Prognostic value and immunological role of cathepsin S gene in pan-cancer

SHENGSHENG LIANG[1*], BOWEN DANG[2*], SHAOHUA CHEN[3] and HUA MI[1]

[1]Department of Urology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530021; [2]Department of Urology, The First People's Hospital of Yulin, Yulin, Guangxi Zhuang Autonomous Region 537000; [3]Department of Urology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China

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Abstract. The cathepsin S (CTSS) gene encodes a lysine cysteine protease and serves an important role in the development of autoimmune diseases, inflammation and nervous system diseases. Furthermore, CTSS is implicated in tumor invasion and metastasis by the induction of tumor angiogenesis and the degradation of the tumor extracellular matrix. Nevertheless, the precise impact of CTSS on predicting pan-cancer prognosis and its influence on the tumor microenvironment and immune infiltration in human cancers remains unknown. This present study employed a comprehensive array of bioinformatic methods to evaluate the expression of CTSS and its associations with prognosis, clinicopathological characteristics, tumor microenvironment, tumor immune infiltration, tumor mutational burden and microsatellite instability across numerous cancer types. The current study demonstrated abnormal expression and distinct genomic alteration profiles of CTSS in many of the cancers tested. Furthermore, CTSS expression exhibited close associations with the prognosis of numerous cancers. High CTSS expression was significantly associated with better overall survival and disease-specific survival in bladder urothelial carcinoma (BLCA) and skin cutaneous melanoma (SKCM) but worse outcomes in brain lower grade glioma (LGG) and uveal melanoma (UVM). Moreover, CTSS demonstrated significant correlations with tumor mutational burden and microsatellite instability in 8 and 12 cancer types respectively, as well as different responses in immunotherapy sub-cohorts, especially in melanoma and bladder cancers. CTSS expression showed a positive correlation with stromal and immune cell scores in the four aforementioned cancers. Moreover, CTSS expression was correlated with the number of infiltrating $CD8^+$ T cells, $CD4^+$ T cells and macrophages. Conversely, CTSS was negatively associated with resting Mast cells, resting NK cells and resting memory $CD4^+$ T cell infiltration in BLCA, SKCM and kidney renal clear cell carcinoma (KIRC). Furthermore, CTSS expression was correlated with immune-related gene expression, notably PDCD1, LAG3, PDCD1 and TIGIT in BLCA, KIRC, SKCM, LGG and UVM. Functional enrichment analysis suggested that CTSS could drive a dynamic adjustment of biological functions and pathways in BLCA, SKCM, LGG and UVM, including immune response regulating signaling pathways, regulation of lymphocyte activation and T cell receptor singling pathways. The current study suggested that CTSS could be an essential biomarker for prognosis and immune infiltration features in multiple cancers.

Correspondence to: Dr Shaohua Chen, Department of Urology, Guangxi Medical University Cancer Hospital, 71 Hedi Road, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China
E-mail: silverchan1994@163.com

Dr Hua Mi, Department of Urology, The First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China
E-mail: mihua2019@163.com

*Contributed equally

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Introduction

The incidence and mortality of cancer continue to rise as the global population grows and ages. In 2020, an estimated 19.3 million new cancer cases and nearly 10 million cancer-related deaths occurred (excluding non-melanoma skin cancer) (1). Current therapeutic approaches for cancer include surgery, radiotherapy and chemotherapy. However, their effectiveness is far from satisfactory (2,3). Immunotherapy has revolutionized the field of cancer treatment, offering a promising direction for tumor treatment research (4). Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that reportedly strengthen T cell-mediated antitumor immunity and improve immune clearance of tumor cells (5,6). Antibodies for programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) (7) have been approved by the US Food and Drug Administration for use in the treatment of malignant tumors and have demonstrated promising results in clinical trials (8). Given that only a small subset of patients treated with these agents derive benefit (7),

it is essential to identify robust indicators for predicting treatment responses to immunotherapy.

Cathepsin S (CTSS), is a lysosomal protease-encoding gene located on the human 1q21 chromosome, which is mainly expressed in immune cells, including B cells, dendritic cells (DCs) and macrophages (9). CTSS has been reported to be associated with the development of numerous diseases, including autoimmune diseases, inflammation, nervous system diseases and cancers (such as pancreatic cancer, breast cancer and glioblastoma) (10,11). Notably, CTSS serves a pivotal role in major histocompatibility complex class II (MHC-II) antigen presentation, thereby influencing autoimmunity (12). Additionally, it can also activate protease-activated receptor 2 (PAR2) to boost the generation of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (13). These findings underscore the critical role of CTSS in regulating inflammatory responses and immune modulation.

Furthermore, CTSS expression is elevated in certain cancers, such as colorectal cancer (14), gastric cancer (15) and breast cancer (16), and has been demonstrated to serve a crucial role in tumor invasion and metastasis by inducing tumor angiogenesis and degradation of the tumor extracellular matrix (ECM) (11,17). Silencing of CTSS expression has been linked to the inhibition of malignant phenotypes in cancer cells and improved clinical outcomes in patients with breast cancer (16-19). Therefore, CTSS could be a potential predictive and therapeutic biomarker for cancer. However, the role of CTSS in tumorigenesis and its association with response rates to antitumor agents remain obscure. Dheilly *et al* (20) reported that gene mutation and amplification could contribute to the elevated expression of CTSS, thus inducing a tumor-promoting immune microenvironment in follicular lymphoma (FL), characterized by $CD4^+$ T cell enrichment and activation. In addition, their two independent clinical studies on FL have reported a correlation between CTSS and PDCD1 expression, further emphasizing its potential as a latent predictive biomarker for responses to anti-PD1 therapy. These observations suggest that CTSS may affect the tumor immune microenvironment (TIME) and could become a latent biomarker to predict patient response to ICIs (21).

The present study performed a comprehensive analysis of CTSS expression and its implications for overall survival (OS), progression-free interval (PFI) and disease-specific survival (DSS) across multiple cancer types. The correlation between CTSS expression and clinicopathological characteristics such as, the tumor microenvironment (TME), tumor immune infiltration, tumor mutational burden (TMB) and microsatellite instability (MSI) were also assessed. The methodology employed in the present study is outlined in Fig. 1, providing an overview of the investigative approach.

Materials and methods

Data acquisition and clinical specimen information. Datasets comprising 33 tumors types [acute myeloid leukemia, adrenocortical carcinoma (ACC), cholangiocarcinoma, bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma (CESC), colon adenocarcinoma (COAD), uterine corpus endometrioid cancer (UCEC), esophageal carcinoma (ESCA), glioblastoma (GBM), head and neck cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), diffuse large B-cell lymphoma (DLBC), liver hepatocellular carcinoma (LIHC), lower grade glioma (LGG), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), skin cutaneous melanoma (SKCM), mesothelioma, uveal melanomas (UVM), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectal adenocarcinoma (READ), sarcoma (SARC), stomach adenocarcinoma (STAD), testicular germ cell tumors (TGCT), thymoma (THYM), thyroid carcinoma (THCA) and uterine carcinosarcoma] were obtained from The Cancer Genome Atlas (TCGA) from the University of California Santa Cruz (UCSC Xena; https://xenabrowser.net/). These datasets contained RNA sequences (HTSeq-FPKM), somatic mutations (VarScan2 Variant Aggregation and Masking), survival (Survival data) and clinicopathological data (Survival_SupplementalTable_S1_20171025_xena_sp) of patients with cancer. The clinical KIRC tissue samples (n=4) were acquired from patients at The First Affiliated Hospital of Guangxi Medical University from December 2022 to March 2023 (additional validation sample added in October). The present study was approved by the Medical Ethics Committee of The First Affiliated Hospital of Guangxi Medical University (approval no. 2022-E387-01; Nanning, China). Informed consent was provided by each patient.

Differential expression and genomic alteration of CTSS in pan-cancer

CTSS expression extraction and integration. CTSS expression levels were extracted and integrated for subsequent analysis from RNA sequences using Perl software. Subsequently, differential CTSS expressions between cancerous and normal tissues in various cancers were analyzed utilizing the R-package 'ggpubr' (version 4.0; https://rpkgs.datanovia.com/ggpubr/) and shown as a box plot with a cut off value of P=0.05.

Assessment of CTSS changes in multiple cancers. CTSS alterations were assessed in multiple cancers using the cBioPortal database v5.4.7 (https://www.cbioportal.org). Data from 32 studies, including 10,953 patients (10,967 samples), were incorporated.

Connection of CTSS expression with prognosis and clinicopathological indicators

Survival analysis. The survival information for each patient was retrieved from the TCGA database and the CTSS expression matrix in tumor tissue was integrated with the survival time utilizing the 'limma' package (version 3.46.0; http://bioinf.wehi.edu.au/limma). Kaplan-Meier curves were made using the 'survminer' (version 0.4.9; https://rpkgs.datanovia.com/survminer/index.html) and 'survival' (version 3.3-1; https://github.com/therneau/survivalpackages) to visualize the connection between CTSS expression with patients' prognosis in terms of OS, PFI, and DSS. Univariate Cox regression analysis was performed to determine the hazard ratio (HR) with 95% confidence intervals and the P-value. Log-rank analysis was performed. A forest plot was delineated utilizing

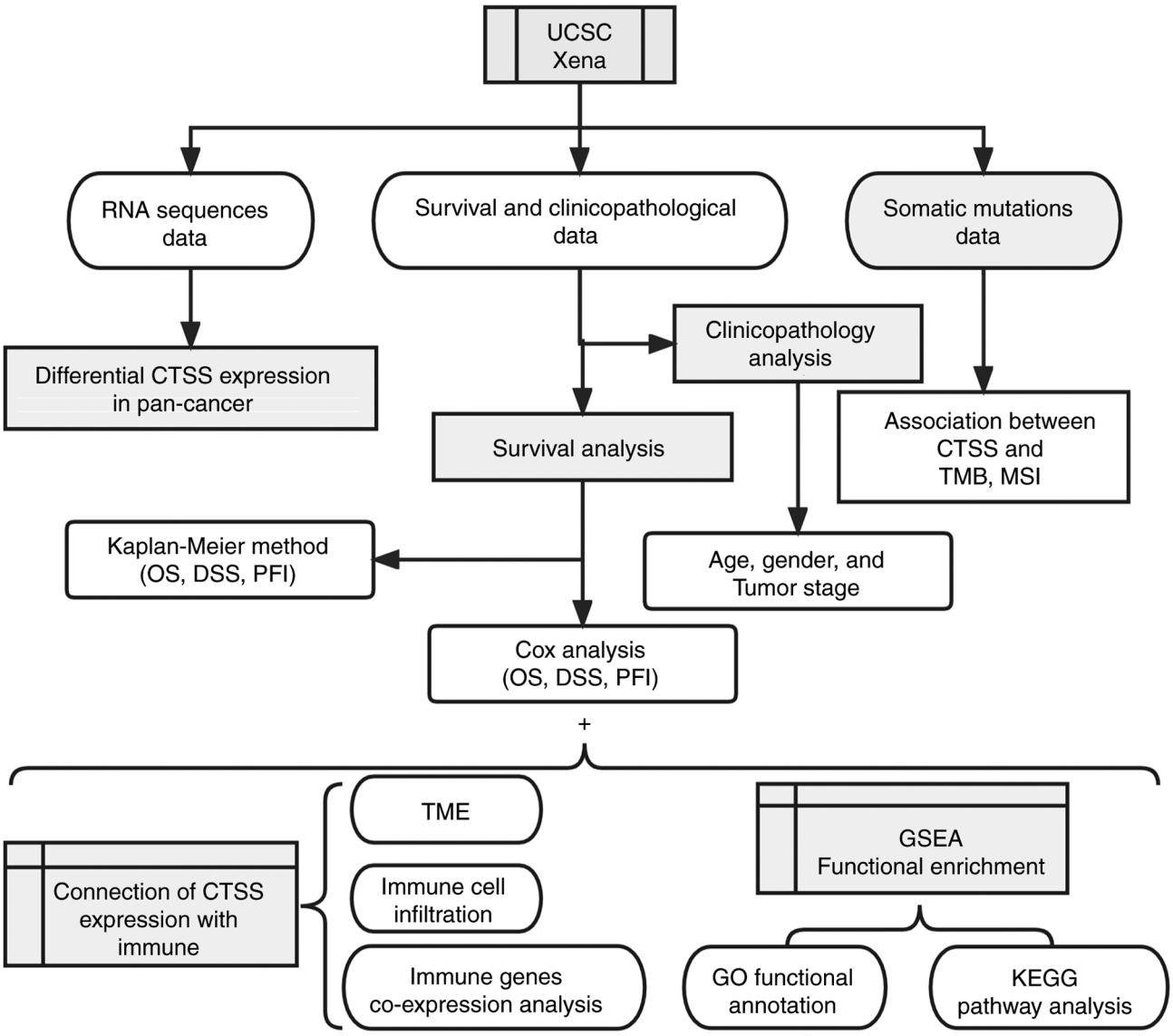

Figure 1. Analytical flow chart of CTSS. CTSS, cathepsin S; UCSC, University of California Santa Cruz; OS, overall survival; DSS, disease-specific survival; PFI, progression free interval; TMB, tumor mutational burden; MSI, microsatellite instability; TME, tumor microenvironment; GSEA, Gene Set Enrichment Analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

the 'survival' and 'forestplot' (version 2.0.1; https://gforge.se/packages/) packages.

Evaluation of CTSS expression with clinicopathological indicators. The connection of CTSS expression with clinicopathological indicators, including age, sex and tumor stage (American Joint Committee on Cancer) (22), was evaluated using the 'limma' and 'ggpubr' packages. CTSS in different cancer types was further analyzed using the 'Gene Outcome' module of TIMER2.0 (http://timer.cistrome.org/). This module used the Cox proportional hazard model to evaluate the outcome significance of CTSS gene expression, optionally adjusted by clinical factors (including age, sex and stage) and a heatmap illustrated the normalized coefficient of the CTSS gene in the Cox model.

Correlation between CTSS and immunotherapeutic response. Somatic mutation and MSI score were obtained from the TCGA database, and used to determine the normalization value of each sample's TMB. The connection of CTSS with TMB and MSI was visualized through radar maps generated using the 'fmsb' package (version 0.7.5; https://minato.sip21c.org/msb/) 'Biomarker evaluation' and 'query gene' modules of the Tumor Immune Dysfunction and Exclusion database (http://tide.dfci.harvard.edu/) were used to evaluate the potential function of CTSS as a biomarker for immunotherapy.

Connection of CTSS expression with TME. The immune and stromal cell scores of CTSS expression data were calculated using the ESTIMATE algorithm, implemented through the R-packages 'limma' and 'estimate' (version 1.0.13; https://r-forge.r-project.org/projects/estimate/). The connection of CTSS expression with stromal and immune scores was visualized utilizing the 'ggplot2' (version 3.3.5; https://ggplot2.tidyverse.org), 'ggpubr' and 'ggExtra' (version 0.10.0; https://github.com/daattali/ggExtra) packages.

Connection of CTSS expression with immune cell infiltration and immune-related genes. The CIBERSORT algorithm

Table I. Antibody information.

Antibody	Host	Dilution	Manufacturer	Cat. no.
CTSS	Rabbit	1:1,000	Cusabio Technology, LLC	CSB-PA10729A0Rb
β-actin	Mouse	1:2,000	Proteintech Group, Inc.	66009-1-Ig
Goat anti-rabbit	Goat	1:5,000	Thermo Fisher Scientific, Inc.	31460
Goat anti-mouse	Goat	1:5,000	Proteintech Group, Inc.	SA00001-1

CTSS, cathepsin S.

was used to analyze the relative proportion of immune cell infiltration in each sample. The connection between CTSS expression and immune cell infiltration was visualized using the 'ggplot2', 'ggpubr' and 'ggExtra' packages. Then, the relationship between CTSS mutation and immune infiltration was further explored using the 'Mutation' module of TIMER2.0 (http://timer.cistrome.org/). Moreover, the correlation between immune-related genes and the CTSS gene was analyzed using the 'limma' package, and the results were visualized in a heatmap created with the 'reshape2' (version 1.4.4. URL: https://github.com/hadley/reshape) and 'RColorBrewer' (version 1.1-3) packages.

Functional enrichment analysis of the CTSS gene. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets were downloaded from the Gene Set Enrichment Analysis site (https://www.gsea-msigdb.org/gsea/downloads.jsp). The GO functional annotation and KEGG pathway enrichment analyses of CTSS were performed and visualized utilizing the 'limma', 'org.Hs.eg.db' (version 3.12.0), 'clusterProfiler' (version 3.18.1; https://yulab-smu.top/biomedical-knowledge-mining-book/) and 'enrichplot' (version 1.10.2; https://yulab-smu.top/biomedical-knowledge-mining-book/) packages.

Validation of differential expression of CTSS

Reverse transcription quantitative PCR (RT-qPCR). Total tissue RNA was extracted from the frozen normal and KIRC tissues using the AxyPrep Total RNA Small Volume Preparation Kit (cat. no. UEL-UE-MN-MS-RNA-50G; Corning, Inc.). The Fast Start Essential DNA Green Master kit (Roche, USA) was utilized for PCR amplification and fluorescence quantification of nucleic acids. The PCR was performed on an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: Initially, a three-step amplification process was employed, comprising 10 sec at 95˚C, 10 sec at 60˚C and 10 sec at 72˚C, for a total of 45 cycles. Subsequently, a melting stage was executed, involving thermal insulation at 95˚C for 10 sec, 65˚C for 60 sec and 97˚C for 1 sec. Finally, the reaction mixture was cooled at 37˚C for 30 sec. The primer sequences used were as follows: CTSS forward (F), 5'-TGACAACGGCTTTCCAGTACA-3' and reverse (R), 5'-GGCAGCACGATATTTTGAGTCAT-3'; and β-actin F, 5'-GTCATTCCAAATATGAGATGCGT-3' and R, 5'-GCTATCACCTCCCCTGTGTG-3'. β-actin was used as an internal control gene and the $2^{-\Delta\Delta Cq}$ method was used to calculate the relative expression level (23,24).

Western blotting. Total tissue protein was extracted using RIPA lysate buffer (Beijing Solarbio Science & Technology Co., Ltd.), containing protease inhibitors (including 1% PMSF and 1% phosphoprotease inhibitors) and the protein concentration was then quantified using the BCA Protein Assay Kit (EpiZyme Scientific). After adding 5x SDS-PAGE protein loading buffer, the protein sample was boiled at 100˚C for 10 min. The proteins (50 μg/lane) were separated by SDS-PAGE on a 12% gel, then transferred to a polyvinylidene fluoride membrane. The membrane was blocked with 5% skimmed milk for 1 h and then washed thrice for 5 min each, in 1x TBST. The membrane was then incubated with primary antibodies at 4˚C overnight, washed in TBST, then incubated with secondary antibodies at room temperature for 1 h. Finally, the blots were visualized using the Immobilon Western Chemiluminescent HRP substrate (Merck KGaA) and analyzed using Image J software 1.8.0 (National Institutes of Health) and GraphPad Prism 9.4.0 (Dotmatics). The antibody information is provided in Table I.

Immunohistochemistry. Immunohistochemical staining images of CTSS protein expression in three normal tissues and malignant tumors tissues were downloaded from the Tissue Atlas and Pathology Atlas of the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/).

Statistical analysis. The Wilcoxon signed-rank test was used to assess differential CTSS expression in tumor and normal tissues. Univariate Cox regression analysis and Kaplan-Meier methods were used to assess the association of CTSS expression with patients' survival. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were processed using R (version 4.0.3), Strawberry Perl (version 10.0.22000.527) and GraphPad Prism (version 9.4.0).

Results

Expression and genomic alteration profiles of CTSS in pan-cancer. To examine the variations in CTSS mRNA levels between cancerous and normal tissues, R software was used to analyze data from the TCGA database. These findings demonstrated that CTSS expression was significantly higher in malignant tissues compared with benign tissues in CESC, GBM, KIRC, KIRP, STAD, THCA and UCEC. Conversely, significantly lower CTSS expression was observed in COAD, LUAD, LUSC, PAAD, PRAD and READ (Fig. 2A). Moreover, changes in CTSS expression were assessed across various cancers. According to

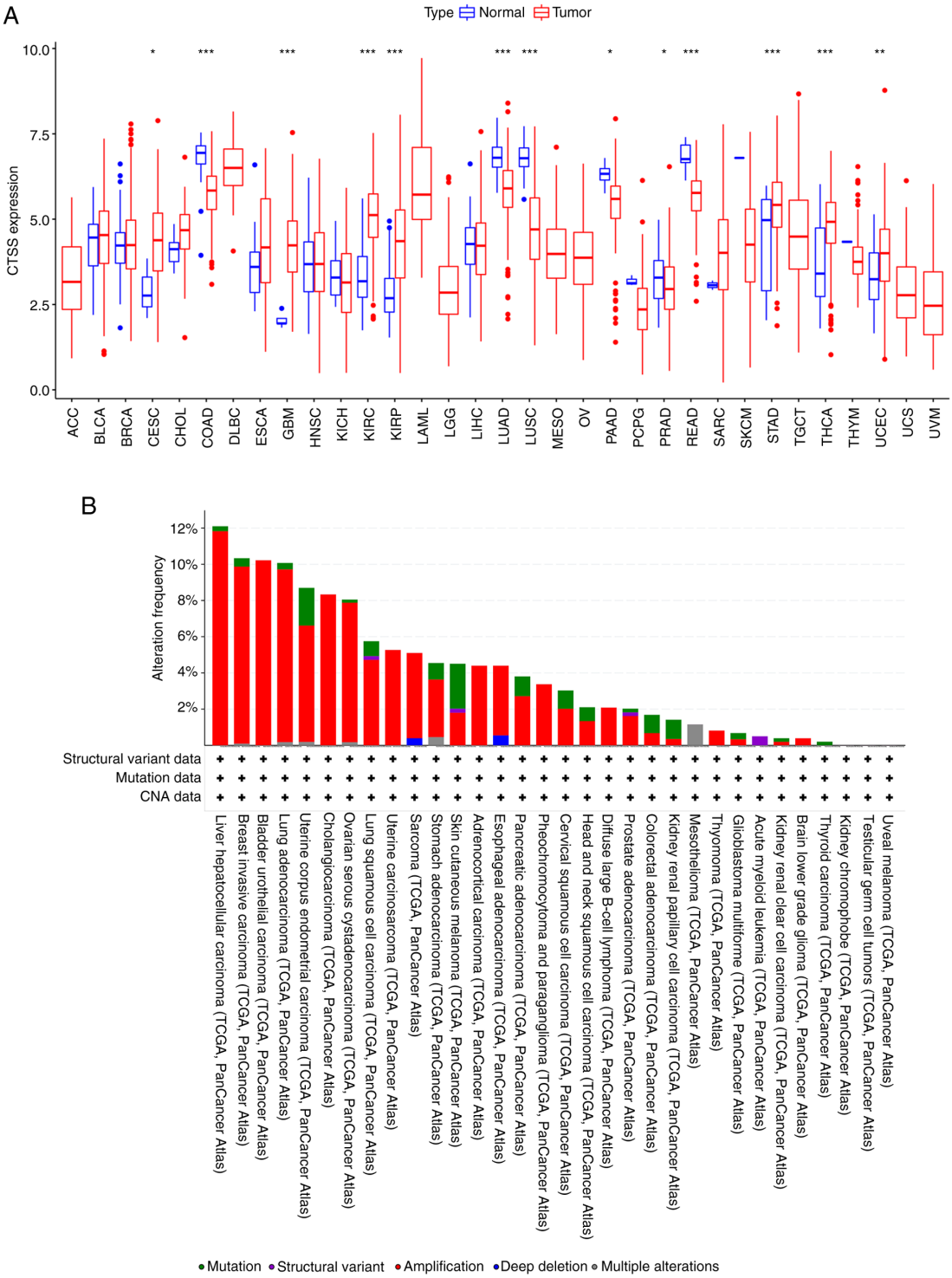

Figure 2. Expression levels and genomic alteration of CTSS gene. (A) CTSS expression in 33 human cancer types. (B) Alteration profiles of the CTSS gene in diverse malignant tumors from the cBioPortal database. *P<0.05, **P<0.01 and ***P<0.001. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma; HNSC, head-neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; TCGA, The Cancer Genome Atlas; CTSS, cathepsin S; CNA, copy number alteration.

the cBioPortal database, there were changes to the CTSS gene in 518 (5%) of 10,953 patients, with amplification being the most common alteration, followed by mutation. Notably, LIHC displayed the highest frequency of CTSS alteration among all cancers assessed, while SKCM had the highest mutation frequency relative to total alterations (Fig. 2B). These results

underscored the abnormal expression and distinct genomic alteration profiles of CTSS in pan-cancer datasets.

Relationship between CTSS expression and prognosis in multiple cancers. To comprehensively assess the correlation between CTSS expression and prognosis in patients with cancer, the relationship between CTSS and survival-related indicators OS, PFI and DSS was analyzed for 33 cancer types using univariate Cox analysis and Kaplan-Meier methods.

OS. The present study demonstrated significant associations between CTSS expression and OS for seven cancer types, including LGG (P<0.001, HR=1.492), BLCA (P=0.003, HR=0.832), THYM (P=0.007, HR=2.487), PAAD (P=0.042, HR=1.213), SKCM (P<0.001, HR=0.818), SARC (P=0.025, HR=0.855) and UVM (P=0.004, HR=1.517) (Fig. 3A).

Kaplan-Meier OS curves demonstrated a significant positive association between OS and CTSS in BLCA (P=0.048), OV (P=0.011), SKCM (P<0.001) and SARC (P=0.007) however, a significant negative association was observed between OS and CTSS in LGG (P=0.006) and UVM (P=0.002) (Fig. 3B).

DSS. CTSS expression was significantly correlated with DSS in BLCA (P<0.001, HR=0.785), CESC (P=0.030, HR=0.785), LGG (P<0.001, HR=1.539), LUAD (P=0.023, HR=0.799), SKCM (P<0.001, HR=0.798), THCA (P=0.004, HR=0.436), and UVM (P=0.017, HR=1.434) (Fig. 4A).

Kaplan-Meier curves of DSS demonstrated that high CTSS expression was significantly associated with a favorable prognosis in BLCA (P=0.038), OV (P=0.005), SARC (P=0.029), SKCM (P<0.001) and THCA (P=0.050), and was significantly associated with an unfavorable prognosis in LGG (P=0.003) and UVM (P=0.008) (Fig. 4B).

PFI. Furthermore, the present study demonstrated significant associations between CTSS expression and PFI in 6 cancer types; BLCA (P=0.003, HR=0.832), COAD (P=0.013, HR=0.739), GBM (P=0.007, HR=1.257), LGG (P<0.001, HR=1.374), SKCM (P=0.018, HR=0.910) and THYM (P=0.041, HR=1.688) (Fig. 5A).

Kaplan-Meier curves demonstrated that higher CTSS expression was significantly correlated with poor PFI in GBM (P=0.007) and LGG (P=0.009) (Fig. 5B).

Summary of patient prognosis indicators. Collectively, these results indicated the significant association between CTSS expression and patient prognosis in various cancer types, including BLCA, SKCM, LGG and UVM. Which supported the potential use of CTSS as a biomarker for predicting patient prognosis.

Relationship between CTSS expression and clinicopathological indicators in pan-cancers. To assess the relationship between CTSS expression and clinicopathological indicators in pan-cancer, CTSS expression was analyzed across different age groups, sexes and tumor stages in patients with cancer.

Age. It was demonstrated that CTSS expression was significantly higher among cancer patients ≥65 years compared with patients <65 years old, in ESCA (Fig. 6A), LUAD (Fig. 6B) and PRAD (Fig. 6C).

Sex. There was significantly lower expression of CTSS in males with LUSC (Fig. 6D). Conversely, CTSS was expressed at significantly higher levels in males with KIRC (Fig. 6E) and SARC (Fig. 6F).

Cancer stage. It was demonstrated that CTSS was significantly upregulated in patients with stage I-II ACC (Fig. 6G), COAD (Fig. 6H) and LUAD (Fig. 6J) compared with patients with the respective cancers at stage III-IV. However, CTSS was significantly downregulated in patients with stage I-II ESCA compared with patients with stage III-IV ESCA (Fig. 6I).

Moreover, the 'Gene Outcome' module analysis results indicated that CTSS was significantly correlated with multiple clinical factors (including age, sex and stage) in KIRC, SARC, SKCM and UVM (Fig. 6K). Collectively, these results indicated that CTSS was strongly related to clinicopathological indicators in multiple cancers, including ESCA, KIRC and SARC.

Relationship between CTSS expression and immunotherapeutic response. To examine the relationship between CTSS and immunotherapy response, the association of CTSS with TMB and MSI in multiple malignant tumors was analyzed. This analysis indicated that CTSS was significantly related to TMB in eight cancer types, with significant positive associations in LGG, BRCA and ESCA and significant negative associations in LIHC, LUAD, LUSC, HNSC and GBM (Fig. 7A).

Furthermore, CTSS was significantly negatively associated with MSI in twelve cancer types; DLBC, HNSC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, PCPG, SKCM and TGCT (Fig. 7B).

Moreover, the significance of CTSS as a biomarker was estimated by comparing it with other established biomarkers, based on their predictive ability of the response in certain immunotherapeutic sub-cohorts. The results indicated that CTSS, whose area under the dose-response curve (AUC) value was >0.5 in 16 out of 25 sub-cohorts (64%), demonstrated a higher predictive value than MSI and B-cell clonality, whose AUC values were >0.5 in 13 out of 24 sub-cohorts (54%) and 7 out of 14 sub-cohorts (50%), respectively (Fig. 7C).

The relationship between CTSS expression and immunotherapy clinical response in certain cancer types was then assessed. It was shown that higher CTSS expression was significantly associated with improved clinical outcomes of OS in colorectal cancer and significantly correlated with improved OS in renal cancer, melanoma, bladder cancer, lung cancer, lymphoma, sarcoma and ovarian cancer. While low CTSS expression was significantly correlated with a poor prognosis in glioma and uveal cancer. Notably, CTSS expression was positively associated with cytotoxic T lymphocytes (CTLs) in the aforementioned cancer cohorts (Fig. 7D). Overall, these findings indicated the intricate interplay between CTSS expression, immune response and clinical prognosis in these specific cancer types.

Relationship between CTSS expression and TME in pan-cancer. Based on survival analysis, it was demonstrated that high CTSS expression correlated with a good prognosis in BLCA and SKCM but correlated with a poor prognosis in LGG and UVM. Therefore, based on the correlation between these cancers and prognosis (25), they were grouped into low-risk cancer (BLCA and SKCM) and high-risk cancer (LGG and

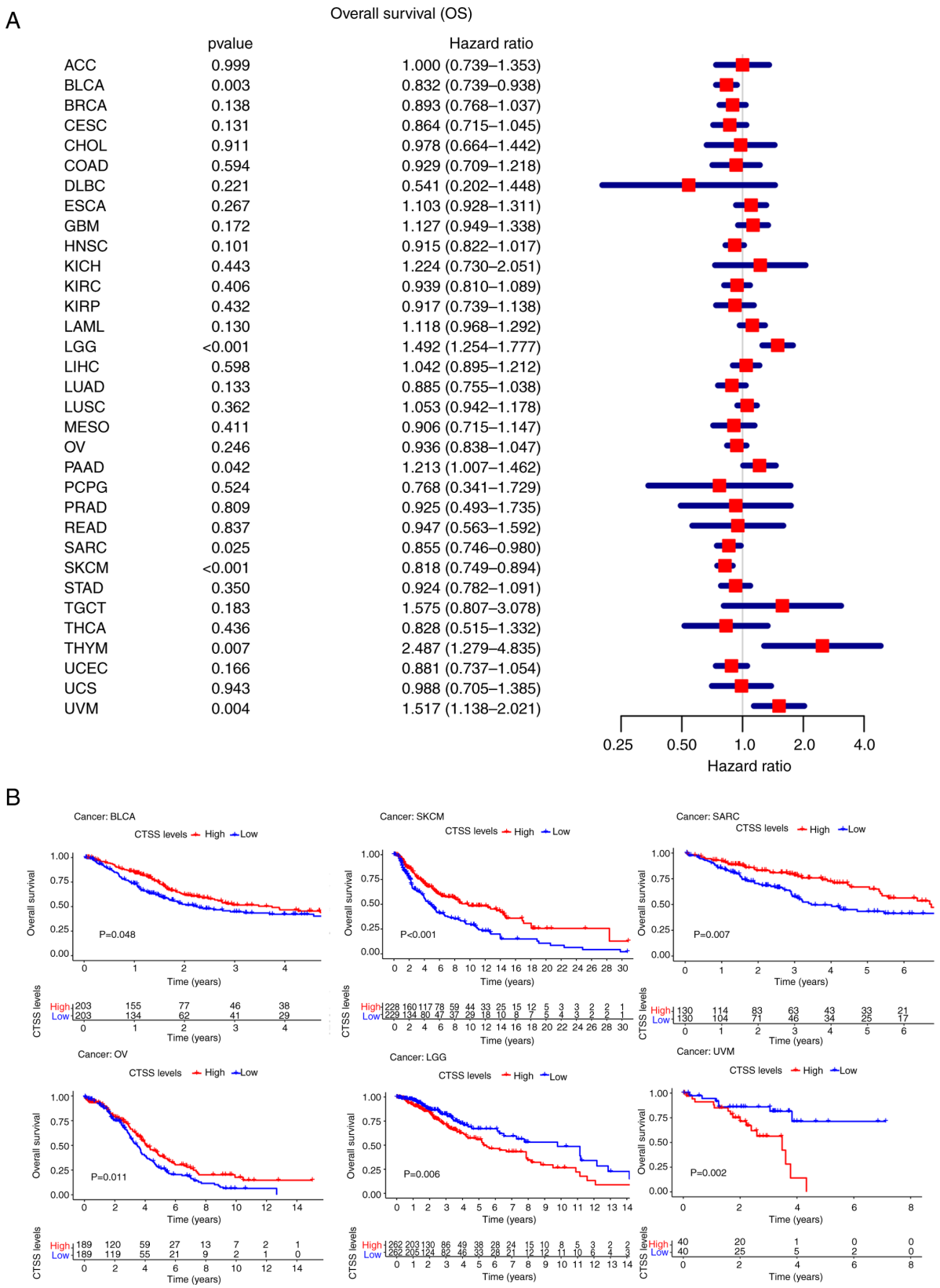

Figure 3. Relationship between CTSS expression and the overall survival (OS) in pan-cancer. (A) Forest plot showing the hazard ratios of CTSS in pan-cancer. (B) Kaplan-Meier curves demonstrate the correlation of CTSS expression with the patients' OS in BLCA, SKCM, SARC, OV, LGG, and UVM. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma; HNSC, head-neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; CTSS, cathepsin S.

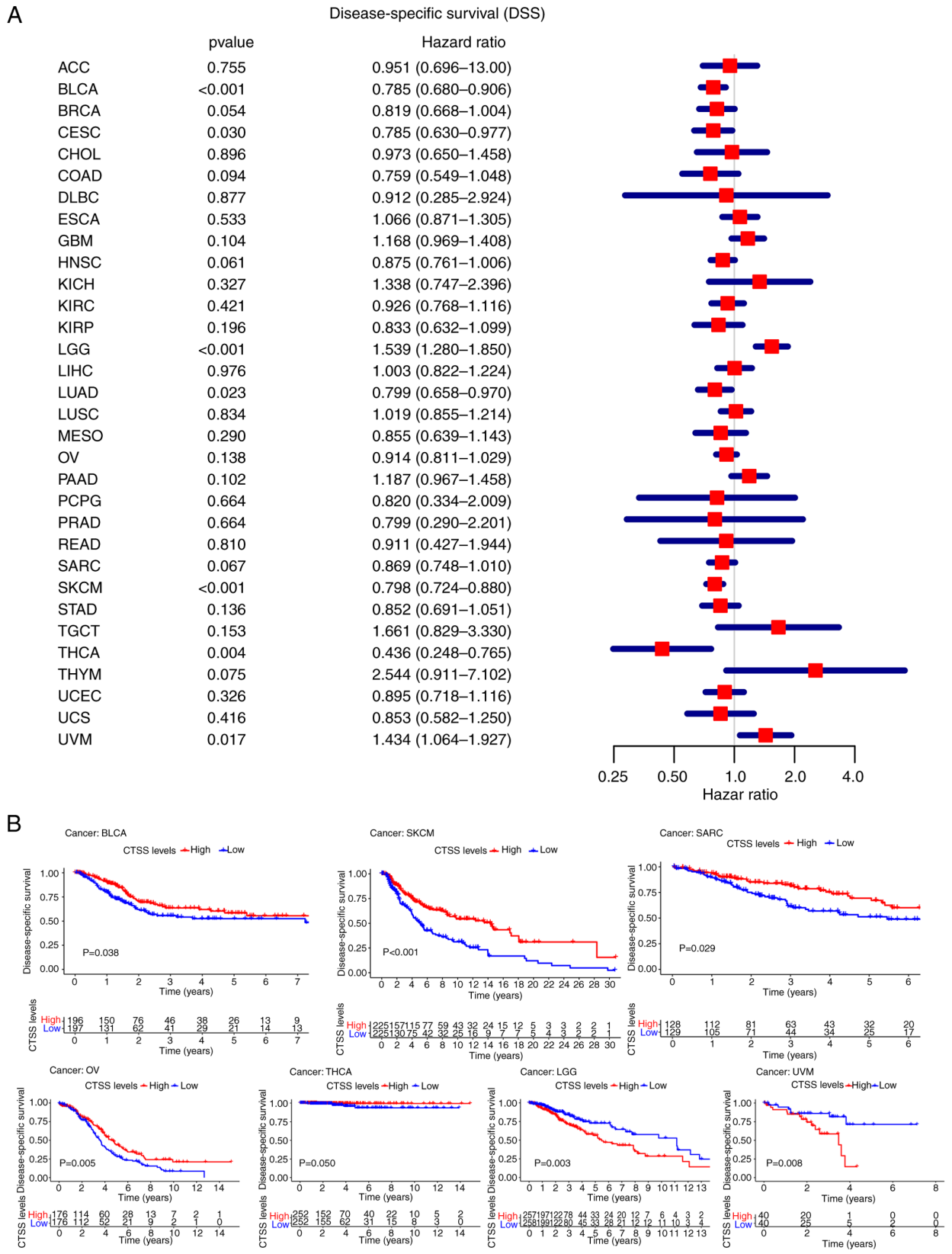

Figure 4. Correlation analysis of CTSS expression with the DSS in pan-cancer. (A) Forest plot showing the hazard ratios of CTSS in pan-cancer. (B) Kaplan-Meier curves demonstrate the connection of CTSS expression with the patients' DSS in BLCA, SKCM, SARC, OV, THCA, LGG, and UVM. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma; HNSC, head-neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; CTSS, cathepsin S.

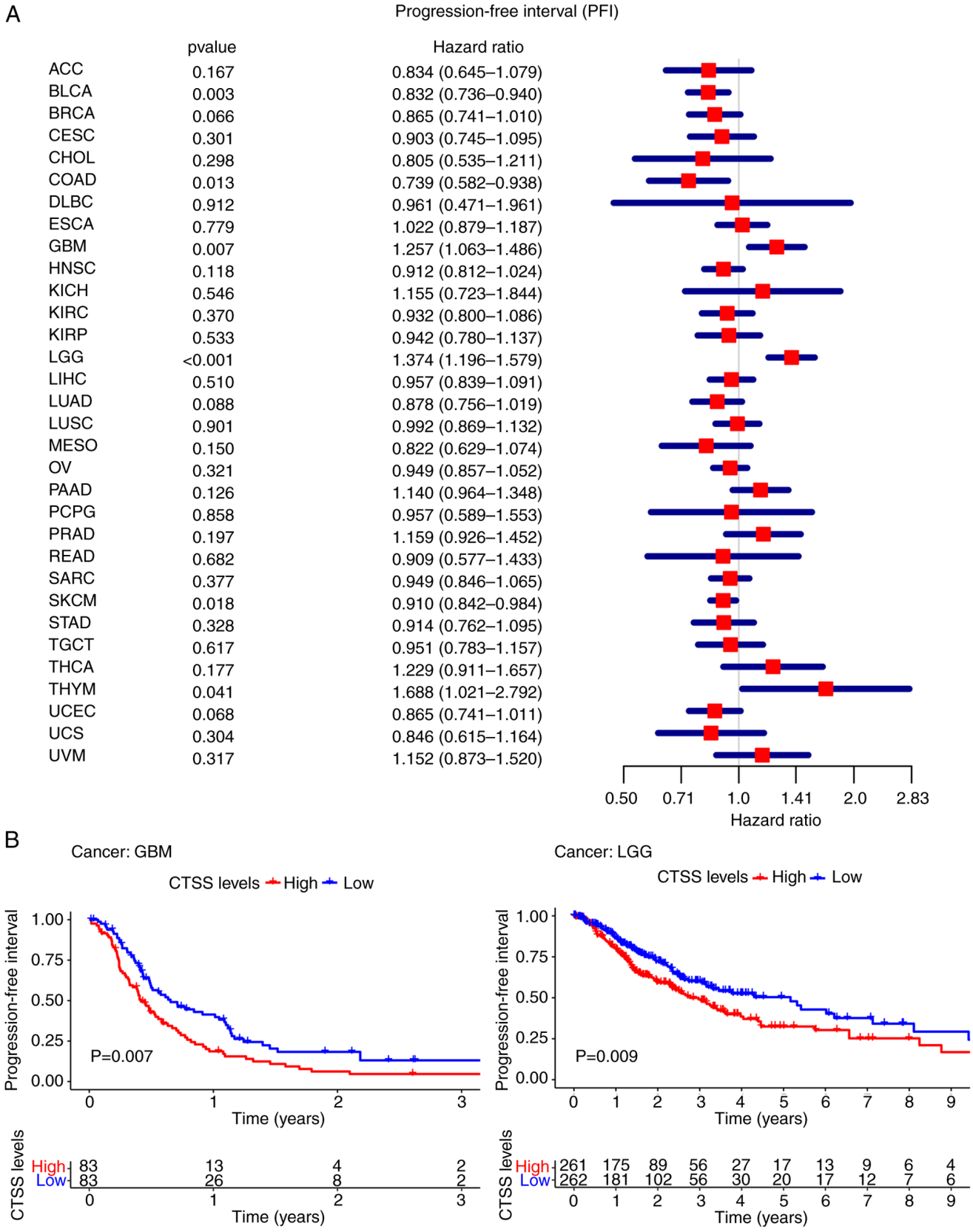

Figure 5. Correlation of CTSS expression with the PFI in pan-cancer. (A) Forest plot showing the hazard ratios of CTSS. (B) Kaplan-Meier curves demonstrate the association between CTSS expression and the patients' PFI in GBM and LGG. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma; HNSC, head-neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; CTSS, cathepsin S.

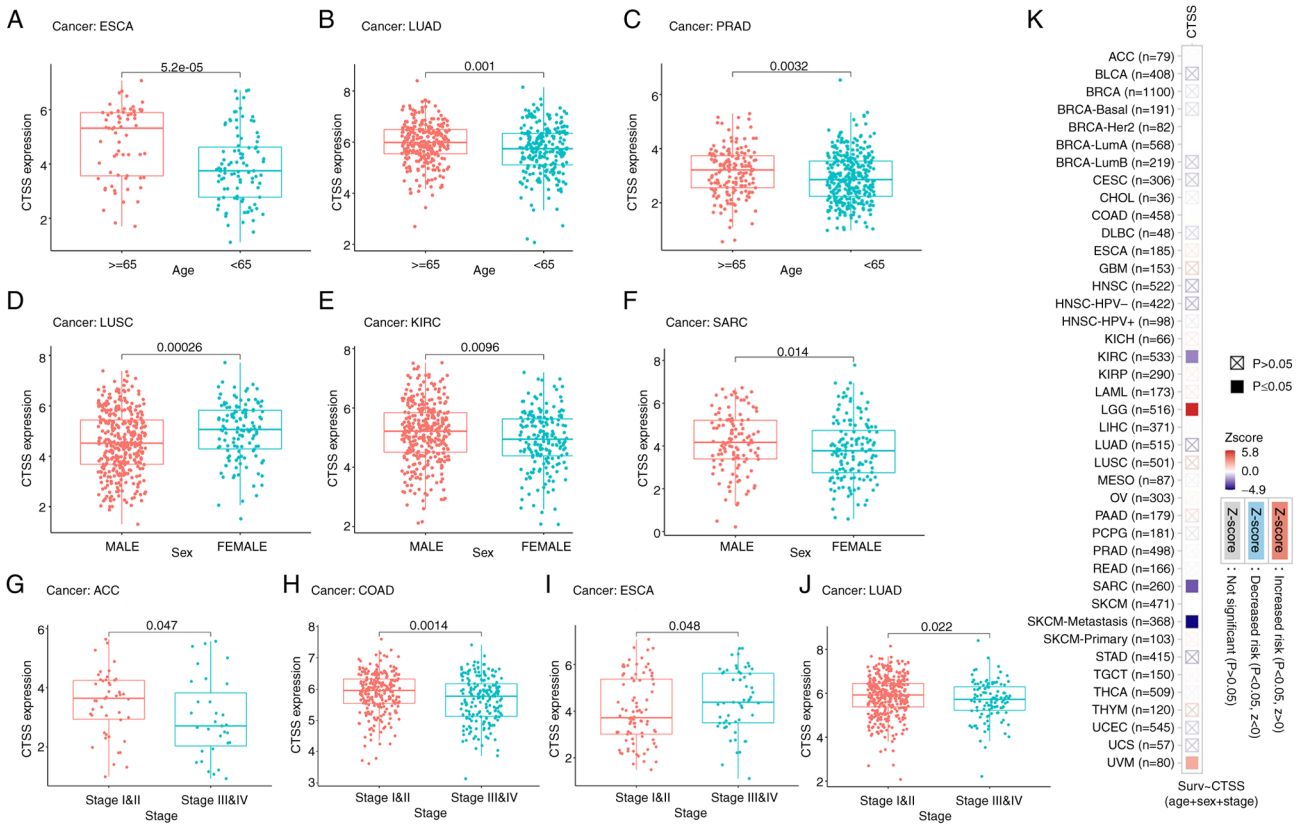

Figure 6. Correlation of CTSS expression with clinicopathological indicators in multiple cancers. Box plots showing the correlation between CTSS expression and (A-C) age, (D-F) sex and (G-J) cancer stage. (K) Heatmap showing clinical correlation (multiple clinical factors including age, sex and stage) of CTSS expression in different types of cancer. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma; HNSC, head-neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; CTSS, cathepsin S; LumA, luminal A; LumB, luminal B; Her2, HER2-enriched, Basal, basal-like; HPV, human papillomavirus.

UVM). To assess the relationship between CTSS expression and TME in these cancer types, the ESTIMATE algorithm was applied to analyze the stromal and immune scores for the four selected cancers. It was found that CTSS was significantly correlated with stromal and immune scores in BLCA, SKCM (Fig. 8A), LGG and UVM (Fig. 8B).

Correlation analyses of CTSS expression with immune cell infiltration and immune-related genes. The CIBERSORT algorithm was used to evaluate the relationship between CTSS expression and the infiltrating levels of immune cells. Analysis showed that CTSS expression was significantly positively associated with the infiltration level of activated memory $CD4^+$ T cells and $CD8^+$ T cells in BLCA, SKCM, and UVM and significantly positively correlated with the infiltration level of $CD8^+$ T cells in LGG (Fig. S1).

Moreover, CTSS expression had a significant positive correlation with activated NK cells, but a significant negative correlation with activated mast cells in BLCA. Furthermore, CTSS exhibited a significant positive association with the infiltration levels of M1 macrophages, monocytes, and activated NK cells but a significant negative association with the infiltration levels of M2 macrophages, activated mast cells and resting NK cells in SKCM. Moreover, CTSS was significantly positively correlated with the infiltration level of follicular helper T cells (TFHs) and M1 macrophages in UVM and significantly negatively associated with activated mast cells in LGG. It is worth noting that resting memory $CD4^+$T cells were significantly positively associated with CTSS expression in LGG (Fig. 9A).

Differential expression analysis and immunotherapeutic assessment demonstrated that CTSS was closely related to the prognosis of immunotherapy and CTLs were differentially expressed in KIRC (Fig. 7D). Therefore, the correlation between CTSS and immune cell infiltration in KIRC was assessed. Results indicated that CTSS had a significant negative correlation with resting NK cell infiltration in KIRC. Conversely, CTSS had a significant positive correlation with resting DCs, activated memory $CD4^+$ T cells and gamma-delta T (γδ T) cell infiltration (Fig. 9B). Moreover, KIRC with CTSS mutation demonstrated significantly higher levels of infiltration of resting NK cells compared with the wild type. TFHs and regulatory T cells (Tregs) in SKCM with CTSS mutation exhibited significantly higher levels of infiltration compared with the wild type (Fig. 9C).

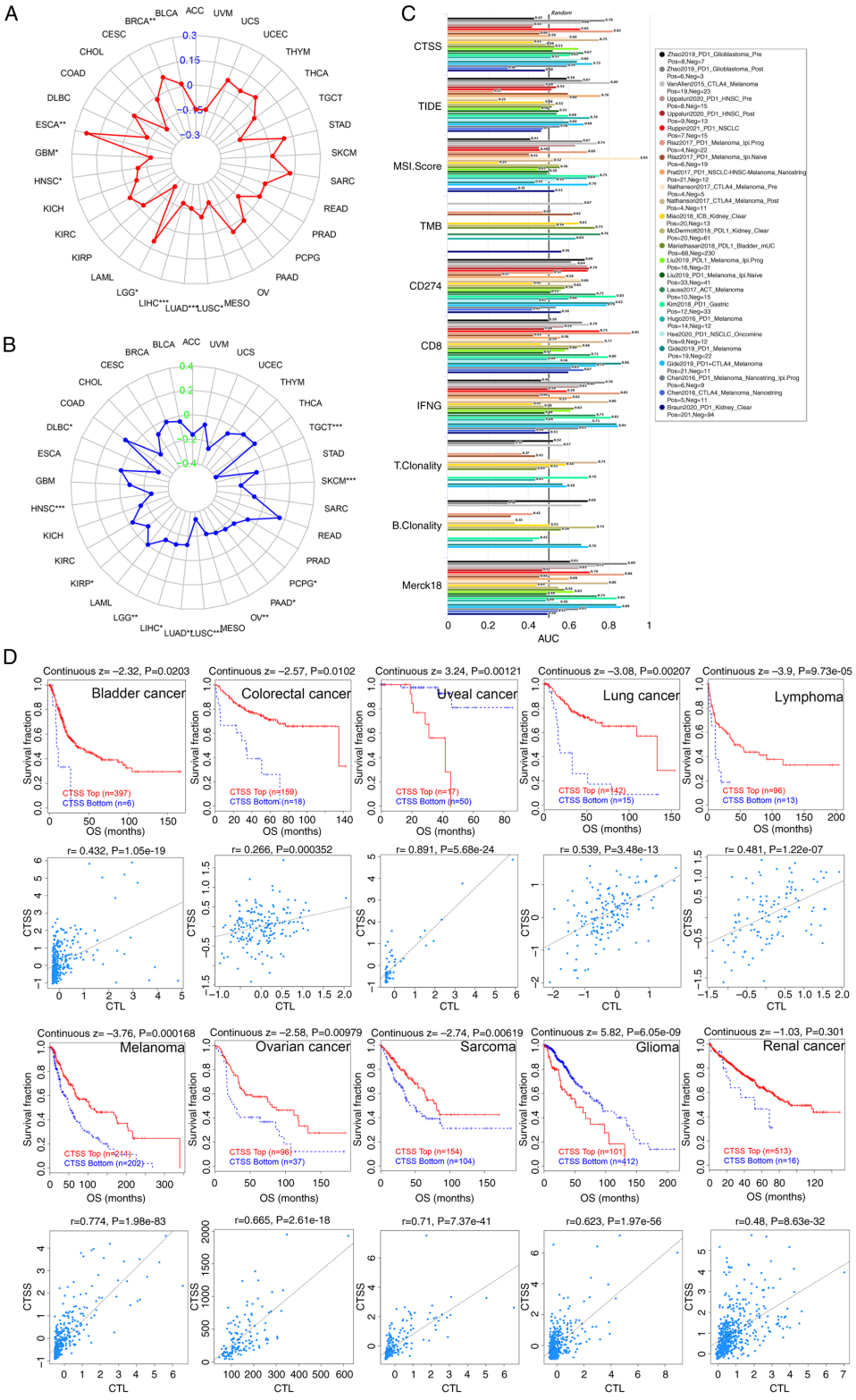

Figure 7. Correlation of CTSS expression with the immunotherapeutic response. Radar chart displaying the connection of CTSS with TMB (A) and MSI (B) in pan-cancer. (C) Bar plot showing the biomarker relevance of CTSS compared with other canonical biomarkers in different immunotherapeutic sub-cohorts. (D) Kaplan-Meier curves (upper panel) showing the connection between survival ratios and CTSS in diverse cancer cohorts. The figure below shows the relationship between CTSS expression and CTL in different cancer cohorts. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma; HNSC, head-neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; CTSS, cathepsin S; CTL, cytotoxic T-cell level; TIDE, Tumor Immune Dysfunction and the Exclusion; MSI, microsatellite instability; TMB, tumor mutational burden; CD274, cluster of differentiation 274; IFNG, Interferon gamma; OS, overall survival; CTL, cytotoxic T lymphocytes.

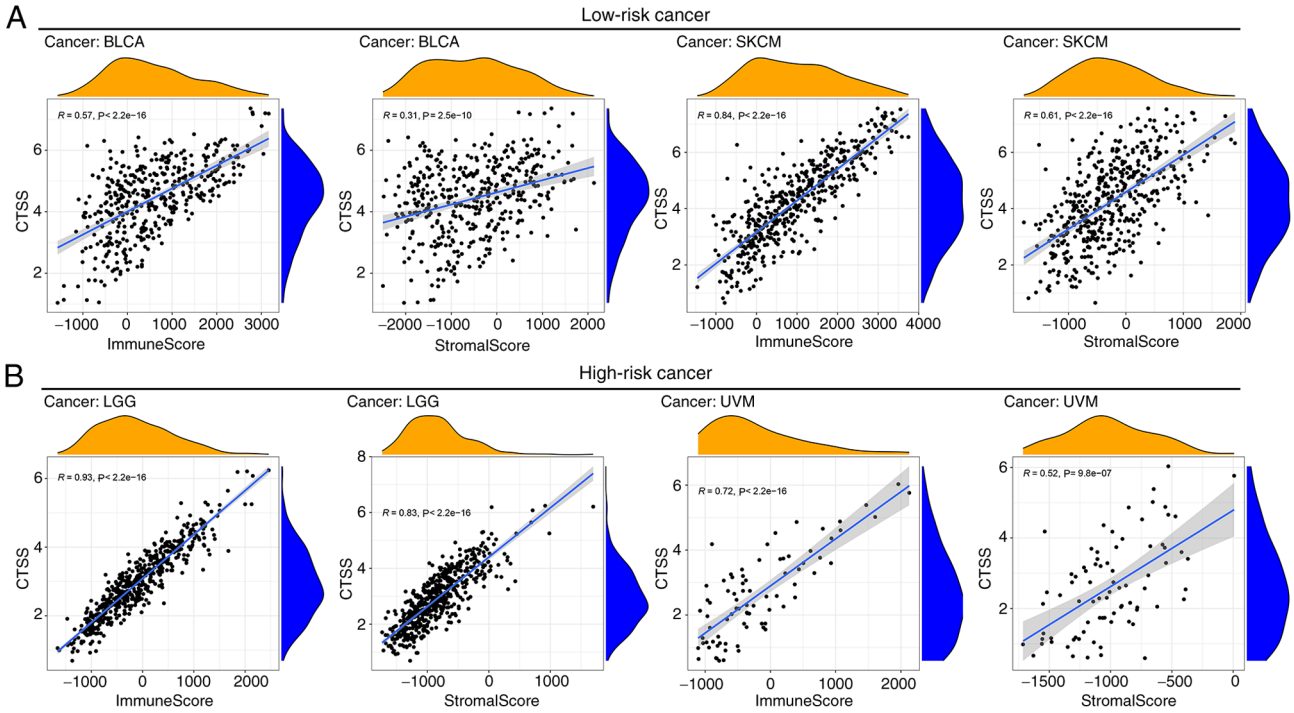

Figure 8. Correlation analysis of CTSS with immune and stromal cell infiltration of TME in various cancers. CTSS expression was positively correlated with the stromal and (A) immune score (B) in these cancers. The cancers were separated into low-risk (BLCA and SKCM) and high-risk cancer types (LGG and UVM). BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; LGG, lower grade glioma; UVM, uveal melanoma; CTSS, cathepsin S.

The relationship between CTSS and 24 immune-related genes in these cancer types was estimated by gene co-expression analysis. This analysis showed that numerous immune-related genes, such as PDCD1, LAG3, CTLA4, TIGIT and LGALS9 exhibited significant positive co-expression with CTSS in all five cancer types assessed (BCLA, KIRC, SKCM, LGG and UVM) (Fig. 9D). Overall, the analysis indicated that CTSS was closely correlated with immune cell infiltration and immune-related genes across different cancer types, which suggested that CTSS may serve an important role in the TIME.

Functional enrichment analysis of CTSS. To evaluate the biological functions of CTSS in multiple malignant tumors, a functional enrichment analysis of the selected cancer types (n=4) was performed. GO functional annotation, demonstrated that CTSS was negatively correlated with the negative regulation of sprouting angiogenesis, mRNA binding and myoblast proliferation in BLCA. Conversely, CTSS positively regulated numerous biological functions in SKCM, LGG and UVM, including immune response-regulating signaling pathways and the regulation of lymphocyte activation (Fig. 10A). Moreover, KEGG pathway enrichment analysis demonstrated that CTSS was positively correlated with numerous crucial biological pathways in BLCA, SKCM, LGG and UVM, including type I diabetes mellitus and T cell receptor singling pathway (Fig. 10B). These findings suggested that CTSS may participate in tumorigenesis by regulating multiple signaling pathways and biological processes across different cancer types.

Differential expression of CTSS across malignant and normal tissues. According to the differential expression and clinical correlation analysis results of CTSS, KIRC was selected for clinical validation. To further verify the differential expression of CTSS in KIRC and normal adjacent tissues, RT-qPCR and western blotting were performed. The results demonstrated significant upregulation of CTSS mRNA and protein expression levels in cancerous tissue compared with normal tissue in patients with KIRC (Fig. 11A-C) which was consistent with TCGA database analysis results. The full-length gels of western blotting can be seen in Figure S2.

Moreover, to further assess the expression of CTSS in these tumors with differential expression and clinical prognostic relevance, immunohistochemistry images of three types of malignant tissue (from renal cancer, melanoma and glioma) and normal tissues (unpaired) were analyzed. A higher level of CTSS antibody staining in malignant tissues compared with normal tissues in the brain, kidney and skin tissue was observed (Fig. 11D). This finding highlights the potential association between CTSS expression and malignancy at these specific anatomical sites.

Discussion

Immunotherapy, especially immune checkpoint therapy, has emerged as an epochal milestone in anti-cancer therapy. Nevertheless, the efficacy of immunotherapy varies greatly among individuals. Therefore, the identification of biomarkers that can predict immunotherapy efficacy is vital. A previous study reported significant breakthroughs in identifying biomarkers as molecular mechanism studies of ICI treatment continue to advance (26), and a recent study reported MSI and TMB as potential biomarkers of response to ICI therapy (27). However, there are ongoing debates

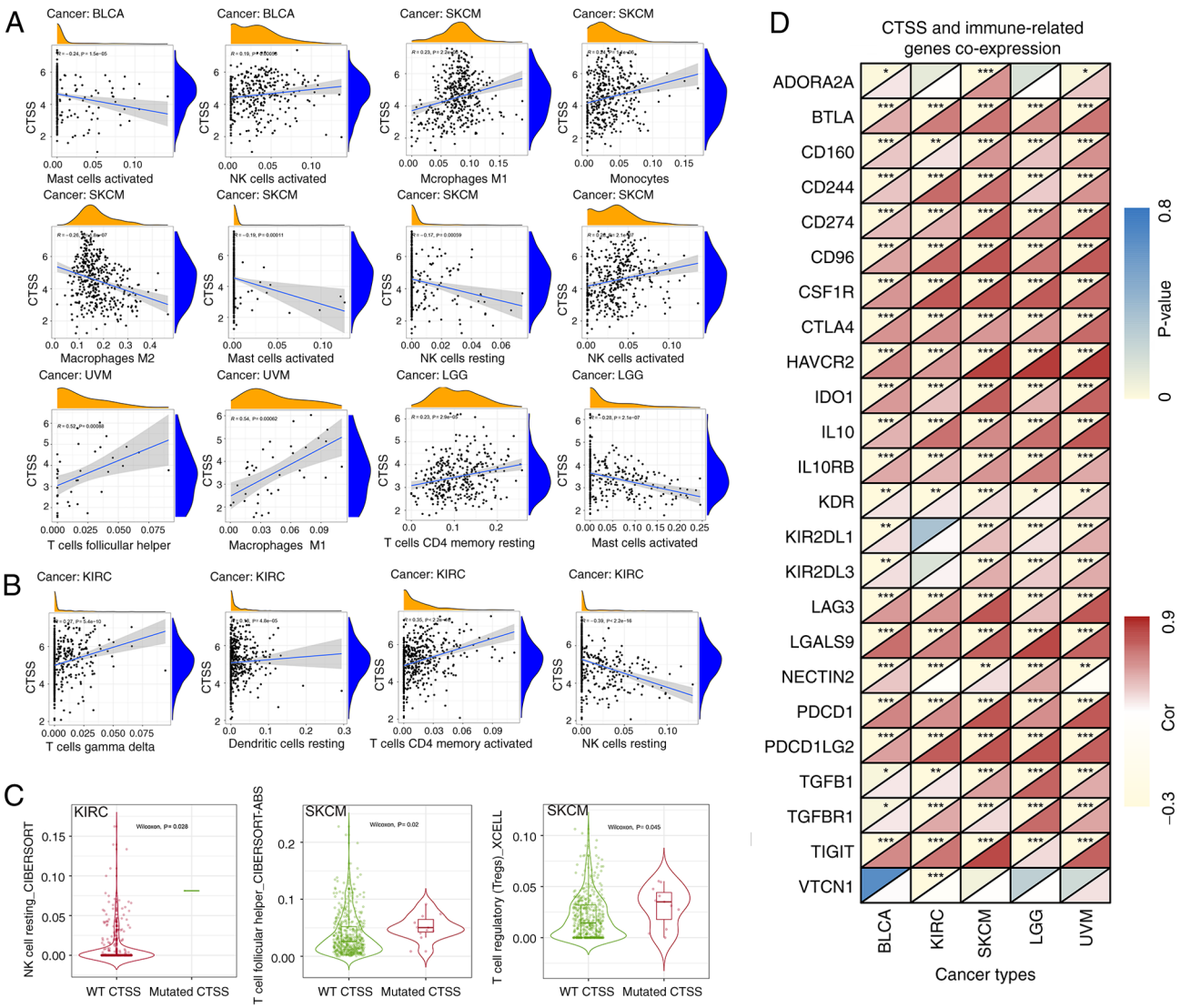

Figure 9. Relationship between CTSS and immune cell infiltration and immune-related genes. (A) Relationship between CTSS expression and various infiltrating immune cells in four cancer types. (B) Relationship between CTSS expression and immune cell infiltration in KIRC. (C) The relationship between CTSS mutation and immune cell infiltration. (D) Co-expression analysis between CTSS and immune-related genes in BLCA, KIRC, SKCM, LGG and UVM. BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; UVM, uveal melanoma; LGG, lower grade glioma; KIRC, kidney renal clear cell carcinoma; CTSS, cathepsin S; NK cell, natural killer cell; WT, wild type.

and challenges in their clinical application. For example, consensus on TMB cutoff values for patient stratification remains elusive and PD-L1 expression is only applicable to certain tumor types (28,29). Therefore, selecting the optimal biomarker that can accurately reflect the effectiveness of immunotherapy and guide combination therapy poses a challenge for cancer treatment. In recent years, pan-cancer analysis has aimed to uncover gene mutations, mRNA variations and immune-related genes across multiple tumor types (30-32). It is widely thought that identifying sensitive biomarkers for early cancer diagnosis and developing novel ideas for personalized treatment strategies for cancer patients are of the utmost importance.

The present study demonstrated the potential of CTSS as a prognostic and immunological biomarker across numerous cancers. The expression of CTSS across multiple human cancer datasets obtained from TCGA was analyzed. The current study demonstrated significant differential expression of CTSS in numerous cancer types and that the upregulation of CTSS was associated with the prognosis of multiple cancer types, including BLCA, SKCM, SARC, OV, LGG and UVM. However, the impact of CTSS varied across different cancer types, high CTSS expression was associated with a good prognosis in BLCA and SKCM but a poor prognosis in UVM and LGG.

Research has also demonstrated that elevated CTSS expression in melanoma effectively enhances the antigen processing and presentation of tumor-related antigens, which helps T cells recognize melanoma cells and trigger immune responses against tumors. Consequently, CTSS serves a crucial role in suppressing melanoma metastasis and exhibits a positive correlation with patient survival rates (33). Furthermore, a previous study reported that CTSS can inhibit Tregs and serve an important role in reducing bladder tumor cell proliferation (34). Moreover, high expression of CTSS may promote tumor occurrence and development in UVM and glioblastoma by promoting

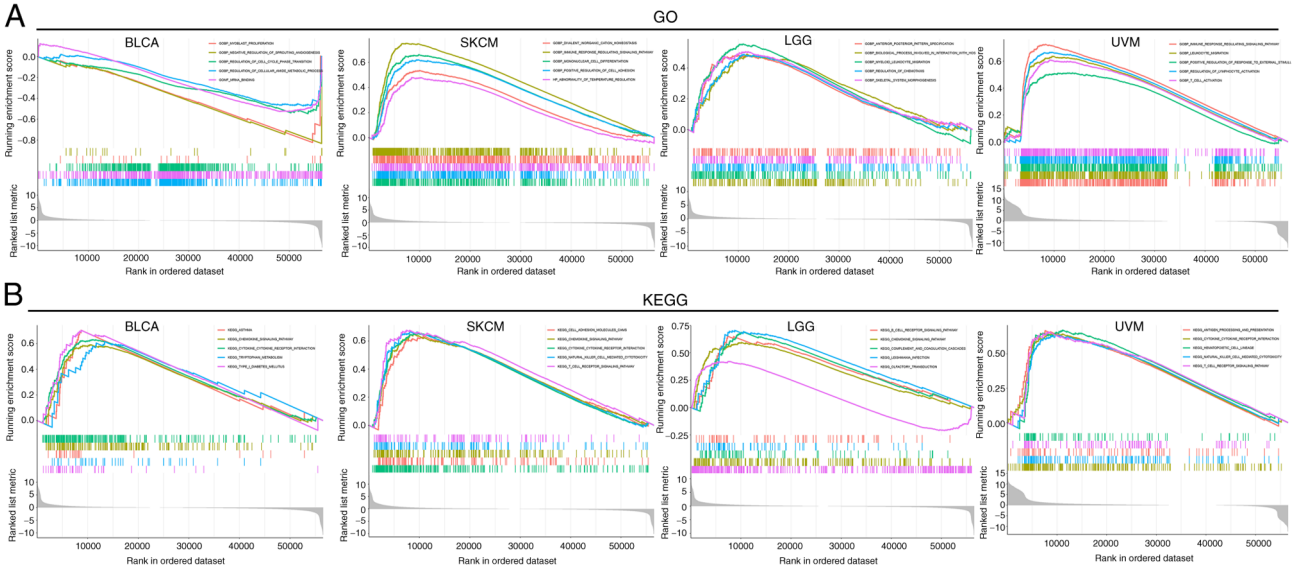

Figure 10. Functional enrichment analysis of the CTSS gene. (A) GO function annotation of CTSS in BLCA, SKCM, LGG and UVM. (B) KEGG pathway enrichment analysis of CTSS in BLCA, SKCM, LGG and UVM. BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; LGG, lower grade glioma; UVM, uveal melanoma; CTSS, cathepsin S; GOBP, Gene Ontology Biological Process; HP, Human Phenotype; GOMF, Gene Ontology Molecular Function; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

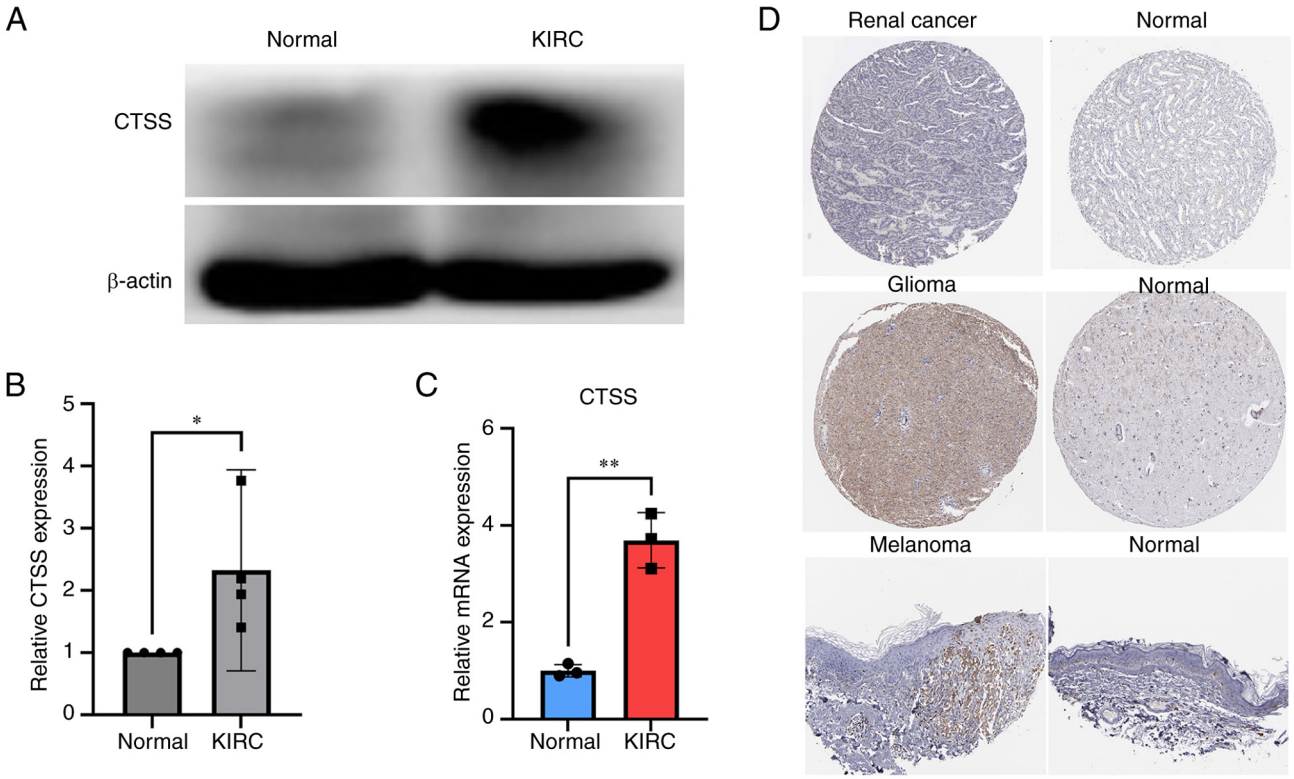

Figure 11. Experimental verification. (A) The differential expression of CTSS in KIRC was verified by western blotting at the protein level. The full-length gels of western blotting can be seen in Figure S2. (B) The gray value analysis of relative CTSS expression. (C) The differential mRNA level of CTSS between cancer and normal tissue in KIRC. (D) The immunohistochemistry images of differential CTSS gene expression in malignant and normal tissues (x5 magnification). Images downloaded from Tissue Atlas and Pathology Atlas of the Human Protein Atlas database (https://www.proteinatlas.org/). *P<0.05 and **P<0.01. KIRC, kidney renal clear cell carcinoma; CTSS, cathepsin S.

angiogenesis and anti-apoptotic mechanisms, and is associated with poor prognosis in patients (35-38). The aforementioned studies overlap in their assertion that CTSS likely influences immune responses, which in turn affects the prognosis of cancer. These findings may partially explain the difference in prognosis observed across different cancers with high CTSS expression.

Additionally, in terms of predicting immunotherapy response, CTSS showed significant associations with TMB

and MSI in eight and 12 cancer types, respectively. Compared with MIS, CTSS exhibited a superior ability to predict response outcomes in different immunotherapy sub-cohorts. Additionally, CTSS was positively correlated with CTL levels in numerous tumors. CTLs serve as pivotal immune cells responsible for combating tumors by recognizing tumor-specific antigens and initiating robust anti-tumor immune responses (39). Studies have demonstrated that glioma cells possess intricate cell surface morphology and release immunosuppressive molecules, enabling them to evade lymphocyte-mediated cytotoxicity (40,41). These immune evasion mechanisms have the potential to attenuate the tumor-killing capacity of CTLs, resulting in a reduction in patient survival. CTSS serves an essential role in tumor biology, including tumor angiogenesis, ECM degradation and genomic alterations (17,42). Bararia *et al* (43) reported that 6% (19/305) of FL patients had CTSS Y132 mutations and 13% (37/286) of FL patients had CTSS amplifications. Consistent with these findings, the present study demonstrated that CTSS alterations primarily involved amplification and mutation. For example, a high rate of CTSS mutations were seen in SKCM, and SKCM with a CTSS mutation showed higher levels of infiltration of TFHs and Tregs cells compared with SKCM with wild type CTSS.

It is now understood that immune cells and related matrix components in the TME can create an inflammatory micro-environment that impedes tumor development (44). However, the TME can also cause the depletion of effector T cells and transform into a tumor-associated microenvironment after numerous stimuli, such as a hypoxic or inflammatory response. This shift in the microenvironment can markedly facilitate cancer progression (45). Thus, identification of new targets or biomarkers that can reverse the immunosuppressive effect of the TME is urgently needed. The present study evaluated the correlation between CTSS and the immune microenvironment in numerous cancers. Results demonstrated that CTSS positively correlated with both stromal and immune cell scores in BLCA, SKCM, LGG and UVM.

Furthermore, previous studies have reported that immune cells can exert anti-neoplastic and tumor-supportive effects, which offer an avenue for interventions in the immunosuppressive state of the TME (46). Immune cell infiltration has been reported to be significantly related to improved survival (47,48). However, the relationship between tumor-infiltrating immune cells and patient survival can vary depending on tumor type. For example, a previous study reported that tumor-infiltrating immune cells were negatively related to survival of patients with KIRC (49), while another study indicated that the differential infiltration of $CD8^+$ T cells yielded contrasting effects on the prognosis of KICH in patients with KIRP (50). These observations provide compelling evidence of the heterogeneity in immune cell infiltration patterns and mechanisms among various tumor types. Kim *et al* (51) demonstrated that increased CTSS in DCs could alter the repertoire of TFHs, change the presentation of antigens to $CD4^+$ T cells and destroy $CD4^+$ T cell epitopes, which may contribute to autoimmune or inflammatory diseases. However, the correlation between CTSS and immune cell infiltration in cancers has not yet been clarified. The present analysis of immune cell infiltration revealed a notable correlation between CTSS and activated memory $CD4^+$ T cells in BLCA, KIRC, SKCM and UVM, as well as $CD8^+$ T cells in BLCA, SKCM, LGG and UVM. Likewise, a previous study reported that in renal autoimmune diseases, CTSS induced immune responses by driving MHC-II-mediated T and B cell activation (52).

In addition, cysteine cathepsin exerts a regulatory influence on the cytotoxicity of NK cells and T cells, serving a pivotal role in modulating their immune responses (53). Macrophages exert a critical role in modulating the immune microenvironment within tumors. M1 macrophages are known to impede tumor growth and secrete pro-inflammatory cytokines, whereas M2 macrophages facilitate tumor progression (54,55). In addition, owing to their remarkable cytotoxic capabilities and capacity to secrete IFN-γ, γδ T cells assume a pivotal role in anti-tumor immunity (56). The immune cell infiltration analysis revealed CTSS expression had a significant positive association with the infiltration level of γδ T cells while exhibiting a significant negative correlation with resting NK cells in KIRC. Additionally, CTSS displayed a positive association with the infiltration levels of M1 macrophages and activated NK cells but showed a negative association with the infiltration levels of M2 macrophages and resting NK cells in SKCM. These findings suggest that CTSS may serve a crucial role in modulating the immune response within the tumor microenvironment, providing further support for the potential relationship between CTSS expression and favorable prognosis in BLCA and SKCM.

Notably, the increased expression of certain immune co-inhibitory proteins on the cell surface, such as LAG-3, TIGIT and CTLA4, can cause the dysfunction of $CD8^+$ T cells (57). Thus, it is important to identify biomarkers that can predict the expression of these immune-related genes in malignant tumors. Gene co-expression analysis showed that CTSS co-expressed with immune-related genes, such as CTLA4, TIGIT, LAG-3, PDCD1 and LGALS9, in BLCA, KIRC, SKCM, LGG and UVM. These findings suggest the predictive role of CTSS in these cancers.

Previous research has suggested that CTSS is significantly associated with pro-inflammation factors and immunity. CTSS can alter the expression of inflammatory cytokines (11) and activate them, and affects psoriasis inflammation (58). CTSS serves a vital role in MHC-II antigen presentation by promoting the degradation of invariant chains (59). However, the relationship between CTSS and inflammatory and immune-related functions and pathways in pan-cancer remains unclear. Functional enrichment analysis indicated a potential impact of CTSS expression on biological functions and pathways, such as immune response regulating signaling pathways, regulation of lymphocyte activation and T cell receptor singling pathways in BLCA, SKCM, LGG and UVM.

The present study relied predominantly on bioinformatics techniques however, due to sample collection feasibility and other limiting factors, validation of these results was only performed experimentally on KIRC, which is a limitation of the study. To bolster the credibility of the present findings, the differential expression of CTSS in cancer was further investigated using publicly available databases. However, these results are exploratory and require further validation. Future research should encompass a broader range of pan-cancer datasets and clinical samples to further corroborate the findings of this study.

To summarize, the present study substantiated the relationship between CTSS expression and prognosis, TME and immune response in numerous cancers. The findings of the present study suggest that CTSS may be a key biomarker for predicting the prognosis of many cancers, such as BLCA, SKCM, UVM and LGG, and the immune infiltration of cancers, such as KIRC and SKCM. These findings provide novel insights that can contribute to advancements in cancer prevention and treatment strategies.

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Availability of data and materials

The bioinformatics datasets analyzed during the current study are available in the University of California Santa Cruz repository (UCSC Xena, https://xenabrowser.net/datapages/), the cBioPortal database (https://www.cbioportal.org), the Human Protein Atlas database (https://www.proteinatlas.org/), the Mutation module of TIMER2.0 (http://timer.cistrome.org/), the Tumor Immune Dysfunction and the Exclusion (TIDE) database (http://tide.dfci.harvard.edu/). The other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HM and SC designed the study. BD prepared the methodology and software. HM collected renal pathological tissue. SL conducted formal analysis and validation. BD and SL wrote the original draft. SC revised the manuscript, and HM was responsible for the supervision, project administration, providing research funding, and coordinating and resolving any issues related to scientific accuracy during the research process. SL and BD confirm the authenticity of all the raw data. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Medical Ethics Committee of The First Affiliated Hospital of Guangxi Medical University (approval no. 2022-E387-01). Informed consent was acquired from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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