Int. J. Morphol.,
42(2):387-401, 2024.

Expression and Clinical Significance of CLCA2 in Pan-Cancer

Expresión y Significado Clínico de CLCA2 en Pan-Cáncer

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ZHAO, X.; ZHOU, L.; XU, Y.; SUI, D. & LU, J. Expression and clinical significance of CLCA2 in pan-cancer. *Int. J. Morphol., 42(2)*:387-401, 2024.

SUMMARY: The calcium-activated chloride channel (CLCA2) performs a vital function in the intricate process of tumorigenesis. Using a bioinformatics analysis system, we conducted a pan-cancer investigation on CLCA2 to explore its association with tumor prognosis and its involvement in immunology. In order to achieve this objective, we examined the prognostic significance and expression level of CLCA2 in multiple cancer types using the TIMER and Sangerbox databases. The analysis of protein interaction networks revealed proteins linked to CLCA2. To investigate the potential biological functions and enrichment pathways of CLCA2 in cancer, the SangerBox and GSCA databases were utilized. Furthermore, the expression of CLCA2 in different cancer subtypes was evaluated during the analysis. Various functional conditions of cancer cells were then compared with CLCA2 in the CancerSEA database. Using online tools like TISIDB and Assistant for Clinical Bioinformatics, the investigation explored the link between CLCA2 and immune subtypes. Additionally, it assessed immune cell infiltration as part of the analysis. In addition, the application of GDSA was employed to investigate the predictive significance of CLCA2 in relation to drug sensitivity. The research outcomes uncovered abnormal expression patterns of CLCA2 in diverse tumor categories, with its expression level demonstrating a correlation with distinct subtypes of tumors. Strong associations have been observed between enhanced patient survival rates and CLCA2 in specific tumor types. There is a noteworthy connection observed among diverse tumor types, immune cell infiltration, immune subtypes, and CLCA2. The enrichment analysis of KEGG indicates that there may exist a connection between the expression of CLCA2 and renin secretion, pancreatic secretion, as well as other pathways in pan-cancer. CLCA2 appears to primarily activate pathways such as EMT (epithelial-mesenchymal transition), RAS/MAPK, RTK, apoptosis, TSC/mTOR, and PI3K/ AKT in pan-cancer. On the other hand, it seems to inhibit pathways like cell cycle, DNA damage, hormone AR, and hormone ER. Through single-cell functional analysis, it has been confirmed that CLCA2 is associated with diverse cellular functional states, encompassing DNA repair, EMT, hypoxia, invasion, metastasis, and quiescence. Furthermore, a substantial correlation has been observed between the expression of CLCA2 and drug sensitivity towards bosutinib, tipifarnib-P1, as well as other therapeutic agents. This research affirms that various cancer types express CLCA2 and its involvement in tumor advancement and immune penetration. CLCA2 possesses the capability to function as a noteworthy biomarker and target for therapeutic intervention in diverse cancer forms.

KEY WORDS: CLCA2; Pan-cancer; Tumor immunity; Enrichment analysis; Prognosis.

INTRODUCTION

Cancer remains a significant worldwide public health issue, leading to a considerable number of annual occurrences and fatalities. It is estimated that in the year 2020, around 19.3 million individuals were diagnosed with new instances of cancer, with approximately 10 million losing their lives to this ailment globally (Sung *et al*., 2021). Cao *et al*. (2021) reported that cancer diagnoses originating solely from China accounted for 24 % of global new cases and an alarming 30 % of cancer-related deaths. The data emphasizes the immediate necessity for efficient interventions and tactics aimed at tackling the escalating cancer burden worldwide. In recent years, pan-cancer analysis has offered fresh vantage points for cancer research and enhanced global regulation of diverse and potential mechanisms in genes. The rapid advancement of sequencing technology, alongside the institution of online databases, has also furnished a considerable amount of novel data, which has served as the foundation for a comprehensive pan-cancer analysis (Liu *et al*., 2018). Tumor microenvironment (TME) cells include pericytes, endothelial cells, cancer-associated

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FUNDING. Bengbu Medical College's Natural Science Foundation (2021byzd031), the Anhui Province College Student Innovation Training Project (S202110367016; S202210367062), the Anhui Province Education Department's Key Project (2022AH051530), and Anhui Key Laboratory of Computational Medicine and Intelligent Health (Bengbu Medical College)(AHCM2023Z005) provided support for this study.

Received: 2023-12-18 Accepted: 2024-02-06

fibroblasts, and several immune cell types. With respect to variables such the organ of tumor origin, innate traits of cancer cells, tumor stage, and patient characteristics, there can be notable variation in the cellular makeup and functional state of the TME (de Visser & Joyce, 2023). Exploring new biomarkers that have a critical function in regulating cancer immunogenicity and the immune microenvironment could offer valuable insight into the development of immunotherapy (Xu *et al*., 2023).

CLCA2, a member of the regulatory calcium-activated chloride channels, has been extensively researched (Purrington *et al*., 2020). Numerous investigations have consistently reported that CLCA2 expression is significantly reduced in different tumor types. CLCA2 inhibits tumor cell proliferation, migration, and invasion through several mechanisms, including hypermethylation inactivation of the promoter region (Li *et al*., 2004), participation in Wnt/b-catenin signaling (Zhang *et al*., 2021), and inhibition of the FAK signaling pathway (Sasaki *et al*., 2012). In addition, CLCA2 inhibits EMT and activates the FAK/ERK1/2 signaling pathway (Qiang *et al*., 2018). Multiple types of cancer have been investigated in connection with CLCA2, for instance breast cancer (Li *et al*., 2004), cervical cancer (Zhang *et al*., 2021), and nasopharyngeal carcinoma (Qiang *et al*., 2018).

Nevertheless, there exists a scarcity of pertinent investigations concerning the manifestation of CLCA2 in pan-cancer, its association with patient prognosis, and its immunologic characteristics. In order to improve our understanding of cancer biology and improve patient outcomes, it is imperative that the function of CLCA2 in various cancer types be thoroughly examined. Several public databases were used to look into the expression levels of CLCA2 mRNA and protein in various pan-cancer tissues in order to accomplish this goal. We assessed the effect of CLCA2 on the survival rates of tumor patients and investigated the relationship between CLCA2, different immunological factors, cancer subtypes, and medication susceptibility. Through these investigations, a comprehensive comprehension of tumor biology was sought after.

MATERIAL AND METHOD

Data Collection. The study utilized publicly available database data and web-based bioinformatics tools for analysis. Figure 1 displays the names and web addresses of the databases utilized in this study. We obtained the TCGA Pan-Cancer dataset by conducting a search using the keyword “CLCA2”. The dataset includes essential information on clinical stage, pathological stage, and patient survival for various tumors.

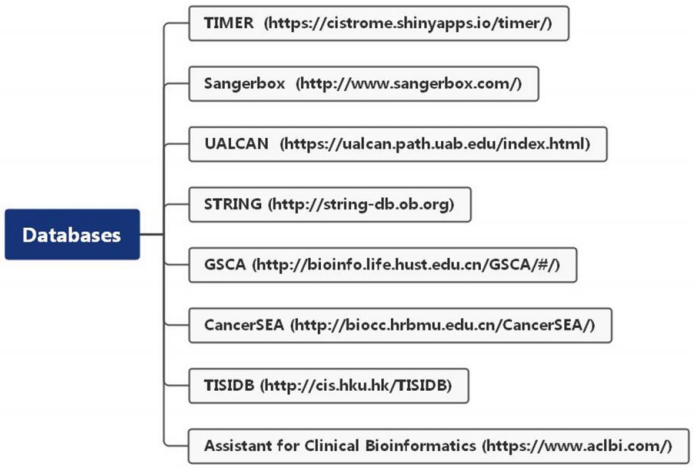

Fig. 1. Databases utilized in this study and their corresponding websites.

Gene Expression. Using the TIMER tool in conjunction with the “Diff Exp” module, CLCA2's expression in 20 tumor samples within the TCGA database was analyzed. To perform pan-cancer analysis of CLCA2, we utilized the single-gene analysis tool available on the Sangerbox platform. This tool effectively integrates the pan-cancer dataset derived from the TCGA database. Annex 1 presents the full names and abbreviations of the cancers investigated in this research. To retrieve the levels of CLCA2 protein expression from the TCGA database, the “CPTAC” tool offered by the UALCAN website was utilized.

Analysis of Survival. We evaluated the effect of CLCA2 on the prognosis of individuals suffering tumors by utilizing the “Gene expression prognostic analysis” feature within the Sangerbox website's “Pan-cancer analysis” module. We generated a forest plot showing associations between CLCA2 expression and overall survival (OS), disease-specific survival (DSS), disease-free interval (DF), and progression-free interval (PF) across cancers. The investigation revealed two patient groups: one for high expression and the other for low expression. This categorization was determined by a predetermined cut-off value, and the results were presented as single-gene survival analysis plots (Kaplan-Meier curves).

Enrichment Analysis. The researchers screened 50 CLCA2-binding proteins using the STRING database. The top ten hub genes with the highest binding were screened using the Cytoscape hubba plugin, and the screened hub genes were further analyzed by GO and KEGG enrichment using the SangerBox online tool. Additionally, Gene Set Cancer Analysis (GSCA) was further utilized to examine the

association of hub genes expression with the functionality of tumor-related pathways across multiple cancer types. This aids in locating relevant data regarding the biological mechanisms behind cancer.

Single Cell Functional Analysis. The CancerSEA database is a specialized resource for researching the distinct functional states of individual cancer cells at the single-cell level. Individual cancer cells are covered in 14 different functional states (Yuan *et al*., 2019). Our effort aimed to ascertain the effect of CLCA2 on particular tumor cells using the CancerSEA database.

Association of gene expression with cancer subtypes. Expression and subtype analysis is utilized to identify changes in gene expression associated with a given subtype. In order to carry out this task, GSCA makes use of clinical data derived from nine different types of cancer tumors, namely HNSC, LUSC, COAD, STAD, LUAD, GBM, BRCA, KIRC, and BLCA. For the purpose of analyzing CLCA2 expression in various cancer subtypes, the GSCA database was utilized in this research study. The specific operation is as follows: enter gene name, select all tumors, and check Expression & Subtype.

Association between Gene Expression and Immune System. The TISIDB portal facilitates the analysis of tumor-immune system interactions by integrating various data types. This study examines the association of CLCA2 with immune subtypes through the TISIDB database. Accurate assessment of the relationship between immune system and CLCA2 expression was made possible by the Assistant for Clinical Bioinformatics. The "Pan-Cancer" module, along with the "Immune Correlation" and "Immune Checkpoint" analysis methods, were selected for this purpose. We then applied these methods to multiple tumor tissues, correlating CLCA2 expression with both the immune infiltration score and immune checkpoint gene expression.

Correlation between genes and drug sensitivity. An investigation was conducted to examine the connection between drug sensitivity and the CLCA2 using data from the GDSC and CTRP. After visiting the GSCA website, the "Drug" module was chosen. The sections pertaining to "GDSC drug sensitivity and expression correlation" and "CTRP drug sensitivity and expression correlation" were examined in this module.

Statistical Methods. In this study, we employed conventional statistical methods in combination with an online database to determine the statistical significance of our findings. We established the threshold for significance at $P<0.05$.

RESULTS

CLCA2 expression in pan-cancer tissues. Twenty distinct tumor types' levels of CLCA2 mRNA expression were examined using the TIMER program (Fig. 2A). For four tumor types (UCEC, HNSC, LUSC, and THCA), the CLCA2 mRNA expression level was higher than that of normal tissues; however, for six tumor types ($P<0.05$), including LUAD, COAD, BRCA, PRAD, READ, and KICH, it was lower than that of normal tissues. The Sangerbox database was integrated with the pan-cancer dataset from the TCGA database. By removing cancer samples with less than 3 in each type, the levels of CLCA2 mRNA expression were obtained for 26 different types of tumors (Fig. 2B). In 7 tumor tissues, including CESC, ESCA, UCEC, HNSC, LUSC, THCA, and CHOL, CLCA2 mRNA expression levels showed an increase compared to those in normal tissues. CLCA2 mRNA expression levels were down-regulated in 9 tumor tissues ($P<0.05$), such as GBM, LUAD, COAD, COADREAD, BRCA, PRAD, READ, PCPG, and KICH. Using the CPTAC database, our analysis discovered that the CLCA2 protein displayed reduced levels of expression in BRCA. In contrast, a noteworthy upsurge in expression was observed in UCEC (Fig. 2C).

The collective findings from these two database analyses showed that CLCA2 was considerably upregulated in UCEC, HNSC, LUSC, and THCA tumors, while significantly downregulated in LUAD, COAD, BRCA, PRAD, READ, and KICH tumors.

Investigating the Link between Tumor Patient Prognosis and CLCA2 Expression. The outcome of individuals with different types of cancers was evaluated by an examination of clinical data from the TCGA database source. Log-rank P-values were calculated, along with hazard ratios (HR) and 95 % confidence intervals (95 % CI), to determine the association. The forest plot for OS revealed an unfavorable prognosis with high expression in KIPAN (p=2.5e-4, HR=1.09[1.04,1.14]), KIRC (p=2.4e-3, HR=1.10[1.03,1.16]), and SKCM (p=1.9e-3, HR=1.08[1.03,1.13]). In COAD (p=0.03, HR=0.91[0.84,0.99]), TARGET-NB (p= 0.05, HR=0.91[0.82,1.00]), and UVM (p=7.1e-3, HR=0.77 [0.64,0.94]), low expression indicates a poor prognosis, as illustrated in Figures 3 (A-G).

DSS analyses indicate a poor prognosis in KIPAN (p=6.0e-4, HR=1.10[1.04,1.17]), KIRC (p=6.6e-4, HR=1.14[1.06,1.23]), LIHC (p=0.03, HR=1.08[1.01,1.17]), and SKCM (p=0.04, HR=1.05[1.00,1.11]) in cases of high expression. Additionally, there is a poor prognosis in PRAD (p=0.02, HR=0.73[0.55,0.96]), LUSC (p=2.4e-3, HR=0.92 [0.87,0.97]), and UVM (p=3.5e-3, HR=0.75[0.62,0.92]) in cases of low expression, as indicated in Figures 4(A-H).

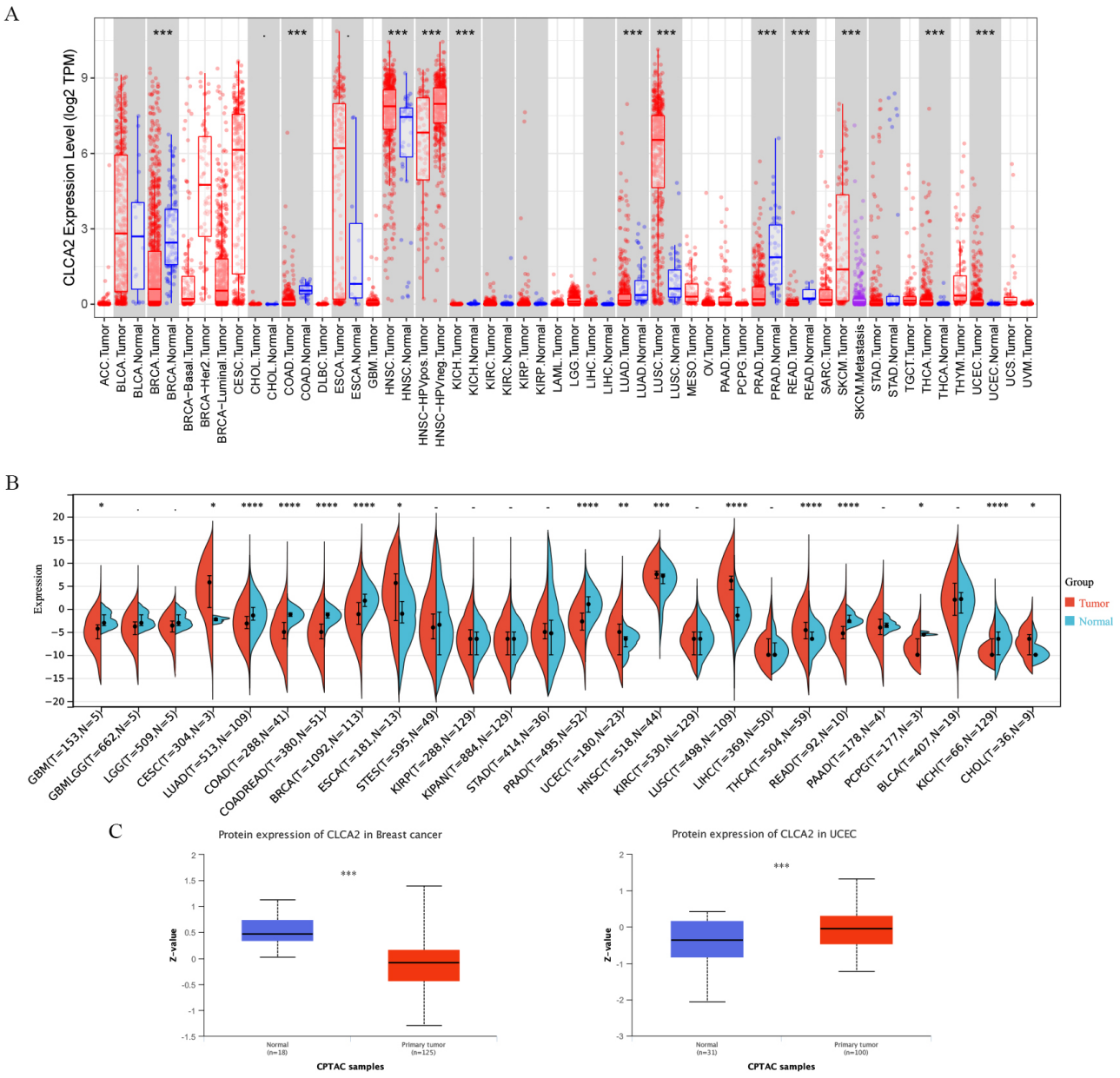

Fig. 2. CLCA2 mRNA expression levels in tumor and normal samples are analyzed. The analysis was conducted using the TIMER database (A) and the SangerBox database (B). (C) CLCA2 protein expression in BRCA and UCEC.

DF analysis showed poor prognosis for high expression in STES (p=6.3e-3,HR=1.06[1.02,1.10]), and poor prognosis for low expression in LUSC (p=5.7e-3,HR=0.91[0.85,0.97]), COAD [p=0.04,HR=0.84 (0.72,0.99)], as shown in Figures 5(A-D). The analysis of PF findings indicate that high expression in STES (p=0.01, HR=1.03[1.01, 1.06]) and KIPAN (p=0.03, HR=1.05 [1.00,1.11]) are associated with a poor prognosis. Figures 6(A-E) shows the details of the findings. Conversely, low expression in LUSC (p=3.6e-3, HR=0.94[0.89,0.98]) and ACC (p=0.03, HR=0.88[0.79,0.99]) are also linked with poor prognosis. The Kaplan-Meier survival curve results show a strong relationship between CLCA2 and the prognosis of patients with relevant malignancies. These results imply that CLCA2 might be used as a predictor of prognosis in these patients.

Functional enrichment analysis. We extracted a total of 50 CLCA2-related genes from the STRING database to investigate the possible relationship between CLCA2 and tumor formation in more detail (Fig. 7A). We then highlighted the top ten hub genes with the strongest associations using the Cytoscape hubba plugin (Fig. 7B),

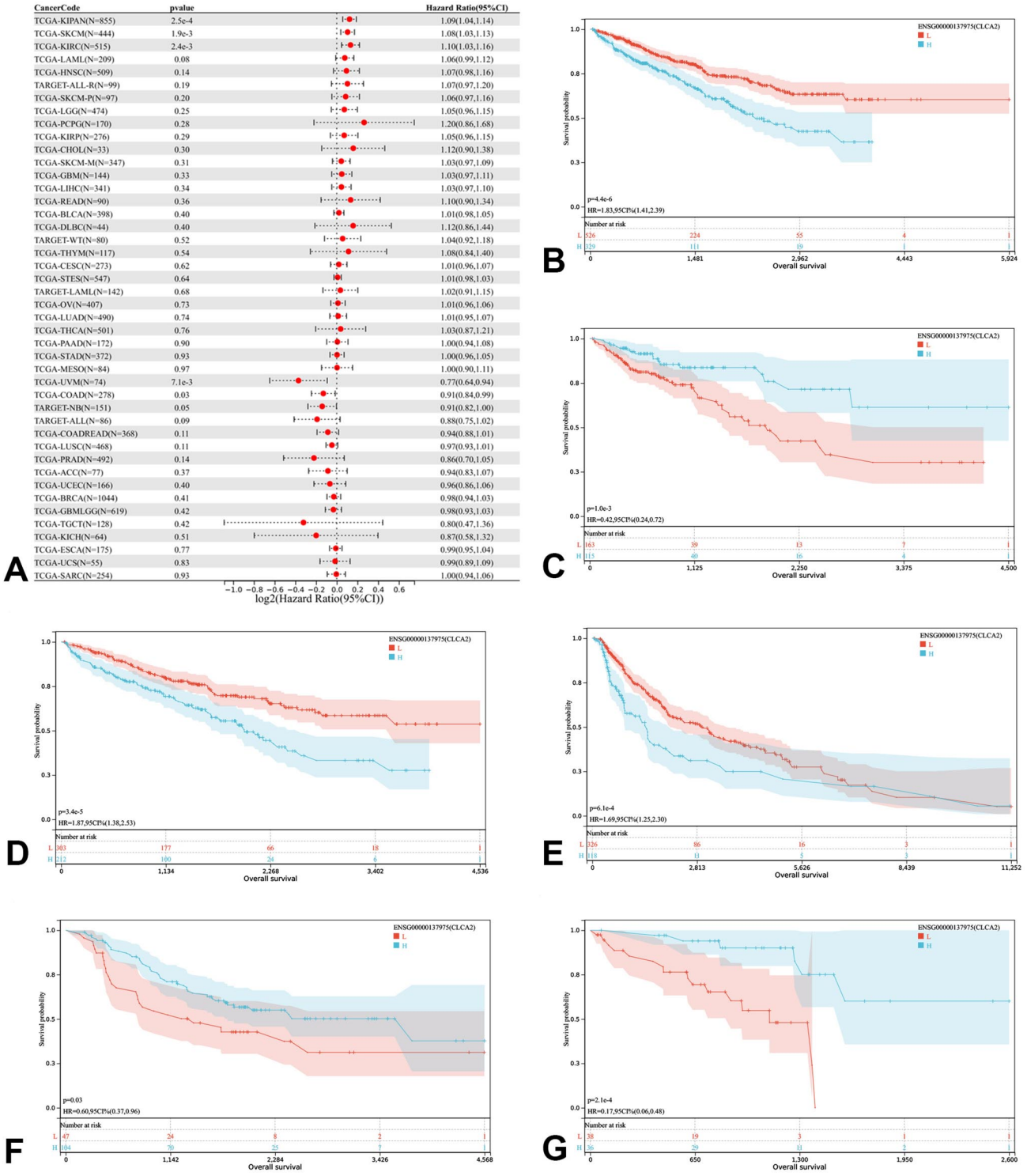

Fig. 3. Correlation analysis concerning CLCA2 and the prognosis of patients. (A) The forest plot demonstrates the prognostic outlook for OS. (B) The OS results of KIPAN are shown. (C) The OS findings for COAD are illustrated. (D) The OS outcomes for KIRC are displayed. (E) The OS results of SKCM are depicted. (F) The OS outcomes for TARGET-NB are presented. (G) The OS findings for UVM are demonstrated.

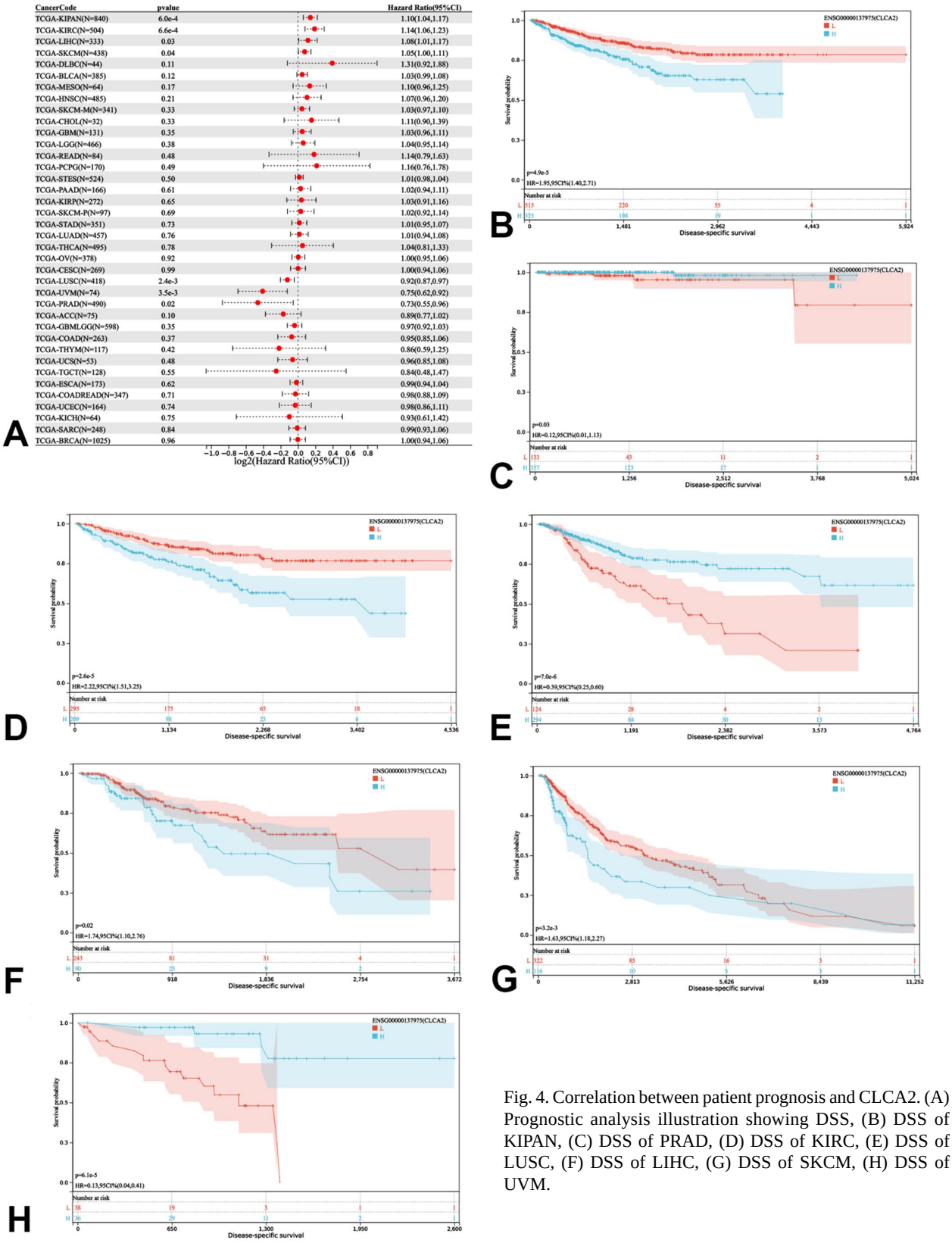

Fig. 4. Correlation between patient prognosis and CLCA2. (A) Prognostic analysis illustration showing DSS, (B) DSS of KIPAN, (C) DSS of PRAD, (D) DSS of KIRC, (E) DSS of LUSC, (F) DSS of LIHC, (G) DSS of SKCM, (H) DSS of UVM.

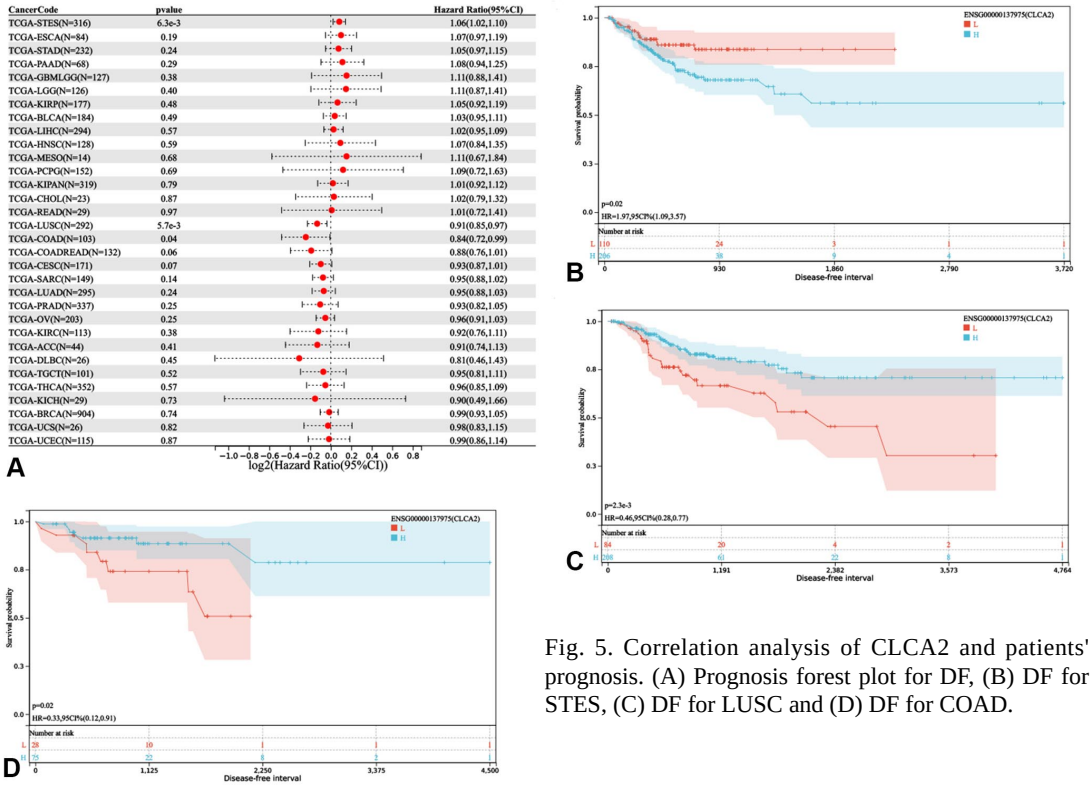

Fig. 5. Correlation analysis of CLCA2 and patients' prognosis. (A) Prognosis forest plot for DF, (B) DF for STES, (C) DF for LUSC and (D) DF for COAD.

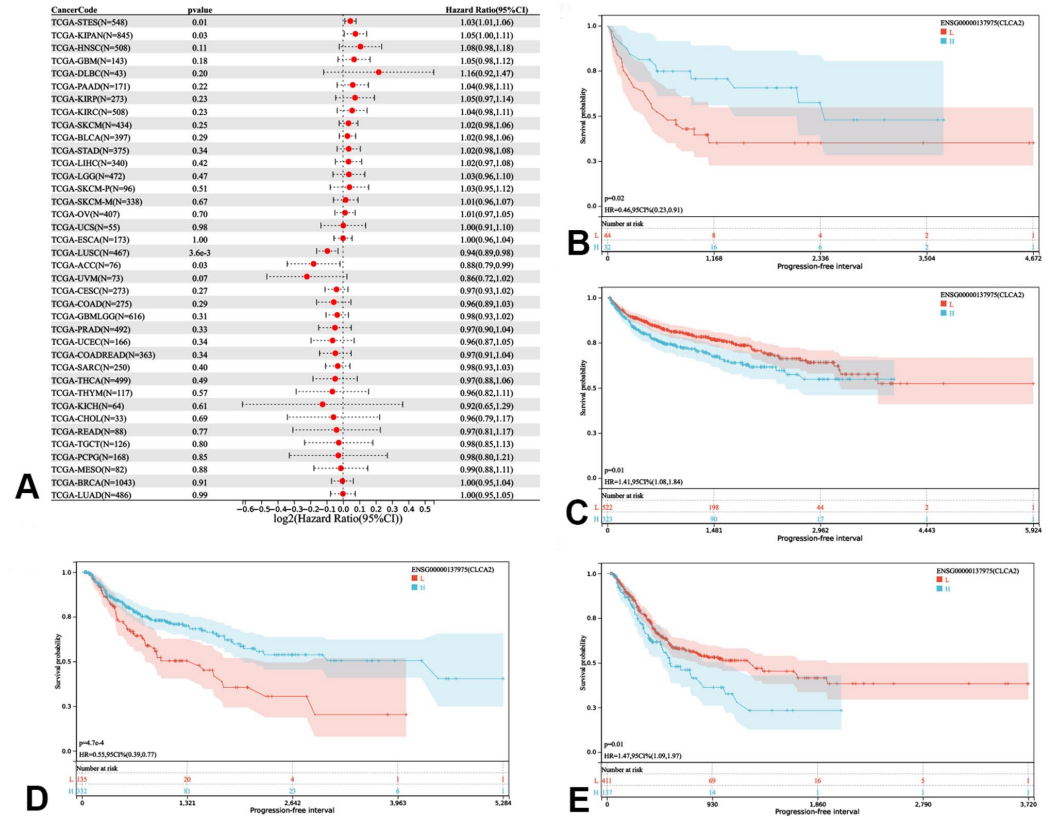

Fig. 6. Examination of the relationship between patient prognosis and CLCA2. (A) Prognostic forest map of PF, (B) PF of ACC, (C) PF of KIPAN, (D) PF of LUSC, (E) PF of STES.

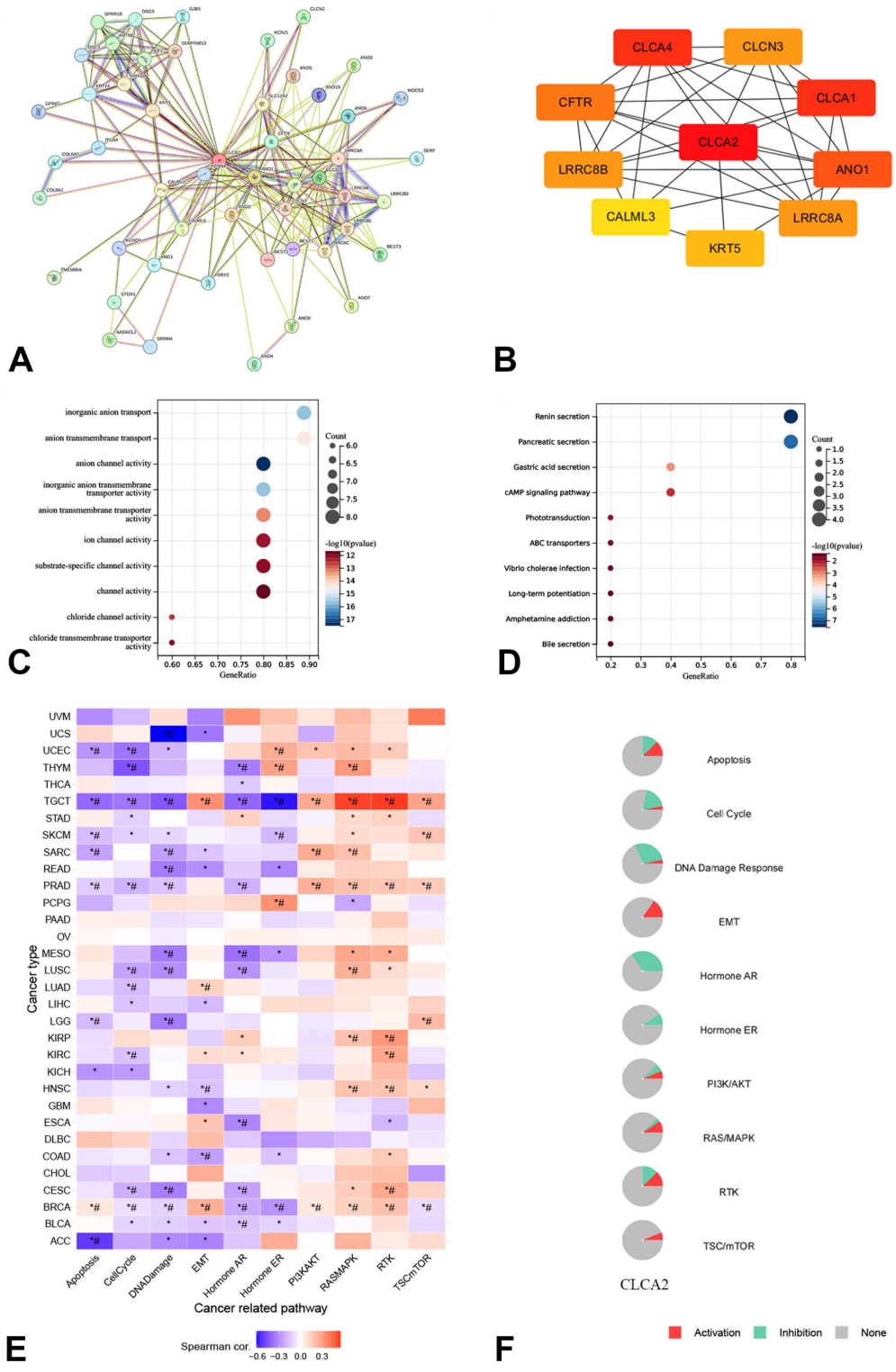

Fig. 7. Analysis of Genes Associated with CLCA2. (A) STRING database analysis showing the genes related to CLCA2. (B) the top ten hub genes screened. GO (C) and KEGG pathways (D) enrichment analysis. (E) Analysis of the relation between the activity of cancer-related pathways and the levels of hub genes in a range of tumor types. (F) Evaluation of the connection between CLCA2 and the activity of cancer-related pathways in a range of tumor types. *: P value <= 0.05; #: FDR <= 0.05.

and proceeded to conduct networked hub gene GO and KEGG enrichment investigations employing the SangerBox online tool. In the process of conducting GO functional enrichment, it was identified that genes related to CLCA2 were enriched for ion channel activity as well as anion transmembrane transport. This was reflected in Figure 7C. Renin secretion and pancreatic secretion are two key pathways in which CLCA2 is involved, as shown in the KEGG pathway analysis (Fig. 7D).

In order to delve deeper into the regulatory mechanism of CLCA2 in pan-cancer, we investigated the association between CLCA2 and the functions of 10 pathways closely related to tumor development using the GSCA database. The results of this study show that CLCA2 stimulates several pathways in pan-cancer. The primary pathways affected by CLCA2 were found to be EMT, RAS/MAPK, RTK, apoptosis, TSC/mTOR, and PI3K/AKT. On the other hand, CLCA2 was found to inhibit the cell cycle, DNA damage response, and the hormone AR and hormone ER pathways (Figs. 7E-F). Understanding these pathways and their interplay can potentially lead to the development of targeted therapies for cancer treatment.

Single-Cell Functional Analysis of CLCA2. Using data from the CancerSEA database, an investigation was conducted to look into the association between CLCA2 and various functional states of cancer cells. According to our investigation, the CLCA2 gene was linked to a number of cellular processes, such as invasion, metastasis, DNA repair, EMT, hypoxia response, and quiescence (Fig. 8A). More importantly, CLCA2 is meaningfully and positively related to cellular hypoxia and metastasis in HNSCC (r=0.38 or 0.34, p< 0.01) (Figs. 8B-C).

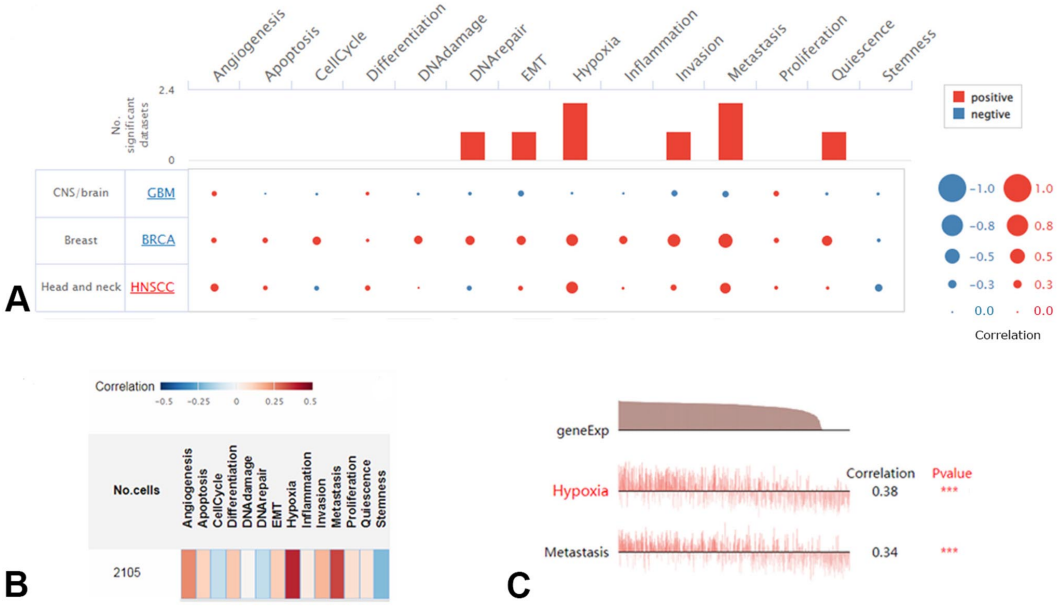

Fig. 8. Association of CLCA2 with functional status of tumor cells based on CancerSEA database. (A) Correlation between CLCA2 and functional states of different tumor cells. (B-C) Explores the relationship between the HNSCC cells' functional states and CLCA2. ***p < 0.001, **p < 0.01, and *p < 0.05.

Correlation between CLCA2 and tumor subtypes. The expression levels of CLCA2 within each of the nine distinct cancer types' subgroups were compared using the GSCA platform (Fig. 9A). Our findings imply significant differences in CLCA2 expression levels within the four LUSC subtypes – Basal, classical, primary and secretory (Fig. 9B).

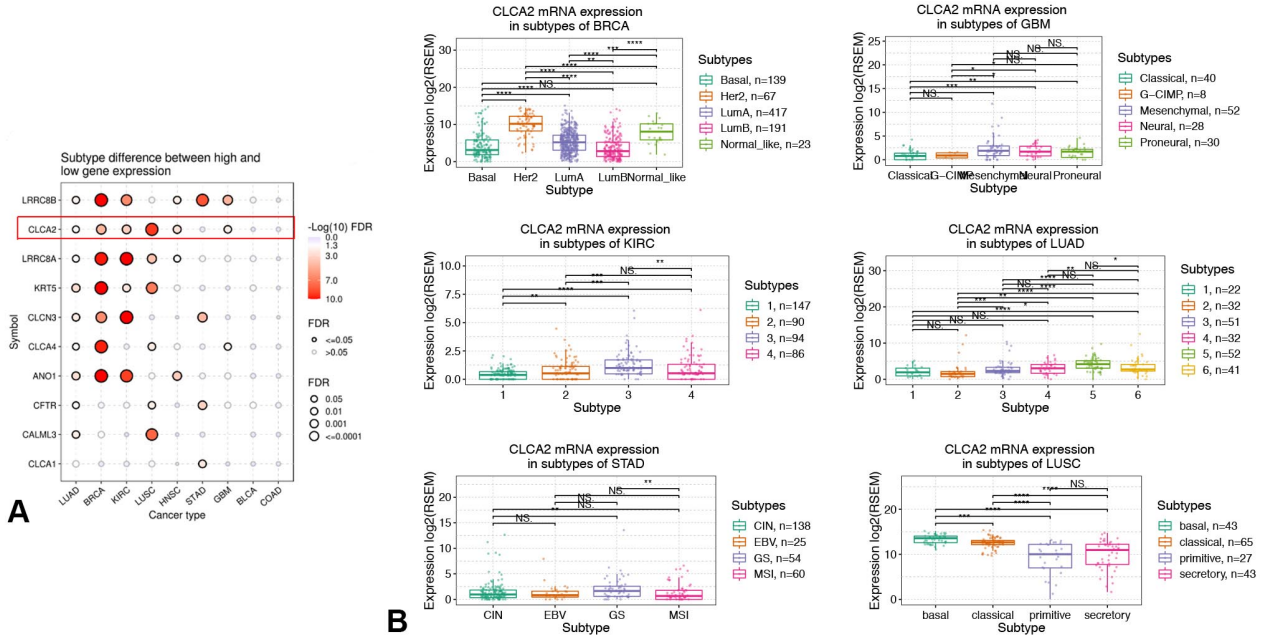

Fig. 9. Association between CLCA2 expression and tumor subtypes. (A) Differential expression of key genes in nine cancer subtypes. (B) Differential expression of CLCA2 mRNA in BRCA, GBM, KIRC, LUAD, STAD and LUSC molecular subtypes.

Association of immune cell infiltration and CLCA2. Using the TISIDB database, the connection between immunological subtypes and CLCA2 in various cancer types was examined (Figs. 10A–B). The study's findings indicate that immunological subtypes and CLCA2 gene expression are strongly correlated across a range of cancer types. In BLCA, BRCA, HNSC, LUSC, SKCM, and UCEC cancers, CLCA2 exhibited a notable correlation with immunological subtypes C1, C2, C3, C4, C5, and C6. Moreover, in the case of CESC, CLCA2 was significantly correlated with C1, C2, and C4. In LGG, CLCA2 exhibited a remarkable association with C3, C4, C5, and C6. Likewise, in PRAD, a substantial correlation existed between CLCA2 and C1, C2, C3, and C4. The potential role of the CLCA2 gene in immune response and its connection to various types of cancer is emphasized by these findings.

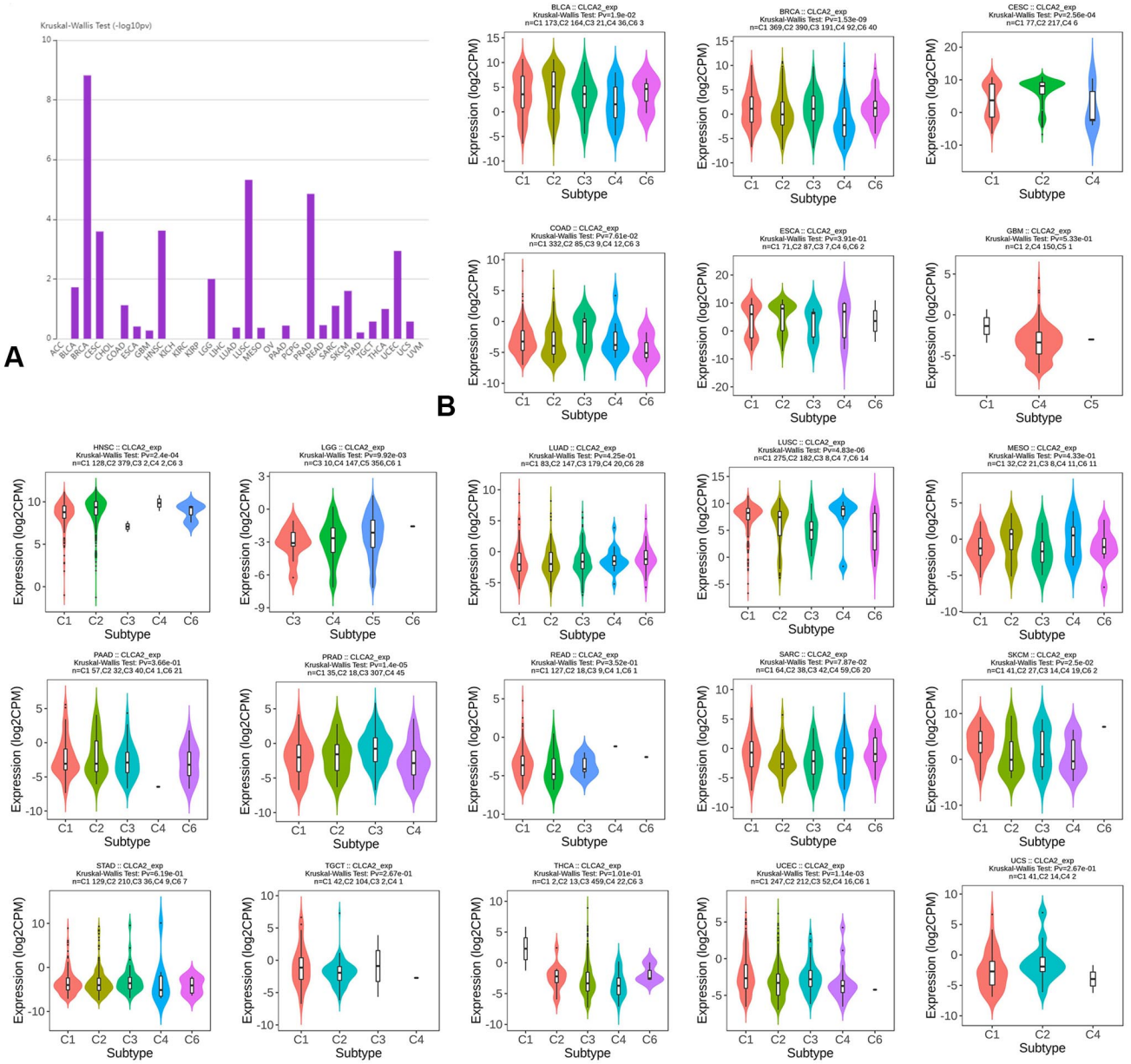

Fig. 10. Distribution of CLCA2 expression among various immune subtypes. (A) The relationship between CLCA2 and immunological subtypes in human cancers is demonstrated. (B) The association of CLCA2 expression with immune subtypes in BLCA, BRCA, CESC, COAD, ESCA, GBM, HNSC, LGG, LUAD, LUSC, MESO, PAAD, PRAD, READ, SARC, SKCM, STAD, TGCT, UCEC, and UCS is illustrated.

To ensure the accurate evaluation of immune correlation, we calculate the relation between CLCA2 and immune infiltration across 33 different types of cancers using the Assistant for Clinical Bioinformatics web application (Fig. 11A). The degree of monocyte infiltration and CLCA2 were found to positively correlate in ACC, CESC, COAD, GBM, HNSC, KICH, OV, PCPG, PRAD, and THCA. Conversely, a negative correlation was seen in the case of BLCA between CLCA2 and the level of monocyte infiltration. The expression of CLCA2 correlated negatively with B-cell plasma in 14 different forms of cancer: BLCA, CESC, ESCA, HNSC, LGG, LIHC, LUAD, LUSC, PRAD, SKCM, STAD, TGCT, THCA, and UCEC in particular. However, in COAD, there was a positive correlation observed between CLCA2 and B-cell plasma infiltration. Furthermore, we have observed a positive correlation between CLCA2 and the infiltration of immune cells in ACC, CHOL, and GBM. The expression of CLCA2 in UCEC has been found to have a negative correlation with the infiltration of immune cells. This shows that immune cell infiltration in different cancer types may be affected by the presence of CLCA2. Thus, depending on the particular cancer type, the link between CLCA2 and immune cell infiltration may be favorable or negative. This discovery emphasizes how intricate the relationship between CLCA2 and the immune system is in the development of cancer. To completely comprehend the mechanisms underlying this link and its consequences for the prognosis and treatment of cancer, more research is required.

In 33 cancers, the association between CLCA2 and immune checkpoint-related genes, including SIGLEC15, TIGIT, CD274, HAVCR2, PDCD1, CTLA4, LAG3, and PDCD1LG2, was analyzed extensively. In 19 of these cancers, including UVM, UCS, THCA, SARC, READ, PRAD, PCPG, PAAD, OV, LUAD, LIHC, LGG, LAML, KIRP, GBM, COAD, CHOL, and BRCA, there was a positive correlation with immune checkpoint gene expression. In 20 cancers, CLCA2 was found to be positively correlated with CD274. Additionally, in SKCM, LUSC, and BLCA, CLCA2 expression was negatively linked to immune checkpoint gene expression (Fig. 11B).

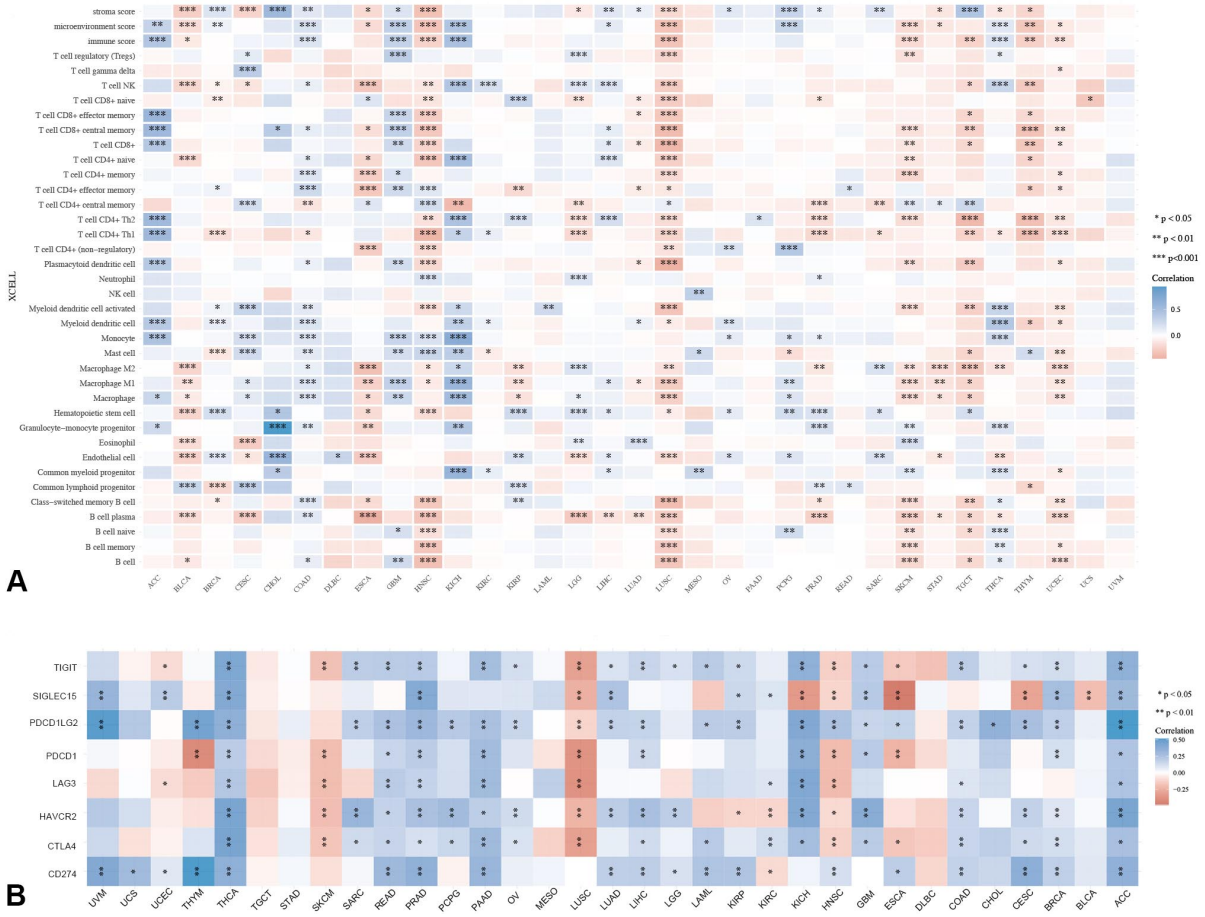

Fig. 11. Investigating the connection between CLCA2 and immune percolation in various tumor tissues. (A) Exploring the relationship between immune percolation score and CLCA2. (B) Association analysis of CLCA2 with immune checkpoint-related genes in various tumors. *P < 0.05, **P < 0.01, ***P < 0.001

Analysis of drug sensitivity. The drug sensitivity of tumors' CLCA2 expression was assessed using GSCA. Figure 12A illustrated a favorable correlation between CLCA2 and the medication THZ-2-49 in the obtained results. On the contrary, there was an inverse relationship observed between CLCA2 and bosutinib, elocalcitol, SNX2112, CR-1-31B, BRD-K66453893, as well as tipifarnib-P1 (depicted in Fig. 12B). This implies that the response to specific medications may be influenced by CLCA2.

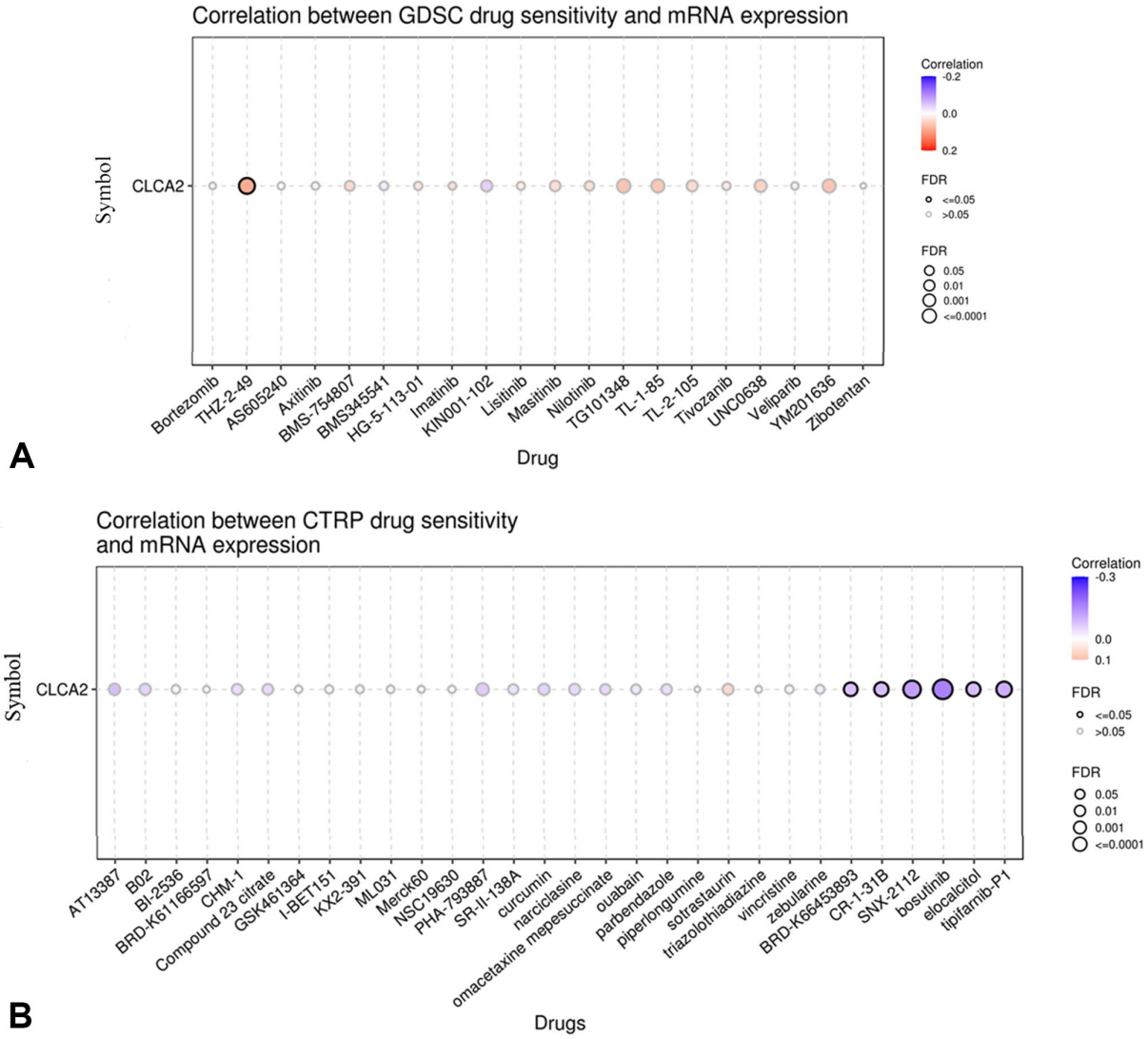

Fig. 12. Correlation between CLCA2 and drug sensitivity in both the GDSC (A) and CTRP (B) databases (top 30).

DISCUSSION

The burden of cancer in the United States is gradually decreasing (Xia *et al*., 2022). However, the cancer situation in China is changing. In China, the burden of cancer is not proportional to its population size. With an increasing trend in cancer occurrence and mortality rates over the last half-century, cancer has become the leading cause of death (Wei *et al*., 2020). Immunotherapy, a promising and innovative therapeutic strategy, has also made significant strides in clinical settings and yielded favorable outcomes (Hwang *et al*., 2022). However, despite its growing acceptance, the clinical adoption of immunotherapy encounters numerous obstacles attributable to the intricate nature of tumorigenesis mechanisms, limited applicability to a specific subset of individuals, and prevailing drug resistance exhibited by a majority of patients (Bear *et al*., 2020). Consequently, the exploration of alternative sensitive biomarkers is of utmost importance.

We utilized multiple databases, including TIMER, Sangerbox, and CPTAC, to conduct an initial analysis of the CLCA2 expression levels in various cancerous tissues. Additionally, we investigated the possible predictive value of CLCA2 for tumor prognosis. The variations observed in CLCA2 expression levels across different tumor types may indicate diverse fundamental functions and mechanisms, and the study revealed that a strong CLCA2 expression was linked to unfavorable results regarding OS and DSS in both KIPAN and KIRC cases. Individuals with low expression of CLCA2 displayed favorable outcomes in relation to OS and DSS. In a similar vein, the study on STES found that individuals who exhibited high expression of CLCA2 had significantly poorer OS prognoses in comparison to those with low expression of CLCA2. Conversely, in COAD, UVM, and TARGET-NB, the low CLCA2 expression group demonstrated poorer OS prognoses and in LUSC, the low CLCA2 expression group exhibited inferior DF and PF outcomes. Based on the examination of survival curves, it has been deduced that CLCA2 possesses the capacity to function as a reliable tumor marker in predicting the prognosis of individuals with tumors. This finding aligns with the prognostic analysis of CLCA2 expression levels in COAD patients, as reported by Pan *et al*. (2019).

Tumor-infiltrating immune cells are crucial components of the intricate TME. The impact of these cells on the pathogenesis of tumors varies depending on the cell type (Domingues *et al*., 2016). Different types of monocytes perform various immune functions related to the tumor. These functions include secreting tumor-suppressive mediators, recruiting lymphocytes, and transforming into macrophages and dendritic cells related to the tumor (Olingy *et al*., 2019). A notable correlation was observed between CLCA2 and monocytes and B-cell plasma in certain types of tumors. B-cells have anti-tumour activity in addition to their tumourigenic effects (Downs-Canner *et al*., 2022). According to Wouters *et al*. (2018), a correlation has been discovered between enhanced prognosis and elevated levels of B-cells in individuals diagnosed with melanoma, sarcoma, breast, oesophageal, lung, colon, or biliary cancers. During this study, it was observed that the CLCA2 gene's expression correlated significantly with immune subtypes across a range of cancer types, encompassing BLCA, BRCA, HNSC, LUSC, SKCM, UCEC, CESC, LGG, and PRAD. Moreover, this study thoroughly explored the relationship between protein expression levels of CLCA2 and immune checkpoint genes in a wide range of cancers, which was demonstrated as positively correlated across 20 different types of cancers. This discovery raises the possibility that CLCA2 regulates immunological checkpoint genes linked to the initiation and spread of cancer. Additionally, the study provides valuable insights into potential targets for future therapeutic interventions that aim to modulate immune checkpoint genes through the regulation of CLCA2 expression.

Aiming to explore the molecular mechanism underlying CLCA2 in various cancers, an investigation was carried out to analyze the enrichment of genes associated with CLCA2 using GO and KEGG pathway approaches. The outcomes demonstrated that renin secretion, pancreatic secretion, and several other pathways were influenced by the involvement of CLCA2. We conducted further analysis on the regulatory mechanisms of CLCA2 in pan-cancer. Based on our research, it is evident that CLCA2 primarily triggers signaling cascades associated with EMT, RAS/MAPK, RTK, Apoptosis, TSC/mTOR, and PI3K/AKT in pan-cancer. Simultaneously, it suppresses pathways linked to Cell Cycle, DNA Damage, Hormone AR, and Hormone ER. Our study outcomes strongly suggest that the regulation of these cellular processes across different types of cancer is significantly influenced by CLCA2. Tumor single-cell functional analysis has confirmed significant correlations of the CLCA2 gene with DNA repair, EMT, hypoxia, invasion, metastasis, and quiescence. EMT is associated with various factors closely related to tumor formation, invasion, recurrence, drug resistance, and numerous other properties, and the EMT process is influenced by various components of the tumor microenvironment, including cancer-associated fibroblasts, angiogenesis, inflammation, hypoxia, and others, which interact with post-transcriptional factors (Pastushenko & Blanpain, 2019). Our findings demonstrate high expression of CLCA2 in tumors including UCEC, HNSC, LUSC, and THCA, among others. A plausible hypothesis suggests that CLCA2 could potentially contribute to the dissemination of cancerous cells through activation of the EMT mechanism, thus presenting significant implications for patient prognosis. Research has demonstrated that breast cancer suppresses CLCA2, which causes the transformation of cancer cells into normal cells. The inhibition of the CLCA2gene triggers EMT and amplifies the invasiveness of breast cancer cells (Walia *et al*., 2012). Our study's results indicate that PRAD has low levels of CLCA2 expression, which is in line with the evidence presented by Porretti *et al*. (2018). Moreover, CLCA2 fosters adhesion of prostate cancer cells, suppresses EMT and triggers CTNNB1 activation. Moreover, it triggers the activation of epithelial indicators like CDH1 while suppressing the expression of mesenchymal indicators such as SNAI2 and TWIST1. Elevated levels of CLCA2 have been shown in recent research to stimulate apoptosis and inhibit the growth, migration, and invasion of cervical cancer cells. Additionally, the activity of CLCA2 hinders the process of EMT through the p38/JNK/ERK pathway, as reported by Xin *et al*. (2022).

Finally, in both the GDSC and CTRP datasets, we identified seven drugs that showed a positive or negative

correlation with CLCA2 expression in cancer cell lines. Specifically, these drugs were bosutinib and tipifarnib-P1. A previous study suggests that bosutinib, a potent dual-kinase inhibitor of SRC/ABL, has the capacity to serve as a promising initial therapy for chronic myeloid leukemia, specifically during the chronic phase (Cortes *et al*., 2018). Concerning tipifarnib-P1, it binds and inhibits farnesyltransferase with effectiveness, which facilitates RAS inactivation. As a result, this agent becomes a pan-targeted therapeutic agent for RAS (End *et al*., 2001). The limitations of this study stem from its reliance on bioinformatics analysis conducted on publicly available databases. In order to fully confirm the significance of the CLCA2 gene in pan-cancer, further experiments must be carried out.

In combination, the pan-cancer analysis of CLCA2 depicts that CLCA2 is differentially expressed in the majority of tumors, has the potential to become an independent prognosticator for various tumors, and could potentially serve as an immunoregulatory factor throughout tumorgenesis and development. These study outcomes offer an initial foundation for understanding CLCA2's function in pan-cancer, its specific biological role necessitates further experimental validation.

ACKNOWLEDGEMENTS. We extend our thanks to the public databases for offering the platform and analyses, as well as to the contributors who uploaded data resources.

AUTHOR CONTRIBUTIONS. Xueying Zhao and Jin Lu generated the initial draft and organised the data. Dongli Sui and Yuanxia Xu analysed and visualised the data. Xueying Zhao and Le Zhou analysed the data and secured the funding. The manuscript's authors actively participated in its writing and revision possessed comprehensive knowledge of its content, and granted their approval for its submission.

DATA AVAILABILITY STATEMENT. The data can be accessed openly within a public repository.

ZHAO, X.; ZHOU, L.; XU, Y.; SUI, D. & LU, J. Expresión y significado clínico de CLCA2 en pan-cáncer. *Int. J. Morphol., 42(2)*:387-401, 2024.

RESUMEN: El canal de cloruro activado por calcio (CLCA2) desempeña una función vital en el proceso de tumorigénesis. Utilizando un sistema de análisis bioinformático, llevamos a cabo una investigación pan-cáncer en CLCA2 para explorar su asociación con el pronóstico tumoral y su participación en la inmunología. Para lograr este objetivo, examinamos la importancia pronóstica y el nivel de expresión de CLCA2 en múltiples tipos de cáncer utilizando las bases de datos TIMER y Sangerbox. El análisis de las redes de interacción de proteínas reveló proteínas vinculadas a CLCA2. Para investigar las posibles funciones biológicas y las vías de enriquecimiento de CLCA2 en el cáncer, se utilizaron las bases de datos SangerBox y GSCA. Además, durante el análisis se evaluó la expresión de CLCA2 en diferentes subtipos de cáncer. Luego se compararon varias condiciones funcionales de las células cancerosas con CLCA2 en la base de datos CancerSEA. Utilizando herramientas en línea como TISIDB y Assistant for Clinical Bioinformatics, la investigación exploró el vínculo entre CLCA2 y los subtipos inmunes. Además, evaluó la infiltración de células inmunitarias como parte del análisis y se empleó la aplicación de GDSA para investigar la importancia predictiva de CLCA2 en relación con la sensibilidad al fármaco. Los resultados de la investigación descubrieron patrones de expresión anormales de CLCA2 en diversas categorías de tumores, y su nivel de expresión demuestra una correlación con distintos subtipos de tumores. Se han observado fuertes asociaciones entre mayores tasas de supervivencia de los pacientes y CLCA2 en tipos de tumores específicos. Se observa una conexión notable entre diversos tipos de tumores, infiltración de células inmunitarias, subtipos inmunitarios y CLCA2. El análisis de enriquecimiento de KEGG indica que puede existir una conexión entre la expresión de CLCA2 y la secreción de renina, la secreción pancreática y otras vías en el pancáncer. CLCA2 parece activar principalmente vías como EMT (transición epitelial-mesenquimatosa), RAS/MAPK, RTK, apoptosis, TSC/mTOR y PI3K/AKT en pan-cáncer. Por otro lado, parece inhibir vías como el ciclo celular, el daño del ADN, la hormona AR y la hormona ER. Mediante análisis funcional unicelular, se ha confirmado que CLCA2 está asociado con diversos estados funcionales celulares, que abarcan la reparación del ADN, la EMT, la hipoxia, la invasión, la metástasis y la inactividad. Además, se ha observado una correlación sustancial entre la expresión de CLCA2 y la sensibilidad al fármaco hacia bosutinib, tipifarnib-P1, así como a otros agentes terapéuticos. Esta investigación indica que varios tipos de cáncer expresan CLCA2 y su participación en el avance tumoral y la penetración inmune. CLCA2 posee la capacidad de funcionar como un biomarcador notable y como un objetivo para la intervención terapéutica en diversas formas de cáncer.

PALABRAS CLAVE: CLCA2; Pan-cáncer; Inmunidad tumoral; Análisis de enriquecimiento; Pronóstico.

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