

Chloride Intracellular Channel 1 (*CLIC1*) as a Prospective Prognostic Biomarker in Pan-cancer: An Integrative Immunoassay and Clinical Value

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Submitted: 28 December 2023 Revised: 7 February 2024 Accepted: 20 February 2024 Published: 1 June 2024

Background: The chloride intracellular channel 1 (*CLIC1*) plays a pivotal role in the progression of various cancers as a significant anion channel within the human body. With the rising global cancer incidence, this study aims to elucidate the regulatory mechanisms of *CLIC1* to prognosis, the tumor immune microenvironment, and immunotherapy.

Methods: We acquired expression matrices and corresponding clinicopathological data for pan-cancer from The Cancer Genome Atlas (TCGA) and UCSC Xena databases. To thoroughly investigate the significance of *CLIC1* in pan-cancer, we conducted analyses encompassing genetic information, mutations, and the distribution of *CLIC1*. Subsequently, we validated differential expression of *CLIC1* through experimental methods. Prognostic evaluation was performed using univariate cyclooxygenase (COX) regression, Kaplan-Meier curves, and two primary algorithms. Employing the median *CLIC1* expression level as a grouping criterion, we explored biological pathways associated with *CLIC1* expression, conducted single-cell transcriptome sequencing, examined immune-infiltrating cells, and investigated immunotherapy. Additionally, we utilized the Connectivity Map (CAMP) to identify potential drugs targeting *CLIC1*.

Results: *CLIC1* is overexpressed in a majority of tumors, with specific validation in stomach and thyroid cancers. Notably, a significant correlation between *CLIC1* and immune checkpoints, tumor mutational burden, and high-frequency microsatellite stability was observed in certain patients ($p < 0.001$), indicating potential for *CLIC1* in immunotherapy. Moreover, *CLIC1* serves as a significant predictor of poor prognosis across various malignant tumors, impacting overall survival, disease-specific survival, disease-free interval, and progression-free interval. Based on *CLIC1*'s expression profile, it was found to be prominently expressed in immunometabolic and biosynthetic pathways. Furthermore, high expression levels of *CLIC1* were identified in monocytes, macrophages, and malignant cells, suggesting its potential as a specific marker for these cell types. Notably, a strong correlation was observed between *CLIC1* expression levels and immune cell infiltration ($p < 0.001$). Lastly, a drug database screened for small-molecule drugs related to *CLIC1*, highlighting potential anticancer applications.

Conclusion: This study represents the inaugural demonstration of *CLIC1* as a prognostic factor across numerous cancers. Furthermore, it elucidates *CLIC1*'s regulatory role in biological pathways, single-cell landscapes, and the tumor immune microenvironment within pan-cancer contexts. Hence, *CLIC1* emerges as a potential biomarker for cancer immunotherapy.

Keywords: chloride intracellular channel 1; pan-cancer; prognosis; single-cell; immunotherapy

Introduction

With the American Cancer Society and the Global Disease Study systematically estimating that over 10 million people worldwide die from cancer annually, addressing the ongoing burden of cancer becomes imperative [1]. Investments directed towards early detection, personalized cancer interventions, and enhanced treatments could substantially alleviate this burden. Currently, robust drug targets play a pivotal role in precision pan-cancer therapy, exemplified by cytotoxic T lymphocyte antigen 4 (*CTLA-4*) and programmed death ligand 1 (*PD-1*). PD-1 has ushered in

cutting-edge therapies for advanced cancer, showcasing favorable safety profiles and tolerability. Moreover, heightened expression of immune checkpoints such as *Axl*, *Rac1*, *Stab2*, *CD28*, and others correlates with cancer cell susceptibility to various anti-cancer drugs [2,3]. Hence, identifying immunotherapeutic biomarkers or immunomodulatory genes further aids cancer patients in tailoring individualized immunotherapy regimens to attain enduring efficacy.

The chloride intracellular channel (*CLIC*) family is distinguished by its ability to exist in both water-soluble and membrane-bound states, exerting its biological functions

primarily through matrix acidification, transmembrane potentials, and cellular secretion [4]. Members such as chloride intracellular channel 1 (*CLIC1*) and *CLIC4* are not only found to be overexpressed in specific tumors and corresponding stroma but also transform from localized and functional hydrophilic cytoplasm into complete transmembrane proteins, acting as ion channels or signal transducers throughout the cell cycle. Multiple studies have shown that *CLIC1* regulates the *MAPK/AKT* pathway to facilitate gastric cancer progression, while *CLIC4* serves as one of the apoptotic factors in colorectal cancer cells under FxOH stress [5,6]. Despite having only one transmembrane structural domain to modulate cellular excitability and ion homeostasis, *CLIC1* can influence normal cells' progression towards carcinogenesis and is closely associated with intracellular localization, tissue compartmentalization, and channel function [7].

CLIC1 plays a pivotal regulatory role in cancer development. Its overexpression serves as an adverse prognostic marker in various cancers including gastric cancer, glioma, squamous cell carcinoma, lung adenocarcinoma, and clear cell renal cancer [8]. Conversely, silencing of the chloride channel gene correlates with a favorable prognosis in benign ovarian and renal tumors [9]. Generally, the mutation rate and promoter DNA methylation of *CLIC1* impact overall survival (OS) in hepatocellular carcinoma (HCC), and the expression level of *CLIC1* is associated with immune cell infiltration [10]. Studies have demonstrated that the immune status of tumors can be discerned through gene expression patterns. Additionally, Russo LC *et al.* [11] localized *ERK1/2* phosphorylation and cytoskeletal perturbation in the nucleus and cytoplasm, elucidating the interaction of *CLIC1* with the breast cancer cell cycle.

Furthermore, chloride intracellular channel proteins play a widespread physiological role in macrophage superoxide production, contributing to both immune component depletion and tumor purity. Despite some exploitation of *CLIC1* function, the regulatory mechanisms in pan-cancer settings necessitate further investigation.

For the first time, we delved into the potential mechanisms and clinical significance of *CLIC1* in pan-cancer. Across 33 cancers and normal tissues, *CLIC1* exhibited varying expression levels, prompting an analysis of its correlation with prognosis via metrics such as overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). Additionally, we elucidated the classical pathways associated with *CLIC1* genomic activation and provided a succinct overview of 32 single-cell scenarios using CellChat. Notably, *CLIC1* emerged as a potential predictor of effective small molecule drugs. Our study sheds light on the involvement of *CLIC1* in cancer immunotherapy.

Materials and Methods

Acquisition of Raw Data

The UCSC Xena platform (<https://xenabrowser.net/datapages/>) offers an extensive public dataset that encompasses The Cancer Genome Atlas (TCGA), GETx, and Target data. It facilitates online access to download RNA-seq expression matrices, mutation data, and clinicopathological data for 33 TCGA pan-cancer and normal cohorts, comprising Tumor (10,327 samples) and Normal (730 samples) datasets. Additionally, transcriptome profiles of 21 cancer cell lines were sourced from the TISCH2 website. Furthermore, high-throughput sequencing expression profiles single-cell RNA sequencing (scRNA-seq) for two cancers were acquired from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). To examine the frequency of *CLIC1* genomic alterations and expression distribution across pan-cancer cohorts, we utilized the cBioPortal for Cancer Genomics tool.

CLIC1 Expression and Western Blot

In a dataset comprising 408 cases of gastric cancer, log₂ (TPM+1) transformation and paired difference analyses were conducted to compare the differential expression of *CLIC1* between normal and tumor tissues. Subsequently, a correlation analysis was performed to examine the relationship between *CLIC1* expression levels and disease-free survival.

We initiated the initial validation step by utilizing RIPA lysis buffer (P0013B, Shanghai Biyuntian Biotechnology Co., Ltd., Shanghai, China) to extract total proteins from various cell lines. These included human normal gastric epithelial cell lines (GES-1, iCell-h062, iCell Bioscience, Shanghai, China), human gastric cancer cell lines (AGS, CC-0401, Cellcook, Guangzhou, China), human thyroid cancer cells (TPC-1, CL-0643, Procell Life Science & Technology Co., Ltd., Wuhan, China), and a human thyroid follicular epithelial cell line (Nthy-ori 3-1, CL-0817, Procell Life Science & Technology Co., Ltd., Wuhan, China). The short tandem repeats (STR) and mycoplasma identification results for the aforementioned cell lines were reported in **Supplementary Material 1**.

The BCA Protein Assay Kit (ZJ101, Shanghai Ya Mei Biopharmaceutical Technology Co., Ltd., Shanghai, China) was utilized to quantify the proteins. Subsequently, the proteins in each sample were separated via SDS-PAGE electrophoresis and transferred onto nitrocellulose filter membranes. These membranes were then incubated overnight at room temperature in a 5% nonfat milk/TBST buffer solution. Primary antibodies, including *CLIC1* Polyclonal Antibody (14545-1-AP, acquired from Wuhan Sanying Biotechnology Co., Ltd., Wuhan, China; dilution: 1:500) and Beta-Actin Polyclonal Antibody (81115-1-RR, Wuhan Sanying Biotechnology Co., Ltd., Wuhan, China; dilution: 1:50,000), were incubated with the complexes on

the membrane. Following this, the membranes were further incubated overnight with secondary antibodies, namely Goat anti-Mouse Poly-HRP Secondary Antibody and Goat anti-Rabbit Poly-HRP Secondary Antibody (SA00001-1, Wuhan Sanying Biotechnology Co., Ltd., Wuhan, China; dilution: 1:10,000). Finally, images were developed using the BioSpectrum 600 imaging system (Ultra Violet Products, Cambridge, UK) with enhanced chemiluminescence working solution (BM101, Biomiga, San Diego, CA, USA), and their grayscale values were calculated using Image J software (version 6.0, Media Cybernetics, Bethesda, MD, USA).

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

In the presence of isopropanol, enzyme-free water, and 75% alcohol, total RNA was purified and washed using Trizol (15596026, Thermo Fisher Scientific Co., Ltd., Shanghai, China). The reverse transcription process was facilitated by a reagent obtained from Nanjing Novozan Biotechnology Co., Ltd. (R021-01, Nanjing, China), enabling the conversion of RNA to cDNA. For RT-qPCR, we prepared enzyme-free water, 2xTaq Master Mix (B541018, B639295-0005, Sangon Biotech Co., Ltd., Shanghai, China), Beta-Actin primer, *CLIC1* primer (QV114, Nanjing Novozan Biotechnology Co., Ltd., Nanjing, China), and reverse transcription products. All primer pairs were annealed at 60 °C for 40 cycles. Gradient RT-qPCRs were conducted using a 7500 Touch Thermal Cycler (Bio-Rad) to ensure optimal annealing temperature for each primer set. The relative expression of *CLIC1* was determined using the $2^{-\Delta\Delta C_t}$ method, wherein ΔC_t represents the difference in cyclic threshold between the target and reference genes. The expression of *CLIC1* was normalized to beta-actin. Detailed primer sequences can be found in **Supplementary Table 1** and previous literature [12].

CLIC1 Genomics and Immune Correlation Analysis

Utilizing the Human Protein Atlas (HPA: <https://www.proteinatlas.org/>) public database, fluorescence immunotechnology was employed to confirm the distribution of *CLIC1* at the organelle level [13]. Immunofluorescent (IF) analysis was conducted using epidermoid carcinoma cell lines (A-431), glioblastoma cell lines (U-251), and osteosarcoma cell lines (U2OS) sourced from the public database.

To elucidate the role of *CLIC1* in predicting the efficacy of immune checkpoint inhibitors, we investigated the relationship between *CLIC1* expression and immune checkpoint genes, tumor mutational burden (TMB), and microsatellite instability (MSI). The Immunophenoscore (IPS), a predictor of anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) and anti-programmed death ligand 1 (PD-1) responses, was employed. The IPS for pan-cancer was derived from the Cancer Immunome Atlas (TCIA:

<https://www.cancerimagingarchive.net/>) database, which provides results from heterogeneity and clonality analysis of tumor second-generation sequencing data (NGS). Our focus was on the PD-1 and CTLA-4 treatment cohorts, along with the *p*-value of subgroup circular scoring.

Prognostic Relevance of CLIC1 in Pan-cancer

To evaluate the relationship between *CLIC1* expression and prognosis, we conducted a univariate cyclooxygenase (COX) analysis considering common clinical indicators. OS time refers to the duration from the initiation of randomization to death from any cause. PFI represents the period of survival without further deterioration. DFI is characterized by disease recurrence, patient death, or a shift in DSS to death from a specific disease. In cases where the *p*-value from the univariate COX analysis for cancer was significant, we proceeded to compare the survival outcomes of patients in high- and low-expression groups using Kaplan-Meier curves. The “survival” and “survminer” R packages (version 3.5.5, University of Auckland, Auckland, New Zealand) were utilized to set the respective thresholds for achieving the risk ratio and 95% confidence interval (CI). Additionally, the Wilcoxon test was employed to ascertain significant differences in *CLIC1* expression among clinically characteristic subgroups.

Biological Signaling Pathways Based on GSEA

Gene Set Enrichment Analysis (GSEA) is a widely employed data mining technique for analyzing transcriptome gene expression. In this study, we investigated the enrichment of biological processes within gene sets among patients categorized into low and high *CLIC1* expression groups. Following the annotation of gene sets, we utilized gene expression matrices and patient grouping labels to compute the activated biological processes in each cancer type. Gene annotation files were obtained from the Molecular Signatures Database (MSigDB) website (<https://www.gsea-msigdb.org/gsea/index.jsp>). The analysis was conducted using the “GSEA” software (version 4.1.0, Broad Institute, Redmond, WA, USA), and the results were visualized in the “multipleGSEA” plot generated using the R package “ggplot2” (version 3.4.4, Systat Software and University of Illinois at Chicago, Chicago, IL, USA).

Single-cell Transcriptome Analyses of CLIC1

The Tumor Immune Single-cell Hub 2 (TISCH2) integrates 76 high-quality cancer datasets, comprising two million cells across 27 types of cancer. To investigate *CLIC1* expression levels across various cell types in different single-cell datasets, we utilized the TISCH2 database, specifying the following parameters: *CLIC1* (gene), major spectrum (cell-type annotation), and all cancers (cancer type). Single-cell dataset searches were conducted to provide cell-type annotation and visualize *CLIC1* expression in the GSE131928 and GSE120575 datasets. Addi-

tionally, CellChat analysis was executed on the GSE125449 and GSE111360 datasets to compare patterns of intercellular communication between the two scRNA-seq datasets. Leveraging the CellChat ligand-receptor (LR) database, which encompasses LR interaction structures, cofactor molecules, and various membrane-bound receptors, we identified changes in cell-to-cell signaling. This approach allows for a more biologically meaningful analysis of the potential significance of *CLIC1* across different cancer types.

Relationship between CLIC1 and Immune Infiltration

TIMER serves as a valuable data source for immune infiltration across various cancers. In this study, we focused on the *CLIC1* gene within the gene module and visualized its expression across different cancer types alongside immune infiltration abundance. Utilizing a heatmap with adjusted Spearman's rho, we explored the relationship between infiltration estimates and gene expression, aiming to dissect tumor immunological and genomic features. Notably, the negative correlation of tumor purity with most immune cells emerged as a significant confounding factor in our analysis. Consequently, we employed the correlation module to generate scatter plots illustrating the relationship between *CLIC1* expression and tumor-infiltrating immune cells (TIICs), such as B cells, CD4+ T cells, CD8+ T cells, monocyte macrophages, and non-specific cells, across breast invasive carcinoma (BRCA), kidney renal clear cell carcinoma (KIRC), and brain lower grade glioma (LGG). Additionally, ESTIMATE was utilized to compute the immune and stromal fractions in these three cancers. A higher ratio of immune/tumor components indicated a richer tumor microenvironment (TME), characterized by a relative abundance of immune/stromal cells and lower tumor purity.

Potential Compounds Correlating with CLIC1 in Pan-cancer

The Connectivity Map (CMap), accessible at <https://clue.io>, serves as a valuable tool for elucidating relationships among diseases, genes, and treatments. It facilitates the uploading of 150 up-regulated genes and 150 down-regulated genes for analysis. In our study, we identified differentially expressed genes (DEGs) by stratifying patients based on *CLIC1* expression levels. Leveraging the pattern matching strategy of the Kolmogorov-Smirnov test, we compared these DEGs with all expression profiles in the database to identify similarities. By ranking the list of similar genes and computing connectivity scores, we aggregated the scores to identify potential novel therapeutic agents relevant to pan-cancer therapy. Finally, we retrieved identification information and structural data of small-molecule compounds from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) to further support our findings.

Statistical Analysis

All data were analyzed using R (version 4.0.3, R Core Team, Rochester, NY, USA) and its companion package, with a significance level set at $p < 0.05$ for two-tailed tests. The Wilcoxon test was employed to compare *CLIC1* expression levels, while validation was performed using a paired *t*-test. Pearson correlation analysis was applied to continuous, normally distributed, and linear data, whereas Spearman correlation analysis was utilized for a broader range of data types, including checkpoints, TMB, MSI, TIME, and immunoreactivity. COX regression, survival forests (SMD), and Kaplan-Meier analysis served as primary algorithms and components for prognostic analysis. The Chi-Square test was utilized to assess differences between datasets. This study adhered to the methods outlined in **Supplementary Fig. 1**.

Results

Comprehensive Genomic Analysis

CLIC1 expression was found to be upregulated in numerous cancer types (Fig. 1A,B), including bladder urothelial carcinoma (BLCA, $p < 0.05$), BRCA, cholangiocarcinoma (CHOL, $p < 0.001$), colon adenocarcinoma (COAD, $p < 0.001$), esophageal carcinoma (ESCA, $p < 0.001$), head and neck squamous cell carcinoma (HNSC, $p < 0.001$), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP, $p < 0.001$), liver hepatocellular carcinoma (LIHC, $p < 0.001$), lung adenocarcinoma (LUAD, $p < 0.001$), prostate adenocarcinoma (PRAD, $p < 0.05$), rectum adenocarcinoma (READ, $p < 0.001$), stomach adenocarcinoma (STAD, $p < 0.001$), thyroid carcinoma (THCA, $p < 0.001$), and uterine corpus endometrial carcinoma (UCEC, $p < 0.05$).

In the Gene Expression Profiling Interactive Analysis (GEPIA) database, a total of 3748 overexpression genes were recorded in STAD, among which *CLIC1* emerged as a potential candidate (Fig. 1C). While *CLIC1* genomic mutations were infrequent in STAD, they represented 6% of deep deletions and 2% of amplifications in mature B-cell neoplasms (Fig. 1D). Elevated levels of *CLIC1* expression were found to be correlated with disease-free survival in gastric cancer (GC) patients, suggesting that *CLIC1* may serve as a predictor of short-term prognosis (Fig. 1E).

Experimental and Database Validation

We isolated proteins from GES-1, AGS, Mthy ori3-1, and TPC-1 cell lines. The findings revealed high expression of the *CLIC1* protein in gastric and thyroid cancer cells, respectively (Fig. 2A,B). Subsequent RNA extraction and DNA amplification, followed by RT-qPCR analysis, demonstrated elevated levels of *CLIC1* expression in gastric cancer cells compared to normal cells (Fig. 2C). Additionally, we utilized fluorescent signals to visualize the localization of target proteins or antigens within cells. By ex-

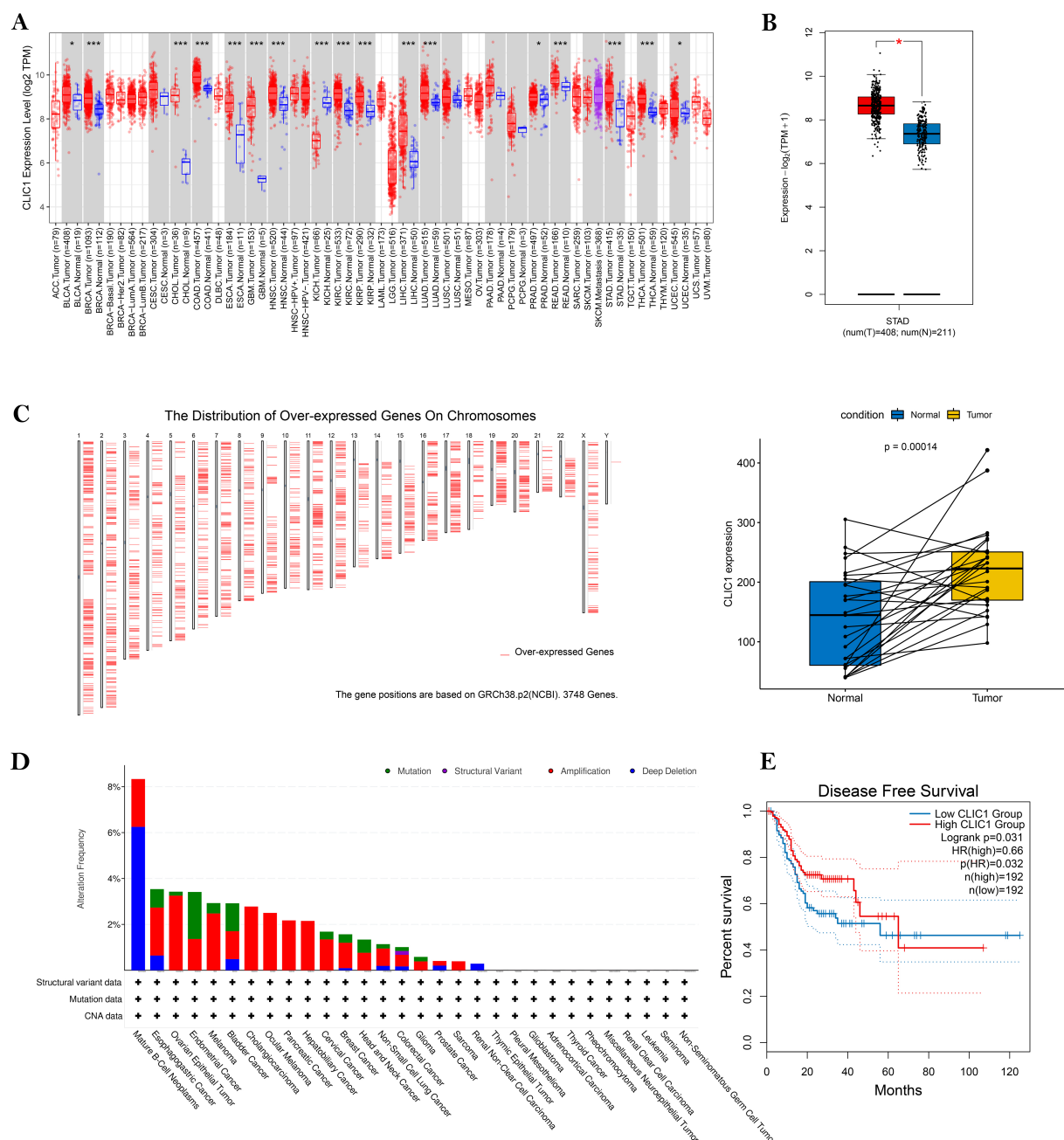


Fig. 1. Identification of pan-cancer related chloride intracellular channel 1 (*CLIC1*) for comprehensive analysis. (A) Differential expression of *CLIC1* between tumor and normal tissues. (B) Boxplots to identify expression between gastric cancer and paraneoplastic tissues. (C) A Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia2.cancer-pku.cn/>) to analyze the chromosomal location of 3748 genes upregulated in gastric cancer, pairing differential *CLIC1*. (D) *CLIC1* alteration frequency profile of the cBioPortal database. (E) Relationship between *CLIC1* expression and disease-free survival in gastric cancer patients. * $p < 0.05$, *** $p < 0.001$.

amining the immunofluorescence localization of *CLIC1*, as well as the nucleus, microtubules, and endoplasmic reticulum, in A-431, U-2OS, and U-251 cells from publicly available databases, we observed predominant cytoplasmic distribution of *CLIC1*. Furthermore, *CLIC1* was notably associated with molecular functions of chloride channels, ion channels, and voltage-gated channels (Fig. 2D).

Correlation of *CLIC1* Expression with Immune Checkpoints

To explore the relationship between immunosuppressive molecules and immune responses in tumor cells, we conducted further investigations into the co-expression of *CLIC1* and immune checkpoints across various cancers. Our findings revealed that patients with *CLIC1* overexpression exhibited heightened expression or enhanced func-

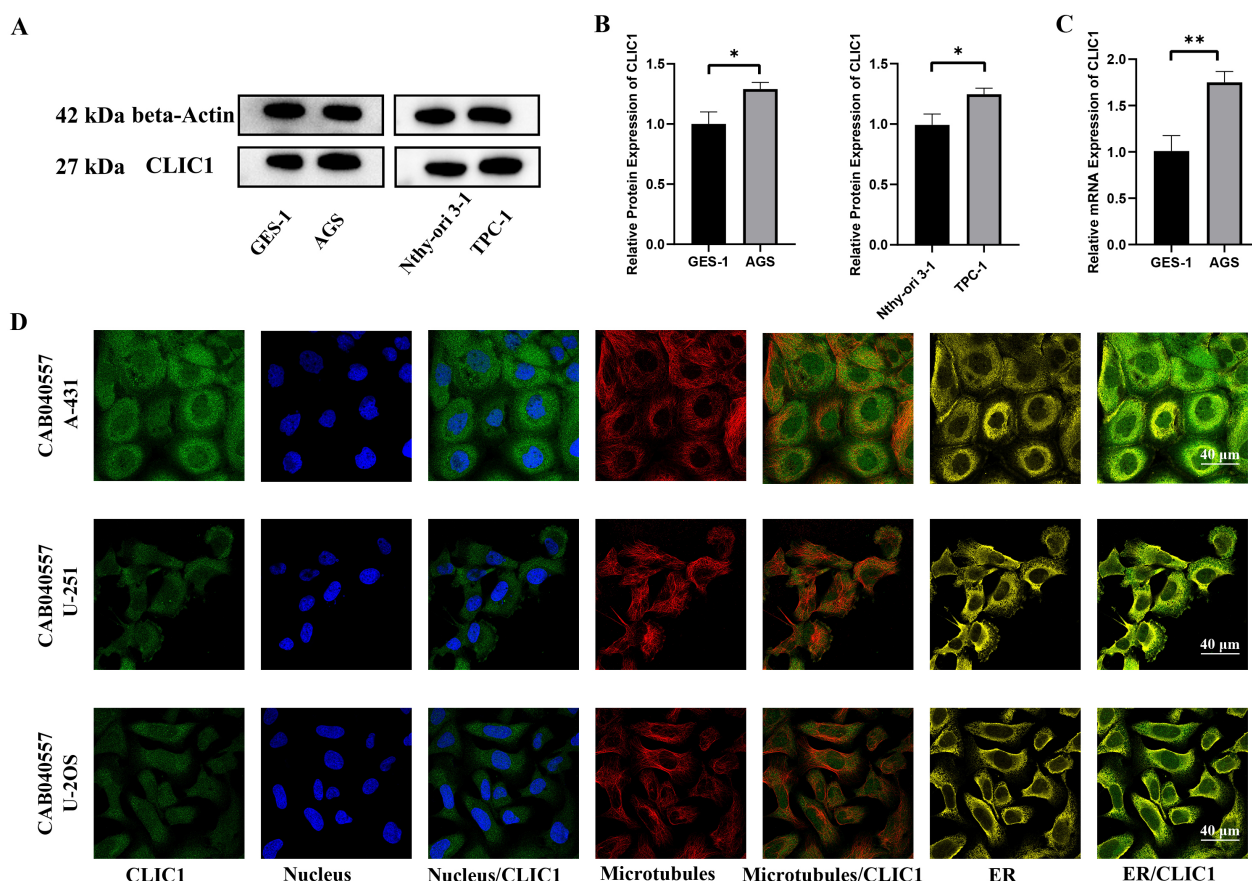


Fig. 2. *CLIC1* at the protein translation, mRNA transcription and subcellular levels. (A) *CLIC1* protein expression of GES-1, AGS, Mthy ori3-1, and TPC-1. (B) Relative protein expression of *CLIC1* in gastric and thyroid cancers, with three replicates per cell line ($n = 3$). (C) Relative mRNA expression of *CLIC1*, three replicates were set for each sample ($n = 3$). (D) Immunofluorescence staining and merged images of *CLIC1* protein localization in the Human Protein Atlas (HPA) public database (<https://www.proteinatlas.org/>). * $p < 0.05$, ** $p < 0.01$.

tionality of immune checkpoints, leading to suppressed immune function and predisposition to immunocompromise or tumor susceptibility (Fig. 3A). For instance, in kidney chromophobe (KICH) and LGG, we observed that over 30 checkpoints were significantly abundant at high *CLIC1* expression levels, notably including *CD276*, *NRP1*, *LGALS9*, and *TNFRSF4*. It is plausible to suggest that tumor cells overexpressing *CLIC1* trigger the activation of immune checkpoints, thereby impeding the antigen presentation process within the immune system, ultimately resulting in immunosuppression and evasion. IPS can accurately assess the response to immunotherapy, encompassing both positive *CTLA-4* and negative *PD-1*, as well as negative *CTLA-4* and positive *PD-1*. Notably, *CLIC1* expression in BRCA and THCA served as a predictive factor for responses to anti-*PD-1* and *CTLA4* antibodies, with higher expression levels correlating with increased responsiveness (Fig. 3B).

Correlation of *CLIC1* Expression with Biomarkers

TMB and MSI are genomic characteristics that predict the efficacy of pan-cancer immunotherapy. TMB refers to the number of mutations per megabase in tumor tissue, while MSI arises from the accumulation of errors during genetic material replication. Increased TMB sites suggest greater genetic instability, potentially leading to heightened immune system recognition and improved treatment efficacy. *CLIC1* expression positively correlates with TMB across 13 cancers (Fig. 3C), including adrenocortical carcinoma (ACC, $p < 0.001$), ESCA, pancreatic adenocarcinoma (PAAD, $p < 0.001$), and STAD ($p < 0.001$). Conversely, it shows a negative correlation in acute myeloid leukemia (LAML, $p < 0.001$) and thymoma (THYM, $p < 0.001$). Additionally, MSI contributes to mutation and enhances the response to immunotherapy. *CLIC1* also emerges as a contributing factor in 12 high-frequency MSI (MSI-H) cancers (Fig. 3D). Notably, patients with HNSC, KIRC, and UCEC exhibiting both MSI-H and high TMB phenotypes may particularly benefit from immunotherapy.

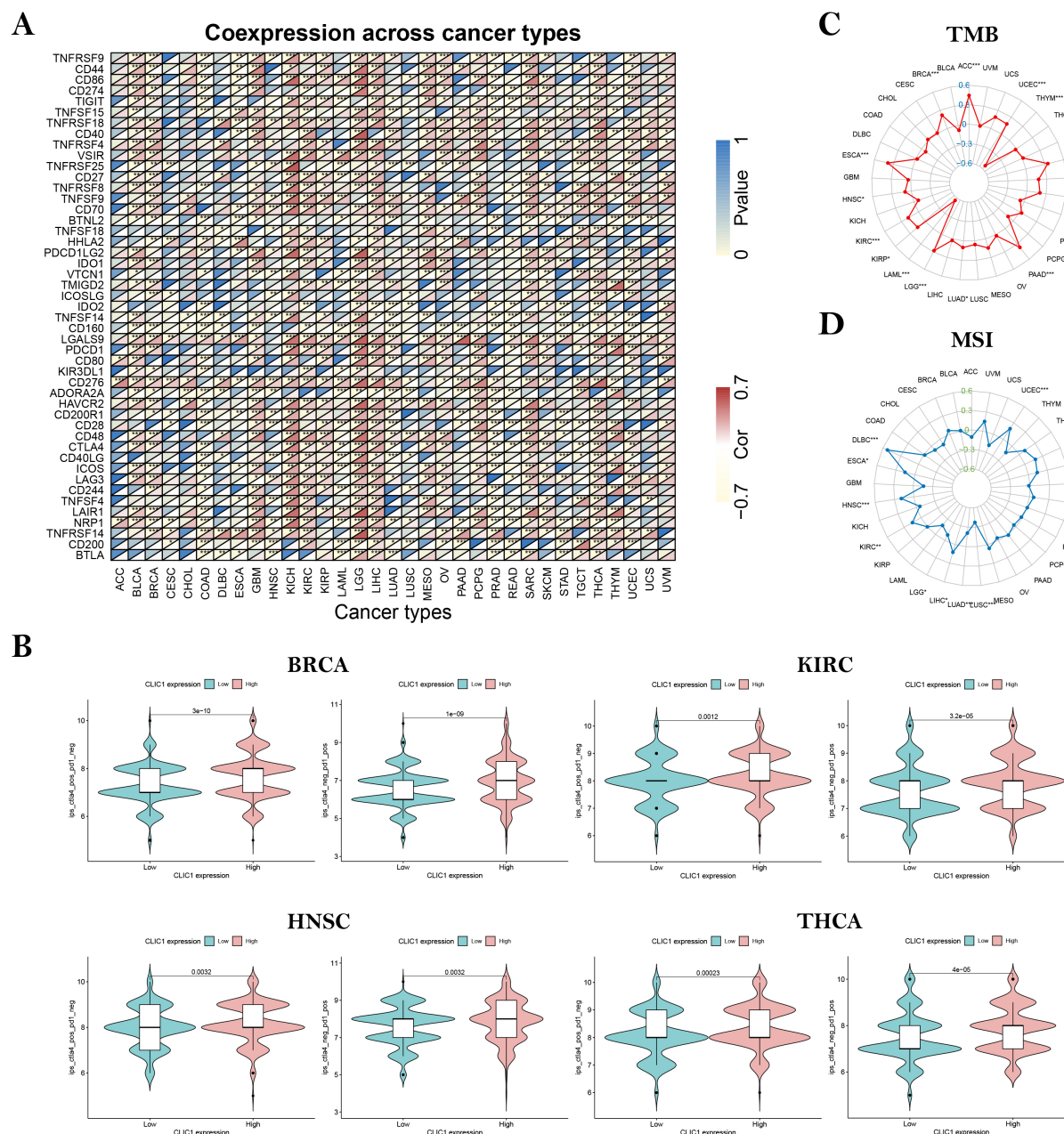


Fig. 3. Immunotherapeutic analysis of chloride intracellular channel 1. (A) Heatmap of *CLIC1* co-expression with 47 immune checkpoints in 33 cancers. (B) Violin diagram of Immunophenoscore (IPS) in high and low *CLIC1* subgroups. The abscissa represents the expression level of *CLIC1*, and the ordinate represents the number of cytotoxic T lymphocyte antigen 4 (*CTLA-4*) and programmed death ligand 1 (*PD-1*) responses. (C,D) Correlation analysis of pre-immunotherapy indicators, including tumor mutational burden (TMB) and microsatellite instability (MSI). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Prognostic Analysis of *CLIC1* Expression in Pan-cancer

OS stands out as the most effective endpoint in oncology clinical trials, with *CLIC1* overexpression identified as a risk factor across most cancers in the univariate COX analysis, yet demonstrating a protective effect for patients with ovarian serous cystadenocarcinoma (OV) and THYM (Fig. 4A,B). Analyses of OS, PFI, and DFI yield relatively accurate results when survival data are available. Elevated

CLIC1 expression, as depicted in the forest plot, is associated with shorter OS in ACC ($p < 0.05$, HR = 1.875 [95% CI, 1.144 to 3.075]), LAML ($p < 0.01$, HR = 2.060 [95% CI, 1.282 to 3.311]), LGG ($p < 0.001$, HR = 2.204 [95% CI, 1.879 to 2.585]), LIHC ($p < 0.01$, HR = 1.310 [95% CI, 1.109 to 1.546]), and PAAD ($p < 0.01$, HR = 1.608 [95% CI, 1.196 to 2.162]). Conversely, in THYM, it correlates with longer survival time ($p < 0.01$, HR = 0.132 [95% CI, 0.029 to 0.607]). However, when focusing on tar-

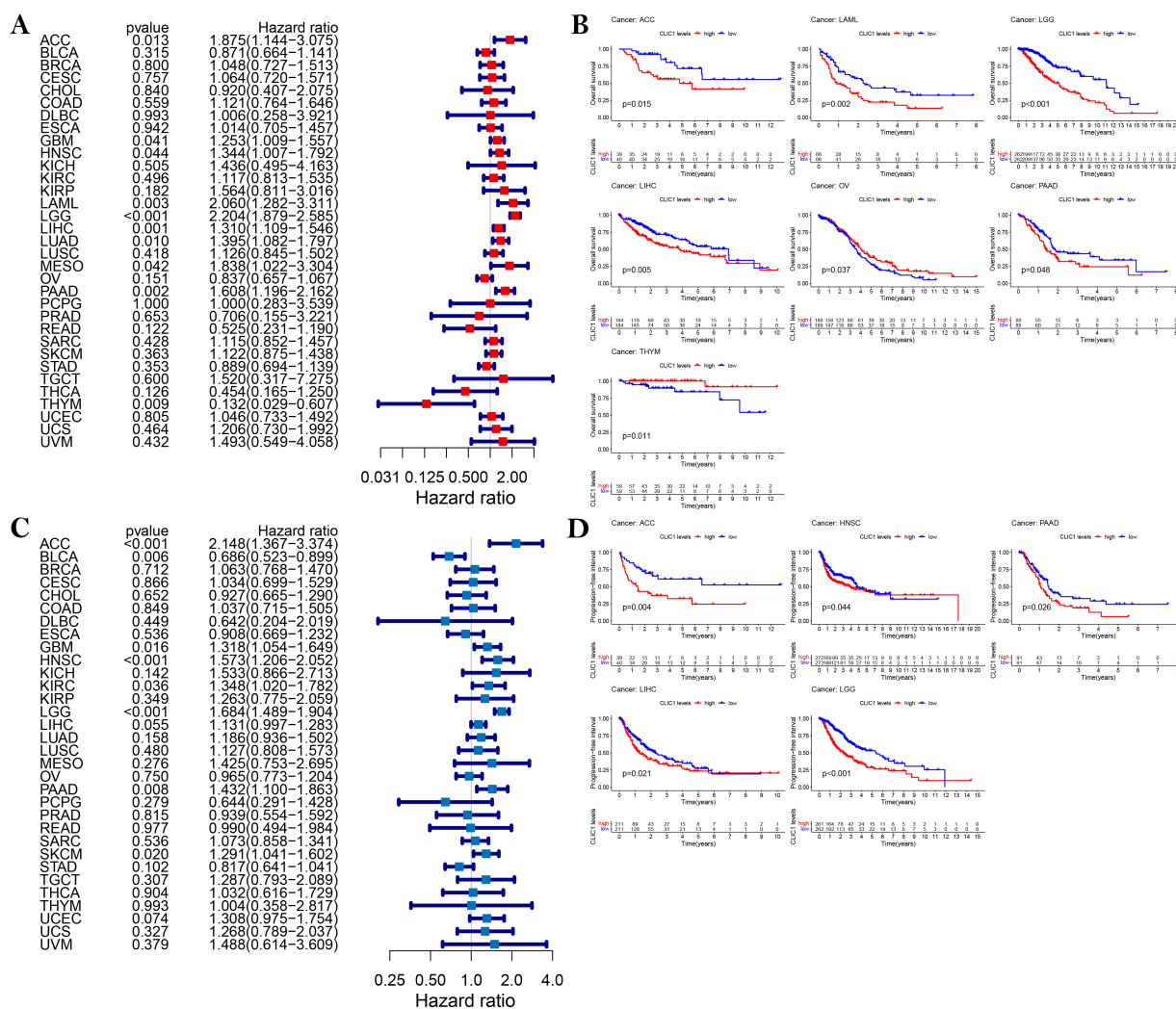


Fig. 4. Univariate cyclooxygenase (COX) regression analyses and Kaplan-Meier models for overall survival (OS) and progression-free interval (PFI). (A,B) Relationship between *CLIC1* expression and overall survival of cancer patients, with shorter OS and a high risk of death. (C,D) Relationship between *CLIC1* expression and clinical benefit, with more pronounced drug toxicities and disease progression.

geted responses to clinical benefit, DSS elevation exhibits a favorable response. The correlations between high *CLIC1* expression and short DSS or OS time remain consistent for ACC, PAAD, LIHC, and LGG (Fig. 4C,D).

Furthermore, PFI and DFI hold greater relevance in tumor studies. Our findings indicate that *CLIC1* expression during the PFI period served as an unfavorable factor for ACC and LGG, whereas it acted as a protective factor for OV (Fig. 5). In summary, the prognostic analysis establishes *CLIC1* as an independent pan-cancer prognostic factor.

In the correlation analysis of clinicopathological features, heightened *CLIC1* expression was observed in patients at advanced American Joint Committee on Cancer (AJCC) stages in COAD, KICH, KIRC, LIHC, LUAD, LUSC, and TGCT. Conversely, decreased levels were noted in THCA at stage II compared to stage I ($p < 0.01$)

(Supplementary Fig. 2A). Additionally, with increasing age (age ≥ 65), *CLIC1* expression exhibited elevation in BRCA ($p < 0.01$) and PAAD patients ($p < 0.05$), while displaying a decrease in LIHC ($p < 0.001$) (Supplementary Fig. 2B). These complementary results suggest that *CLIC1* expression bears varying clinical relevance across different types of cancer.

GSEA of *CLIC1* in Pan-cancer

Differentially expressed genes (DEGs) across multiple cancers were extracted from subgroups categorized by *CLIC1* expression levels to elucidate *CLIC1* activation pathways. We observed prominent *CLIC1* expression in immunometabolic and biosynthetic pathways, with the former encompassing LGG, mesothelioma (MESO), pheochromocytoma and paraganglioma (PCPG), THCA, and uveal melanoma. Particularly noteworthy in LGG and

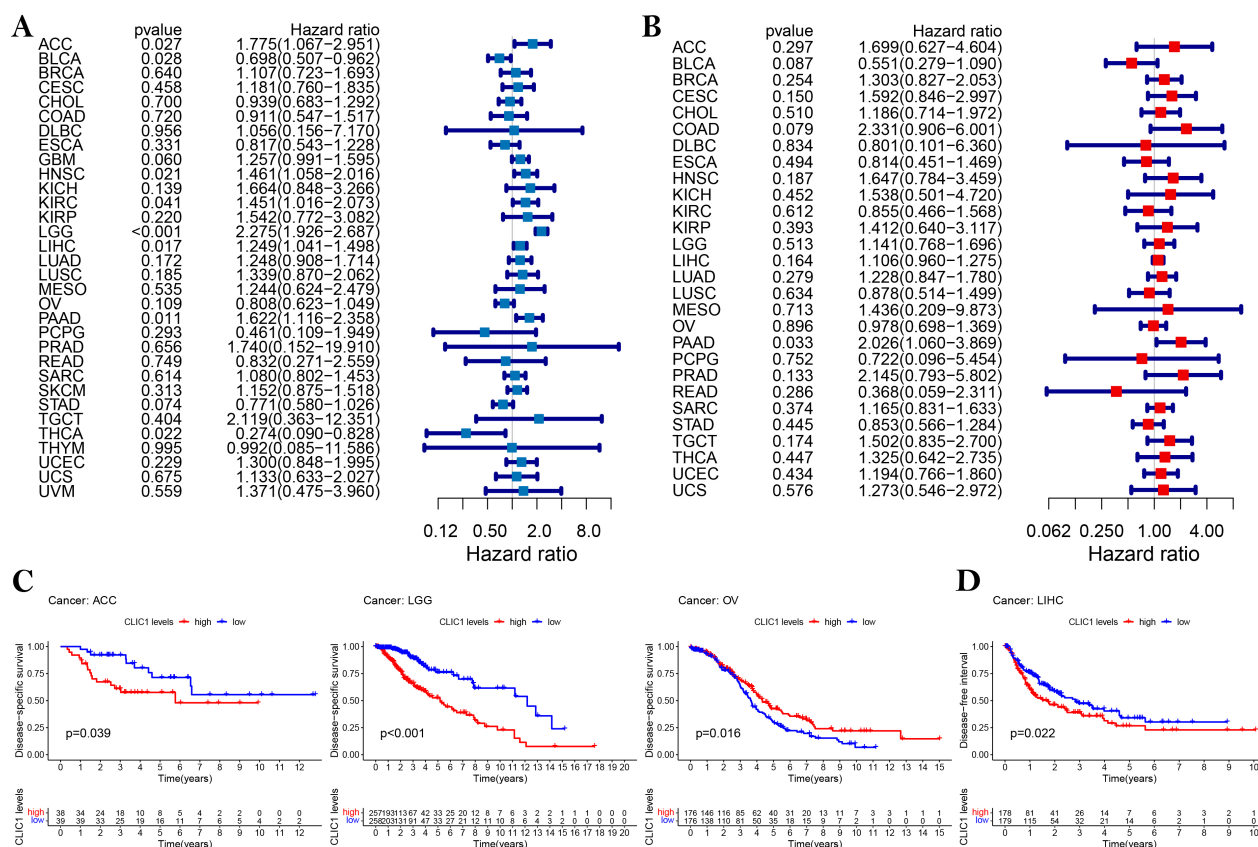


Fig. 5. Univariate COX regression and Kaplan-Meier models for prognosis-related disease-specific survival (DSS) and disease-free interval (DFI). (A,B) Relationship between *CLIC1* expression and specific tumor-directed death, with shorter DSS and high risk of death. (C,D) Relationship between *CLIC1* expression and time to disease recurrence or patient death.

PCPG, *CLIC1* activation facilitated transendothelial migration of leukocytes and natural killer cell-mediated cytotoxicity. Several biosynthetic pathways were identified in ESCA, glioblastoma multiforme (GBM), HNSC, KIRP, PAAD, READ, sarcoma (SARC), STAD, testicular germ cell tumors (TGCT), and uterine carcinosarcoma (UCS). These pathways exhibited activity in oxidative phosphorylation, pyrimidine metabolism, ribosome function, and protease synthesis (Fig. 6).

Interestingly, oxidative phosphorylation (OXPHOS) emerged as a noteworthy marker pathway across various cancers, possibly influenced by chloride channels regulating oxidase carcinogenesis. Furthermore, overexpression of *CLIC1* was associated with immune activation signals, including NOD-like receptors, B-cell receptors, and apoptosis. In summary, the GSEA provides valuable insights for further exploration through single-cell analyses, examination of the tumor microenvironment, and small-molecule investigations.

Role of *CLIC1* in the Single-cell Landscape

Due to its regulatory role in cancer immunity, we investigated the primary cell types expressing *CLIC1* within the cancer microenvironment. Fig. 7A illustrates the ex-

pression levels of *CLIC1* across 32 cell types from 59 datasets, encompassing immune cells, stromal cells, cancer cells, and functional cells. Notably, *CLIC1* exhibited predominant high expression in immune cells, particularly in monocytes and macrophages, as well as in malignant cells. For instance, in the GSE131928 glioblastoma dataset, *CLIC1* was markedly expressed in malignant cells such as AC-like, MES-like, NPC-like, and OPC-like malignant cells, suggesting a potential correlation with the degree of malignancy (Fig. 7B,C). On another front, analysis from GSE120575 elucidated the immune cell dynamics associated with melanoma checkpoint immunotherapy. Within this context, both specific and non-specific immunity constituted the microenvironment for *CLIC1* expression, with maximal expression observed during T cell proliferation (Fig. 7D,E). Furthermore, we assessed the quantity and intensity of cellular communication, along with specific LR interactions, using CellChat. In the scRNA-seq analysis of hepatocellular carcinoma, we observed communication between hepatocytes and immune cells to some extent (Fig. 8A). High expression of *CLIC1* in the TME was also evident across various cell types including cancer cells, immune cells, fibroblasts, and stromal cells. Notably, ligand-receptor pairs identified in endothelial cells and fi-

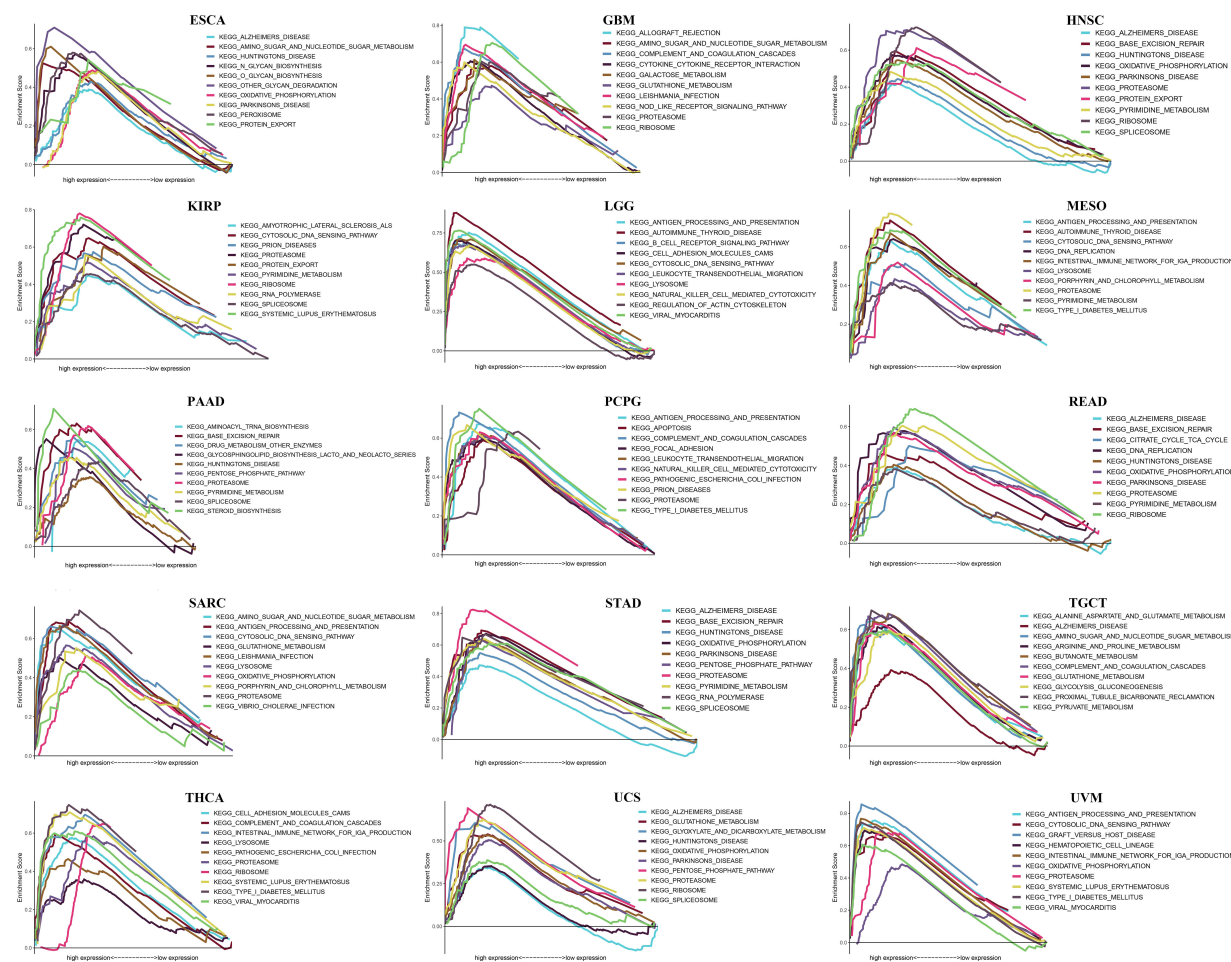


Fig. 6. The Gene Set Enrichment Analysis of *CLIC1* in pan-cancer. The median *CLIC1* expression was used as a cut-off value to enrich the top 10 active biological processes in each cancer.

broblast subpopulations displayed potent immunomodulatory properties, potentially linked to the heightened expression of *CLIC1* (Fig. 8B). Additionally, TME alterations necessitated *CLIC1* regulation within cells, with cancer progression associated with the activation of monocytes, macrophages, dendritic cells, and T cells (Fig. 8C). Importantly, TNF-positive dendritic cells and SPP1-positive macrophages were found to play pivotal roles in identifying therapeutic targets (Fig. 8D).

Immune Cell Infiltration Analyses in Pan-cancer

In comparison to low *CLIC1* expression, high *CLIC1* expression showed significant positive correlations with neutrophils, monocytes, macrophages, dendritic cells, and myeloid-derived suppressor cells (MDSCs), while the opposite trend was observed in CD8⁺ T cells, CD4⁺ T cells, B cells, mast cells, and NK cells (Fig. 9). Notably, in KICH, LGG, and SKCM, both adaptive immune response cells (CD8⁺ T cells, CD4⁺ T cells) and innate immune response cells (neutrophils, monocytes, macrophages, and dendritic cells) exhibited profound levels of infiltration.

Furthermore, utilizing Spearman correlation coefficients, we compared the variability and correlation of TICs across several solid tumors (Supplementary Fig. 3). The high-*CLIC1* subgroups exhibited inverse associations with CD4⁺ resting memory cells in BRCA ($R = -0.24, p < 0.001$) and KIRC ($R = -0.22, p < 0.001$), while demonstrating a positive association in LGG ($R = 0.32, p < 0.001$). Similar trends were observed for CD8⁺ T cells, activated dendritic cells, and activated NK cells concerning *CLIC1* expression values, though not evident in LGG. Additionally, it appears that different macrophage subtypes interact with *CLIC1* in divergent manners, with BRCA showing correlation with macrophage M0 ($R = 0.14, p < 0.001$), KIRC with macrophage M2 ($R = -0.16, p < 0.001$), and LGG with macrophage M0 ($R = 0.35, p < 0.001$) (Supplementary Fig. 3A). Supplementary Fig. 3B illustrated that the immune/tumor component of KICH, LGG, and SARC displayed high stromal scores alongside elevated *CLIC1* expression. Consequently, the TIME likely serves as a favorable prognostic and therapeutic mediator in cancer.



Fig. 7. Detailed gene annotation of Tumor Immune Single-cell Hub 2 at the single-cell level. (A) Summary of *CLIC1* expression in 32 cell types from 59 single-cell RNA sequencing (scRNA-seq). (B,D) Scatter plots showing the distribution of immune and malignant cell subtypes of GSE131928 and GSE120575. (C,E) *CLIC1* expression in immune and non-immune cells. The depth of the color represents expression, ranging from 0 to max.

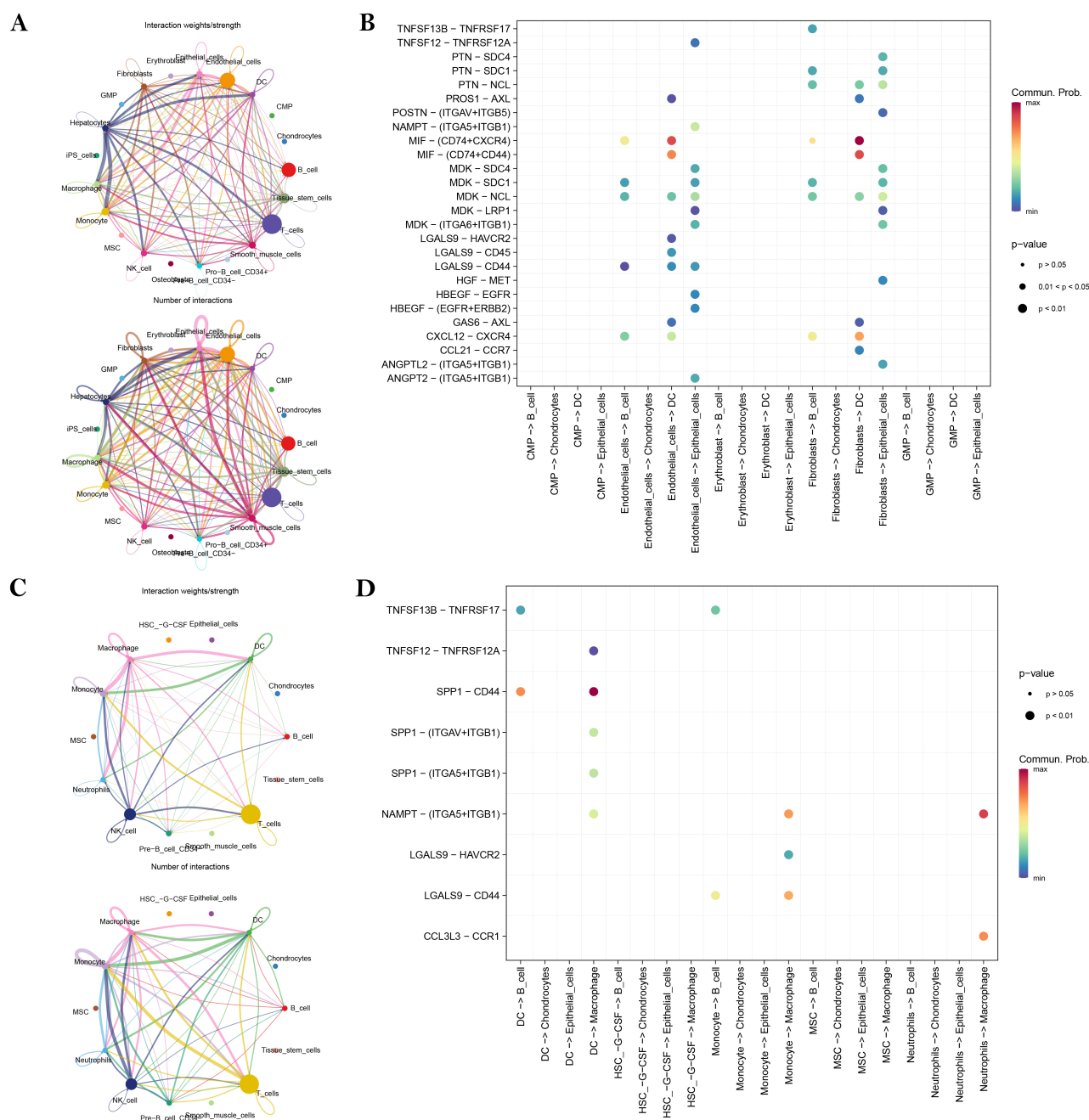


Fig. 8. Single-cell analysis and ligand-receptor level communication. (A,C) CellChat is used to visualize the number and strength of interactions between different cell populations of GSE125449 and GSE111360. The line connecting the types represents their interaction, with the line thickness indicating the strength of the association between types. (B,D) Bubble charts of all interactions between cell clusters. The color of the bubbles indicates the likelihood.

Small-molecule Compounds Related to *CLIC1*

The pharmacogenomic findings across multiple cancers were summarized in a heatmap representing the Connective Map, revealing numerous associations between small-molecule compounds, genes, and diseases (Fig. 10). We focused on drugs or compounds related to *CLIC1* that targeted at least two cancer types. Dimethylsulfoxide emerged as the most prevalent compound across 28 tumors, while crizotinib was notably concentrated in nine tumors,

particularly in UCS. Additionally, vemurafenib, AMG-232, vorinostat, enzastaurin, and veliparib were enriched in at least seven tumors. Among these, AMG-232 stood out as the compound with the most significant negative total normalized connectivity score ($ncs = -13.0469$), while pentoxifylline had the largest sum ($ncs = 1.8060$). The PubChem CIDs for AMG-232 and pentoxifylline are 58573469 and 4740, respectively. The corresponding structures are associated with inducing p53-mediated apoptosis in cancer cells



Fig. 9. The immune cell infiltration landscape of partial correlation with *CLIC1* expression in pan-cancer, including adaptive and innate immune responsive cells. The red color represents a positive correlation, while the blue color represents a negative correlation.

and inhibiting autoimmune responses such as Th1-mediated inflammation. However, further validation is required to ascertain the efficacy of small-molecule drugs in controlling cancer cell proliferation and survival.

Discussion

Ion channels play diverse roles in different types of cancers. Currently, potassium-selective pores and chloride ion permeation are recognized as active conduits in tumor

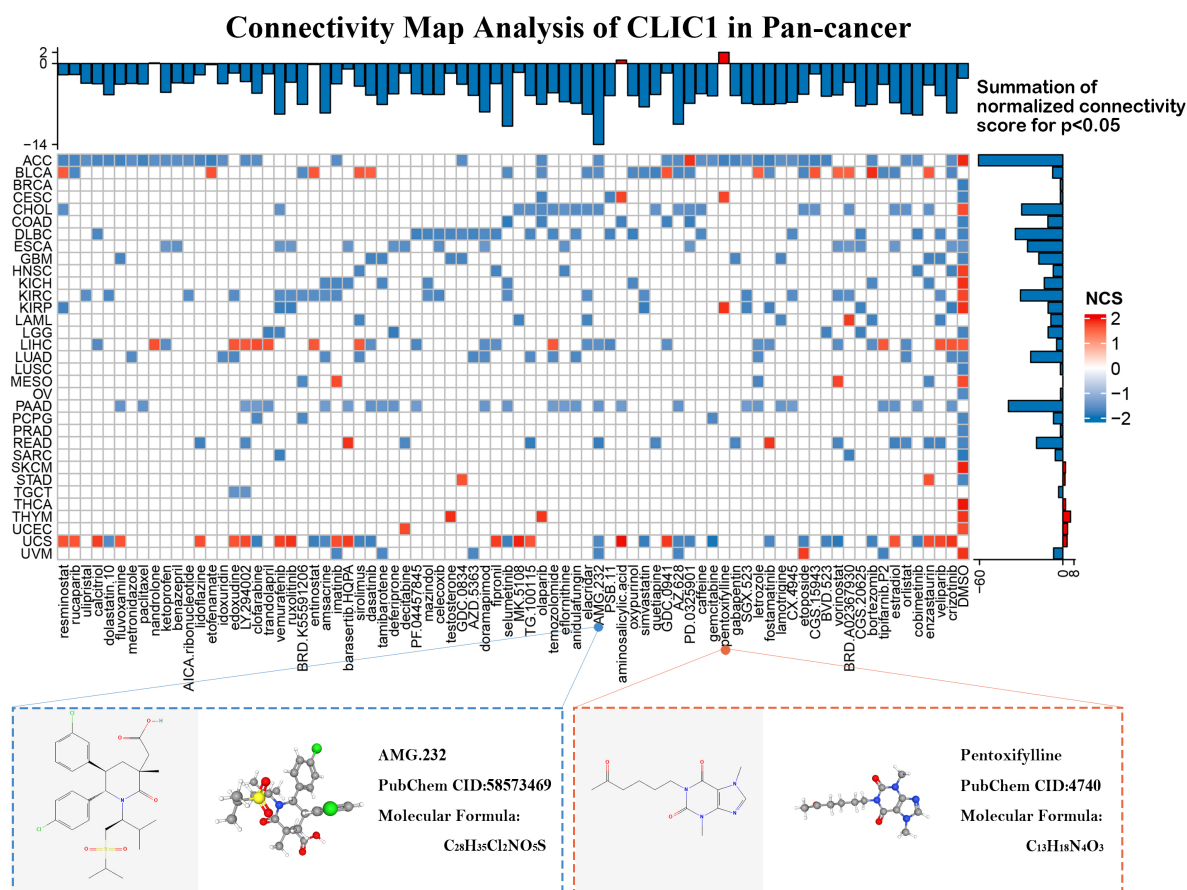


Fig. 10. Normalized connectivity scores of the drugs with the highest pan-cancer enrichment in the Connectivity Map (CMap) heatmap. Organic small-molecule bioactivity data were retrieved for AMG-232 and pentoxifylline, respectively.

development. Due to the varied mechanistic functions of exchange and transport proteins, they serve as partially beneficial targets for anti-tumor interventions and offer numerous opportunities for unique drug designs. Several members of the *CLIC* family have demonstrated efficient regulation of molecular mechanisms involved in anti-tumor activity, including transient *CLIC* membrane translocation and membrane insertion regulated by redox [14]. Among these members, *CLIC1* emerges as a potential prognostic biomarker and immune target for pan-cancer, playing a role in cell-cell communication and immune signaling. In this study, we conducted the first exploration of the oxidative phosphorylation metabolic pathway, immune infiltration, and drug synergistic mechanisms of chloride intracellular channel protein 1 in pan-cancer.

The study presented the first comprehensive analysis of the potential mechanisms of *CLIC1* in pan-cancer. Initially, raw data along with corresponding clinicopathological characteristics were collected for 33 cancers comprising 10,327 samples. *CLIC1* demonstrated overexpression in 16 cancers, with only KICH showing significant low expression. Comparative analysis revealed that *CLIC1* overexpression was positively correlated with immune checkpoint correlation, MSI, and TMB. Key intracellular chlo-

ride channel proteins exhibited the ability to predict prognosis and identify risks for solid tumors, while expression data could effectively derive clinical outcome endpoints with relative accuracy. Following *CLIC1* gene set enrichment analysis, we observed *CLIC1*'s modification of TME development at both immune and malignant cell levels. Finally, the utilization of CellChat, immunoreactive algorithms, and potential LR connections provided guidance in filtering small-molecule drugs and uncovering functional links between genes and disease states.

Microarray and tissue validation have identified elevated expression levels of *CLIC1* in pan-cancer as a critical feature. Previous research has highlighted the role of tumor-derived EVs carrying c-Myc increased *CLIC1* expression and activating the *PI3K/AKT* axis, thereby driving GC progression [15]. Moreover, alterations in exosome-derived *CLIC1* have been observed in mature B-cell neoplasms, suggesting that controlling its amplification could be a potential antitumor strategy targeting of TME in multi-subtype malignancies [16]. Additionally, *CLIC1* positivity reaches as high as 87.5% in renal clear cell carcinoma. Therefore, leveraging *CLIC1* expression frequency and mutation data enables the individualized determination tumor grading, prognostic outcomes, and heterogeneity assess-

ment [17]. Given its expression pattern and immune profile in pan-cancer, *CLIC1* holds promise as a biomarker for predicting cancer patient prognosis.

Aberrant expression of *CLIC1* confers membrane-targeting properties to cancer cells, influencing processes such as tumor invasion, migration, and metastasis. In our evaluation of pan-cancer prognosis, we found that overexpression of *CLIC1* posed a risk factor for survival in ACC, LGG, and LIHC. Specifically, elevated *CLIC1* expression was associated with increased aggressiveness in various LGG subgroups, leading to poorer overall survival outcomes [18]. Similarly, downregulation of *CLIC1* in hepatocellular carcinoma resulted in a reduced percentage of apoptotic cells and lymphatic metastasis, suggesting a potential role in recruiting *PIP5K* to the plasma membrane for directed migration and tumor metastasis [19].

In summary, we generalized that high *CLIC1* expression serves as a molecular biomarker for poor prognosis in ACC, LAML, LGG, LIHC, and PAAD, contrasting with primary ovarian and thymic carcinoma outcomes. Notably, we proposed for the first time that *CLIC1* expression consistently correlates with survival indicators in adrenocortical carcinoma and thymic carcinoma, potentially affecting invasion through the regulation of tumor angiogenesis. Additionally, *CLIC1* expression was linked to various clinicopathological features across pan-cancer. Moving forward, our future investigations aim to refine the analysis of samples and explore upstream *has-miRs* to enhance our understanding of *CLIC1* in pan-cancer, particularly its involvement in aggressive and progressive tumor behaviors [20].

The metabolic pathway of oxidative phosphorylation was markedly enriched in several cancers, suggesting that dysregulated *CLIC1* may be involved in the anti-inflammatory response, oxidative stress, and endothelial damage [21]. *CLIC1* operates through a bio-oxidative mechanism that regulates chloride conductance, and its differential expression has diverse implications in tumor signaling pathways [22]. Teixeira A *et al.* [23] found that *TNFRSF* artificially modulates the immunosuppressive environment to enhance tumor performance. We hypothesized that most necrotic immune genes may antagonize *CLIC1* in the oxidative phosphorylation environment, a concept further explored in the subsequent single-cell analysis. Additionally, the deletion of *CLIC1* in ESCA induces apoptosis via the *TLR2/JNK* pathway, thereby eliminating abnormally proliferating cells. In PCPG, there remains the possibility that the blockade of a chloride channel protein exchange enhances cell death and suppresses early signaling for various molecules [24]. GSEA demonstrates that *CLIC1* expression positively correlates with pyrimidine metabolic signaling in HNSC, which may support the findings of MSI and TMB.

Another crucial finding of our study is the correlation between *CLIC1* expression and immune components in the TIME. On one hand, *CLIC1* was found to enhance the infil-

tration of most immune cell types, including CD8+ T cells, CD4+ T cells, B cells, macrophages, and dendritic cells. However, it was observed to particularly impair the function of CD8+ T cells, promoting their apoptosis. Notably, *CLIC1*-associated channel blockers have been shown to influence immune processes in macrophages and dendritic cells, with demonstrated efficacy in autoimmune diseases [25].

On the other hand, ESTIMATE analysis identified *CLIC1* as a potential marker for the assessment of tumor purity and immune composition. This was exemplified in ovarian cancer, where *CLIC1* expression, along with CA125, served as a diagnostic biomarker of malignancy by indicating the proportion of stromal or cancer cells [26]. These findings suggest promise in developing *CLIC1* as a positive indicator of the pan-cancer immune microenvironment.

CLIC1 has been found to influence the extent of immune cell infiltration, particularly affecting monocytes, macrophages, and resulting in depleted CD8+ cells. It has been shown that tumor-associated macrophages (TAMs) and CD8+ Tex cells are spatiotemporally correlated in cancer [27]. TAMs in the *CLIC1* high-expression subgroup may contribute to the depletion of CD8+ T cells in an intratumoral hypoxic environment.

Ion channels are likely regulated by ligand binding to stimulate the immune system and combat tumors. For instance, chloride channels are implicated in VEGF-mediated alteration of GC aggressive phenotype [28], while intracellular *CLIC5* has been shown to co-localize with ferritin and *ANGPTL2*, mediating apoptotic lysosomal activity in B cells [29]. Additionally, excessive programmed cell death ligand 1 has been found to inhibit T cell ion channels [30]. While *CLIC1* has the potential to modulate monocytes, macrophages, and CD8+ Tex cells, it struggles to effectively support the activation of numerous LR interactions. We hypothesize that *CLIC1* overexpression stimulates cell signaling and immune cascade responses at the local plasma membrane of TAMs, making *CLIC1* an attractive target for immunotherapy [31]. Anti-*CLIC1* antibodies or inhibitors may therefore hold promise in the treatment of multiple cancers by targeting the TME.

Despite the availability of immunotherapy for the treatment of various solid tumors, our study explored the clinical application of *CLIC1* in pan-cancer. Based on our results, AMG-232 emerges as a promising candidate in READ, serving as a central integration point between the *CLIC1* interface and the mutagenesis/peptide repression pathway. This immune small molecule drug has demonstrated efficacy in cancer treatment [32]. Furthermore, the administration of pentoxifylline (PTX), which inhibits intracellular *CLIC1* gene transcription, shows potential efficacy in human renal cell carcinoma [33].

Additionally, Wang *et al.* [34] have utilized dimethyl sulfoxide (DMSO) to inhibit the progression of multiple tu-

mors, indicating its potential as a dose-dependent *CLIC1*-related drug candidate. Furthermore, *CLIC1*-specific inhibitors such as indanyloxyacetic acid 94 and DIDS can functionally and indirectly inhibit apoptotic events regulated by high *CLIC1* expression [35].

However, due to the toxicities of drug designs that directly target *CLIC1* membrane proteins, the efficacy of *CLIC1* inhibitors in tumors remains to be further improved. Nevertheless, the accumulation of small compounds suggests that research related to *CLIC1* is progressing towards immunogenetic and precision-targeted therapies.

The study has several limitations that should be acknowledged. Firstly, the credibility of samples sourced from public databases may be inferior compared to those obtained from sufficient in-house sources. Secondly, the study lacked validation of *CLIC1* expression in pan-cancer using body fluid samples. Thirdly, due to technical constraints, there was a lack of *in vivo* and *in vitro* experiments to investigate the regulatory mechanisms of *CLIC1*. Finally, further research is needed to explore the direct cross-talk of *CLIC1* with small-molecule compounds. These limitations underscore the need for additional studies to validate and expand upon the findings of this research.

Conclusion

We conducted the inaugural comprehensive analysis of *CLIC1* across various cancer types, revealing its potential as a prognostic biomarker. The expression level of *CLIC1* demonstrated correlations with prognosis, immune function, immune cell infiltration, and the tumor microenvironment. In summary, *CLIC1* emerges as a promising therapeutic target applicable to a broad spectrum of cancers.

Availability of Data and Materials

The results shown in the study are partly based upon data generated by the GTEx, cBioPortal, GEO, TCGA, and TISCH2. Each dataset supporting the findings of the pan-cancer analysis has a serial number (e.g., GSE131928), and the databases containing GTEx Portal at <https://gtexportal.org/home/>, cBioPortal at <https://www.cbioportal.org/>, Gene Expression Omnibus at <https://www.ncbi.nlm.nih.gov/gds/>, the Cancer Genome Atlas at <https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>, the Human Protein Atlas at <https://www.proteinatlas.org/>, and TISCH2 at <http://tisch.comp-genomics.org/>.

Author Contributions

YL, SL, MJ, WX, and HW were involved in the conceptualization and design of the study. YL and MJ performed data compilation and formal analysis. WX and HW provided help and advice on WB and PCR experiments. SL participated in the analysis and interpretation of the data.

All authors were involved in writing, editing and revising the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

The research was supported by the Guangxi Natural Science Foundation (Grant ID #: 2022JJA140340).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.400>.

References

- [1] GBD 2019 Cancer Risk Factors Collaborators. The global burden of cancer attributable to risk factors, 2010-19: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* (London, England). 2022; 400: 563–591.
- [2] Jiang X, He JIN, Wang Y, Liu J, LI X, He X, *et al.* A pan-cancer analysis of the biological function and clinical value of BTLA in tumors. *Biocell*. 2023; 47: 351–366.
- [3] Cheng L, Weng B, Jia C, Zhang L, Hu B, Deng L, *et al.* The expression and significance of efferocytosis and immune checkpoint related molecules in pancancer samples and the correlation of their expression with anticancer drug sensitivity. *Frontiers in Pharmacology*. 2022; 13: 977025.
- [4] Cromer BA, Morton CJ, Board PG, Parker MW. From glutathione transferase to pore in a CLIC. *European Biophysics Journal: EBJ*. 2002; 31: 356–364.
- [5] Yokoyama R, Kojima H, Takai R, Ohta T, Maeda H, Miyashita K, *et al.* Effects of CLIC4 on Fucosanthin-Induced Apoptosis in Human Colorectal Cancer Cells. *Nutrition and Cancer*. 2021; 73: 889–898.
- [6] Geng HY, Feng ZJ, Zhang JJ, Li GY. Exosomal CLIC1 released by CLL promotes HUVECs angiogenesis by regulating ITGβ1-MAPK/ERK axis. *Kaohsiung Journal of Medical Sciences*. 2021; 37: 226–235.
- [7] Peretti M, Angelini M, Savalli N, Florio T, Yuspa SH, Mazzanti M. Chloride channels in cancer: Focus on chloride intracellular channel 1 and 4 (CLIC1 AND CLIC4) proteins in tumor development and as novel therapeutic targets. *Biochimica Et Biophysica Acta-Biomembranes*, 2015; 1848: 2523–2531.

- [8] Chen CD, Wang CS, Huang YH, Chien KY, Liang Y, Chen WJ, *et al.* Overexpression of CLIC1 in human gastric carcinoma and its clinicopathological significance. *Proteomics*. 2007; 7: 155–167.
- [9] Ye Y, Yin M, Huang B, Wang Y, Li X, Lou G. CLIC1 a novel biomarker of intraperitoneal metastasis in serous epithelial ovarian cancer. *Tumor Biology*. 2015; 36: 4175–4179.
- [10] Huang JJ, Lin J, Chen X, Zhu W. Identification of chloride intracellular channels as prognostic factors correlated with immune infiltration in hepatocellular carcinoma using bioinformatics analysis. *Medicine*. 2021; 100: e27739.
- [11] Russo LC, Araujo CB, Iwai LK, Ferro ES, Forti FL. A Cyclin D2-derived peptide acts on specific cell cycle phases by activating ERK1/2 to cause the death of breast cancer cells. *Journal of Proteomics*. 2017; 151: 24–32.
- [12] Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*. 2012; 13: 134.
- [13] Uhlen M, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhori G, *et al.* A pathology atlas of the human cancer transcriptome. *Science (New York, N.Y.)*. 2017; 357: eaan2507.
- [14] Mao DY, Kleinjan ML, Jilishitz I, Swaminathan B, Obinata H, Komarova YA, *et al.* CLIC1 and CLIC4 mediate endothelial S1P receptor signaling to facilitate Rac1 and RhoA activity and function. *Science Signaling*. 2021; 14: eabc0425.
- [15] Chen J, Li K, Chen J, Wang X, Ling R, Cheng M, *et al.* Aberrant translation regulated by METTL1/WDR4-mediated tRNA N7-methylguanosine modification drives head and neck squamous cell carcinoma progression. *Cancer Communications (London, England)*. 2022; 42: 223–244.
- [16] Geng HY, Feng ZJ, Zhang JJ, Li GY. EExosomal CLIC1 released by CLL promotes HUVECs angiogenesis by regulating ITGβ1-MAPK/ERK axis. *Kaohsiung Journal of Medical Sciences*. 2021; 37: 226–235.
- [17] Nesi A, Cimpean AM, Ceausu RA, Adile A, Ioiart I, Porta C, *et al.* Intracellular Chloride Ion Channel Protein-1 Expression in Clear Cell Renal Cell Carcinoma. *Cancer Genomics & Proteomics*. 2019; 16: 299–307.
- [18] Wang L, He S, Tu Y, Ji P, Zong J, Zhang J, *et al.* Elevated expression of chloride intracellular channel 1 is correlated with poor prognosis in human gliomas. *Journal of Experimental & Clinical Cancer Research: CR*. 2012; 31: 44.
- [19] Li RK, Zhang J, Zhang YH, Li ML, Wang M, Tang JW. Chloride intracellular channel 1 is an important factor in the lymphatic metastasis of hepatocarcinoma. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2012; 66: 167–172.
- [20] Li S, Liu Y, Yao C, Xu A, Zeng X, Ge Y, *et al.* Prognostic prediction and expression validation of NSD3 in pan-cancer analyses. *Biocell*. 2023; 47: 1003–1019.
- [21] Xu Y, Zhu J, Hu X, Wang C, Lu D, Gong C, *et al.* CLIC1 Inhibition Attenuates Vascular Inflammation, Oxidative Stress, and Endothelial Injury. *PLoS ONE*. 2016; 11: e0166790.
- [22] Fan N, Zhou Y, Wu L, Wang X, Li P, Liu Z, *et al.* Probing Iron-Mediated Synergistic Change of Cl[−] and HClO in Liver Cancer Cells with a Dual-Color Fluorescence Reporter. *Analytical Chemistry*. 2022; 94: 10659–10668.
- [23] Teijeira A, Garasa S, Etxeberria I, Gato-Cañas M, Melero I, Delgoffe GM. Metabolic Consequences of T-cell Costimulation in Anticancer Immunity. *Cancer Immunology Research*. 2019; 7: 1564–1569.
- [24] Geng H, Feng C, Sun Z, Fan X, Xie Y, Gu J, *et al.* Chloride intracellular channel 1 promotes esophageal squamous cell carcinoma proliferation via mTOR signalling. *Translational Oncology*. 2023; 27: 101560.
- [25] Salao K, Jiang L, Li H, Tsai VW, Husaini Y, Curmi PMG, *et al.* CLIC1 regulates dendritic cell antigen processing and presentation by modulating phagosome acidification and proteolysis. *Biology Open*. 2016; 5: 620–630.
- [26] Singha B, Harper SL, Goldman AR, Bitler BG, Aird KM, Borowsky ME, *et al.* CLIC1 and CLIC4 complement CA125 as a diagnostic biomarker panel for all subtypes of epithelial ovarian cancer. *Scientific Reports*. 2018; 8: 14725.
- [27] Kersten K, Hu KH, Combes AJ, Samad B, Harwin T, Ray A, *et al.* Spatiotemporal co-dependency between macrophages and exhausted CD8⁺ T cells in cancer. *Cancer Cell*. 2022; 40: 624–638.e9.
- [28] Xia J, Wang H, Li S, Wu Q, Sun L, Huang H, *et al.* Ion channels or aquaporins as novel molecular targets in gastric cancer. *Molecular Cancer*. 2017; 16: 54.
- [29] Neveu B, Spinella JF, Richer C, Lagacé K, Cassart P, Lajoie M, *et al.* CLIC5: a novel ETV6 target gene in childhood acute lymphoblastic leukemia. *Haematologica*. 2016; 101: 1534–1543.
- [30] Chirra M, Newton HS, Gawali VS, Wise-Draper TM, Chimote AA, Conforti L. How the Potassium Channel Response of T Lymphocytes to the Tumor Microenvironment Shapes Antitumor Immunity. *Cancers*. 2022; 14: 3564.
- [31] Randhawa K, Jahani-Asl A. CLIC1 regulation of cancer stem cells in glioblastoma. *Current Topics in Membranes*. 2023; 92: 99–123.
- [32] Rew Y, Sun D, Yan X, Beck HP, Canon J, Chen A, *et al.* Discovery of AM-7209, a potent and selective 4-amidobenzoic acid inhibitor of the MDM2-p53 interaction. *Journal of Medicinal Chemistry*. 2014; 57: 10499–10511.
- [33] Tong Z, Dai H, Chen B, Abdoh Z, Guzman J, Costabel U. Inhibition of cytokine release from alveolar macrophages in pulmonary sarcoidosis by pentoxifylline: comparison with dexamethasone. *Chest*. 2003; 124: 1526–1532.
- [34] Wang B, Wu J, Wu Y, Chen C, Zou F, Wang A, *et al.* Discovery of 4-(((4-(5-chloro-2-(((1s,4s)-4-((2-methoxyethyl)amino)cyclohexyl)amino)pyridin-4-yl)thiazol-2-yl)amino)methyl)tetrahydro-2H-pyran-4-carbonitrile (JSH-150) as a novel highly selective and potent CDK9 kinase inhibitor. *European Journal of Medicinal Chemistry*. 2018; 158: 896–916.
- [35] Wang P, Zhang C, Yu P, Tang B, Liu T, Cui H, *et al.* Regulation of colon cancer cell migration and invasion by CLIC1-mediated RVD. *Molecular and Cellular Biochemistry*. 2012; 365: 313–321.