 can regulate the interactions among immune cells, and is significantly and positively correlated with the invasiveness and poor prognosis of GC patients[45]. The differentiation of TIICs in GC may predict the prognosis of GC patients, and become a key influential factor for GC treatment[46].

In this study, we demonstrated the overexpression of CTHRC1 in GC using multiple databases, and elucidated that CTHRC1 may affect clinicopathological factors and be a reliable prognostic marker in GC patients. Further analysis revealed that CTHRC1 overexpression could affect the grade and stage of GC. Additionally, our analysis indicated that CTHRC1 expression in GC could affect the level of immune cell infiltration and different immune regulation. This study further demonstrated the significance of CTHRC1 expression in the immune microenvironment of GC. These results provide theoretical support for assessing the role of CTHRC1 in tumor immunity and its application as a biomarker for GC. We also obtained genes co-expressed with CTHRC1 by co-expression method and performed functional analysis. CTHRC1 co-expressed genes were found to be enriched in many cancers. We also used immunoregulatory molecules associated with CTHRC1 to construct prognostic models. Firstly, four genes were screened from 40 immunomodulator molecules to establish a prediction model, and the risk value of each patient was measured using a risk scoring formula, and GC patients were assigned to low- and high-risk groups based on the average risk score of the patients. Second, the survival curve of GC patients showed that the survival rate of GC patients in high-risk group was low. The risk curve and scatter plot demonstrated that the mortality of high-risk patients was remarkably higher than that of low-risk patients. The AUC value of the risk scoring model was 0.725, indicating that the prognostic models could predict the prognosis of GC patients. Third, to establish a clinically relevant quantitative method for estimating 1-, 2-, and 3-year survival, we constructed a nomogram integrating prognostic models with clinicopathological risk factors. Calibration plots demonstrated that the prediction of 1-, 2-, and 3-year survival was more accurate than the ideal model.

Although there are some limitations, this study provides us with a better understanding of the relationship between CTHRC1 and GC. First of all, the data collected in this paper are from the online database and need to be confirmed by both in vitro and in vivo tests. Moreover, this study primarily utilized data from the gastric cancer cohort in the open-access TCGA database, where the majority of patients are of European and American descent. This inevitably introduces some bias to the research findings. Further expansion of patient numbers and distribution across regions is necessary. Second, the roles of CTHRC1 gene in the diagnosis and treatment of GC have not been explored. Therefore, future studies are needed to further determine whether CTHRC1 can be an effective therapeutic target or diagnostic marker. We did not discuss the detailed molecular mechanism of CTHRC1 gene in GC. Hence, the exact mechanism of the relationship between CTHRC1 gene and GC needs to be further explored.

In summary, the correlation between CTHRC1 gene and GC was systematically analyzed. The expression of CTHRC1 was upregulated in GC, and patients with CTHRC1 overexpression had a poor prognosis. The relationships among CTHRC1, immune cell infiltration and immunomodulatory molecules were also explored. The findings demonstrated that CTHRC1 could affect the infiltration of various immune cells. Therefore, CTHRC1 may become a new cancer biomarker and therapeutic target for GC patients.

## Conflict of interests

The author has no conflicts with any step of the article preparation.

## Consent for publications

The author read and approved the final manuscript for publication.

## Ethical statement

Human specimens and animals were not used in this study.

## Informed consent

The authors declare that no patients were used in this study.

## Author contribution statement

Guo Tao's main contributions are data collection, software download, data visualization, experimental validation, article writing and article revision. The major contributions of Xie QiXin, Hong Deng and Changjun Yu are article revisions. Fang Changyi's main contribution is article revision and financial support.

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