Int. J. Morphol.,
41(6):1764-1774, 2023.

Comprehensive Analysis of CLCA1 Expression, Immune Infiltration and Immune Checkpoints in Colon Adenocarcinoma

Análisis Completo de la Expresión de CLCA1, Infiltración Inmune y Puntos de Control Inmunológico en Colonadenocarcinoma

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ZHAO, X.; CHEN, Y.; SUI, D.; WANG, L. & LU, J. Comprehensive analysis of CLCA1 expression, immune infiltration and immune checkpoints in colonadenocarcinoma. *Int. J. Morphol., 41(6)*:1764-1774, 2023.

SUMMARY: Colon adenocarcinoma (COAD) is a prevalent disease worldwide, known for its high mortality and morbidity rates. Despite this, the extent of investigation concerning the correlation between COAD's CLCA1 expression and immune cell infiltration remains insufficient. This study seeks to examine the expression and prognosis of CLCA1 in COAD, along with its relationship to the tumor immune microenvironment. These findings will offer valuable insights for clinical practitioners and contribute to the existing knowledge in the field. In order to evaluate the prognostic significance of CLCA1 in individuals diagnosed with colorectal cancers, we conducted a comprehensive analysis using univariate and multivariate Cox regression models along with receiver operating characteristic curve (ROC) analysis. This study was performed on the patient data of COAD obtained from The Cancer Genome Atlas (TCGA) database. Nomograms were developed to anticipate CLCA1 prognostic influence. Furthermore, the CLCA1 association with tumor immune infiltration, immune checkpoints, immune checkpoint blockade (ICB) response, interaction network, and functional analysis of CLCA1-related genes was analyzed. We found that Colon adenocarcinoma tissues significantly had decreased CLCA1 expression compared to healthy tissues. Furthermore, the study revealed that the group with high expression of CLCA1 demonstrated a significantly higher overall survival rate (OS) as compared to the group with low expression. Multivariate and Univariate Cox regression analysis revealed the potential of CLCA1 as a standalone risk factor for COAD. These results were confirmed using nomograms and ROC curves. In addition, protein-protein interaction (PPI) network analysis and functional gene enrichment showed that CLCA1 may be associated with functional activities such as pancreatic secretion, estrogen signaling and cAMP signaling, as well as with specific immune cell infiltration. Therefor, as a new independent predictor and potential biomarker of COAD, CLCA1 plays a crucial role in the advancement of colon cancer.

KEY WORDS: Colon adenocarcinoma; CLCA1; Bioinformatics analysis; Immune infiltration; Immune checkpoints; Survival analysis.

INTRODUCTION

According to 2019 oncology statistics, gastrointestinal cancers remain the primary reason behind the occurrence of fresh instances and fatalities, with colorectal cancer positioning at the forefront in terms of newly diagnosed cases and deaths. It surpasses other types of gastrointestinal cancers with a relatively higher prevalence (Siegel *et al*., 2019). According to the latest statistics provided by GLOBOCAN (Sung *et al*., 2021), colorectal cancer ranks third in terms of incidence rate and second in terms of mortality rate. The exact cause of COAD remains uncertain, yet it is believed to have connection with various factors such as genetics, nutrition, inflammation, immunity, and microbiome (Birt & Phillips, 2014). Currently, surgery, radiation, and targeted medication therapy make up the majority of clinical management of COAD (Yangnok *et al*., 2022). However, certain individuals diagnosed with advanced colon adenocarcinoma (COAD) exhibit unresponsiveness towards radiation or chemotherapy treatment. It is for this reason that immunotherapy has emerged as one of the most popular treatment options for these individuals (Li *et al*., 2021). Immune-based interventions offer new therapeutic avenues for enduring clinical responses across a variety of cancer types (Thomas *et al*., 2018), so finding good candidate targets for immunotherapy is crucial for the diagnosing, treating, and predicting the prognosis of individuals with tumors.

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The funding for this research was provided by the Key Project of Natural Science Research from the Anhui Provincial Department of Education (2022AH051530) and the Key Project of Natural Science Foundation from Bengbu Medical College (2021byzd031).

Received: 2023-03-17 Accepted: 2023-09-03

CLCA1, a member of the calcium-activated chloride channel regulatory factor (CLCA) family, is involved in the process of mucogenesis specifically in the cup cells found in the respiratory epithelium (Liu & Shi, 2019). Earlier research has shown that CLCA1 primarily participates in multiple respiratory disorders like asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pneumonia, and other related conditions (den Dekker *et al*., 2019; Higham *et al*., 2022). The regulation of airway mucus secretion is controlled by CLCA1 through TMEM16A (Patel *et al*., 2009; Centeio *et al*., 2021), while also facilitating the initiation and progression of asthma via its mediation of IL13 (Xu *et al*., 2022). Further analysis of the whole gene transcriptome of the lung showed that respiratory mucin CLCA1 and tightly connected, inflammation-related genes were differentially expressed in different asthma inflammatory phenotypes (Tan *et al*., 2019). In addition, CLCA1 also contributes to the formation of gastrointestinal disorders distinguished by excessive mucus production, encompassing colitis, intestinal mucosal ailments associated with cystic fibrosis, ulcerative colitis, and gastrointestinal parasitic infestations (Wang *et al*., 2021a). The study carried out by Nyström *et al*. (2019), proposed that CLCA1 has a significant role in controlling the structural organization of mucus, thus contributing to the regulation of mucus processing. More importantly, it has been discovered in various research investigations that the expression of CLCA1 exhibits a strong association with the progression of cancer (Yang *et al*., 2013, 2015). The expression of CLCA1 RNA and protein is low in malignant colon tissues (Praus *et al*., 2023). These results propose that CLCA1 could serve as a promising prognostic indicator for individuals diagnosed with colorectal carcinoma (Hu *et al*., 2019).

However, there are few reports correlating immune cell infiltration with CLCA1 in COAD, which makes the role of CLCA1 in tumor immunity inconclusive. A comprehensive analysis of CLCA1 expression in COAD needs to be performed. In this study, the TCGA database along with various commonly used open datasets were utilized to examine the correlation between CLCA1 expression and the prognosis as well as medical characteristics of COAD. Additionally, the study examines the association between CLCA1 and tumor immune infiltration, immune checkpoints, and ICB responses, thus providing new insights into the treatment and prognosis of COAD.

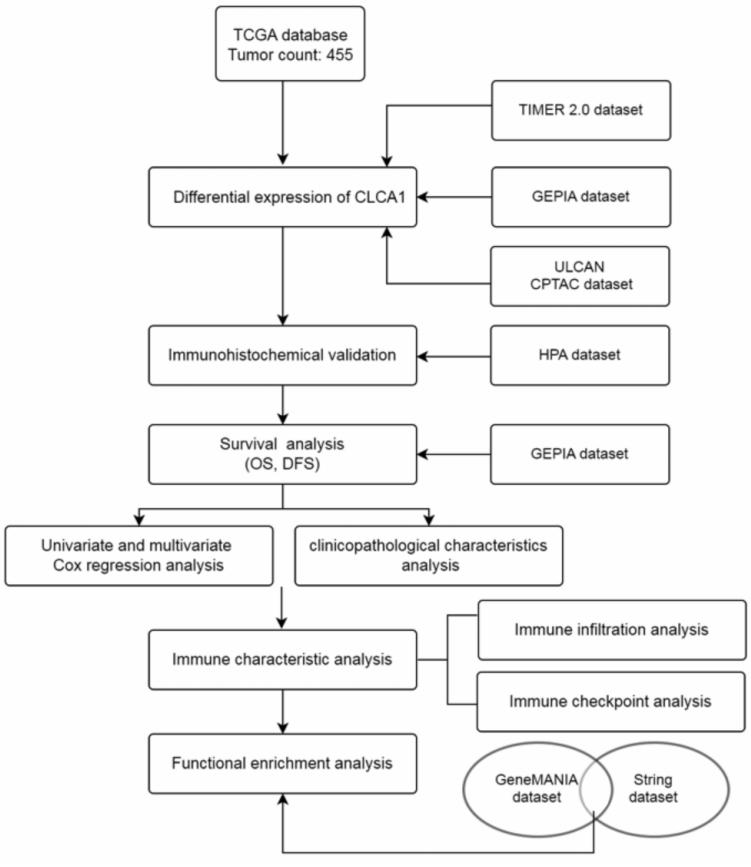


Fig. 1. The flow chart of this study.

MATERIAL AND METHOD

Data collection. The clinical information and RNA-seq expression profiles of COAD patients were collected from the TCGA database (https://cancer genome.nih.gov/). This data included relevant factors such as age, sex, tumor stage (T-stage), lymph node involvement (N-stage), distant metastasis (M-stage), TNM-stage, and survival information. Data on 455 cases of TCGA colon adenocarcinoma, 240 males and 215 females; age 31-90 years, median age 68 years. The study analysis flowchart is shown in Figure 1.

Differential expression of CLCA1. The TIMER2.0 module Gene_DE (http://timer.cistrome.org/) can be employed to examine the differential gene expression of interest between malignant and neighboring healthy tissue in all TCGA tumors. The gene expression level distribution is shown in box plots. Statistics were calculated using the Wilcoxon test.

Expression verification and survival prognosis analysis of CLCA1 gene. Using the GEPIA dataset (http://gepia.cancer-pku.cn/), we analyzed the variation in CLCA1 gene expression between COAD and the adjacent tissues. The study also

assessed the relationship between CLCA1 expression and the prognosis of COAD patients by analyzing OS and disease-free survival (DFS) rates. The center line of the boxplot is the median, the box boundaries are 25 % ~ 75 %, and the whiskers are 5 % ~ 95 %. If $P < 0.05$, the gene was considered a gene with prognostic value. CLCA1 protein expression was further explored using the Ulcan (http://ualcan.path.uab.edu) dataset, and these results were obtained from the Ulcan website.

Immunohistochemical validation. To further validate this result by clinicopathological analysis, we employed the Human Protein Atlas (HPA, https://www.proteinatlas.org/) database to evaluate the immune response towards CLCA1 protein in both normal and COAD tissues, acquire histochemical (IHC) images of CLCA1 protein. The study examined the relationship between clinicopathological features of COAD and CLCA1 expression. The TCGA data were analyzed using R software v4.0.3, and the results showed a significant difference at a significance level of $P<0.05$. The Wilcox test showed significant differences in two sample groups, while the Kruskal-Wallis test showed significant differences in three sample groups. Box plots were generated using the R package ggplot2.

Univariate and multivariate Cox regression analysis. To determine the significance of various factors as independent risk factors for colorectal adenocarcinoma, we conducted a study using univariate Cox regression analysis. This allowed us to assess the impact of CLCA1 expression level, age, sex, race, and TNM stage on the prognosis of patients. If a variable showed a significant association with prognosis, it indicated that it could be considered as a potential risk factor for colorectal adenocarcinoma. Furthermore, if the variable demonstrates significance in the multivariate Cox regression analysis, it can be considered an autonomous predictive factor. In R, the "forestplot" package was employed to show each variable (HR, P-value, and 95 % CI); Finally, variables with significant predictive variations were extracted depending on the multivariate Cox regression analysis outcomes, and a Nomogram was constructed to provide guidance for the clinical prognosis.

Immune infiltration analysis. TIMER 2.0 offers an extensive analysis and visualization of tumor-infiltrating immune cells (Li *et al*., 2020). Using the TIMER 2.0 platform, the relationship between immune infiltration and the expression of the CLCA1 gene in COAD was explored through investigation.

Immune checkpoint analysis. CD274, CTLA4, SIGLEC15, TIGIT, PDCD1, HAVCR2, PDCD1LG2, and LAG3 genes are involved in immune system checkpoints. To monitor the gene expression related to the immune checkpoint, We extracted these eight gene expression levels. The Tumor Immunodeficiency and Elimination (TIDE) algorithm were employed to anticipate a patient's potential response to COAD therapy. And correlations between non-normal quantitative variables were interpreted using Spearman's correlation analysis. The above results were obtained using the R packages ggpubr and ggplot2.

Functional enrichment analysis of CLCA1-related genes. We used the GeneMANIA (http://genemania.org) and String (https://string-db.org/) datasets to search 20 major genes associated with CLCA1 and analyze the protein-protein interaction (PPI) network. Then, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis of the retrieved genes were conducted utilizing the Metscape online analysis means (https://metascape.org/gp/index.html#/main/) to assess the potential functions associated with CLCA1.

RESULTS

CLCA1 differential expression in cancers and healthy tissues from TCGA database. Statistically significant differences in CLCA1 gene expression between BRCA (breast invasive carcinoma), COAD (colon adenocarcinoma), ESCA (esophageal carcinoma), READ (rectum adenocarcinoma), STAD (stomach adenocarcinoma), THCA (thyroid carcinoma), UCEC (uterine corpus endometrial carcinoma), and adjacent normal tissues were indicated based on data analysis from the TCGA database (Fig. 2).

CLCA1 gene expression validation in COAD tissue and prognosis analysis of individuals with COAD. Further, we verified CLCA1 gene expression in COAD using the online tool GEPIA2.0. Our study revealed a significant discrepancy ($P<0.031$) in CLCA1 expression between colon cancerous tissues and healthy tissues (Fig. 3A), aligning with the findings from TIMER2.0 analysis. This substantiates the notion that CLCA1 expression is downregulated in colon malignant tumors compared to healthy tissues. After conducting further analysis on the CPTAC dataset available on the ULCAN website, we discovered that the expression of CLCA1 protein was found to be lower in COAD tissues as compared to healthy colon tissues ($P<0.01$) (Fig. 3B). Subsequently, the connection of CLCA1 with DFS and OS was examined in COAD patients using the GEPIA. Therefore, there was a notable increase in the chances of survival for individuals displaying elevated levels of CLCA1 expression compared to those with lower CLCA1 expression

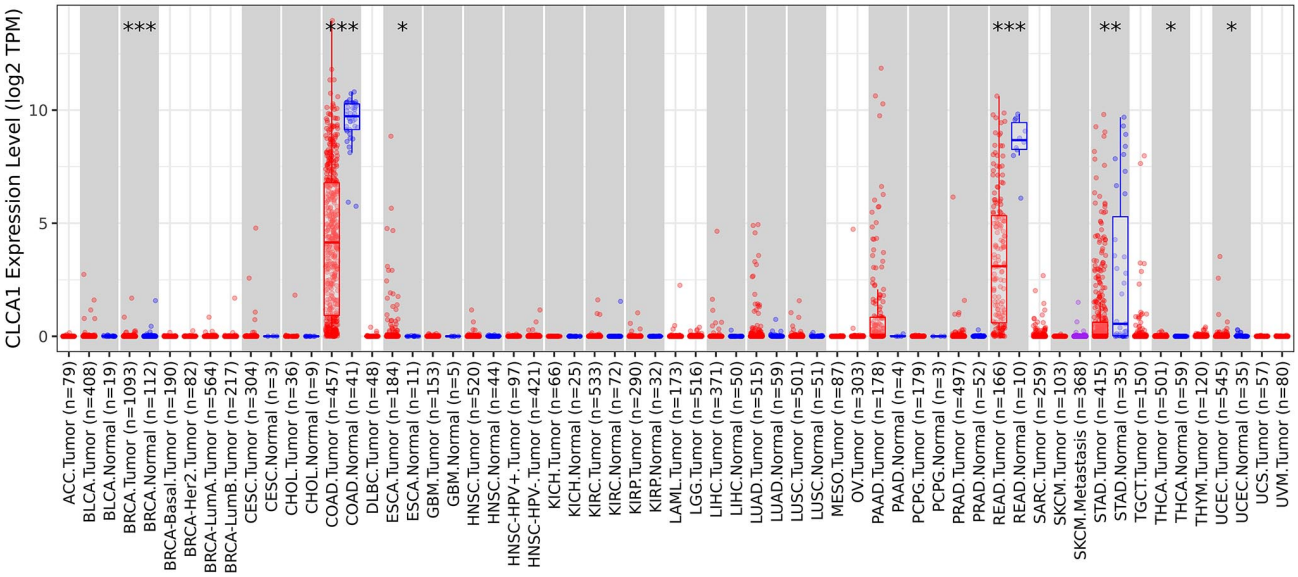
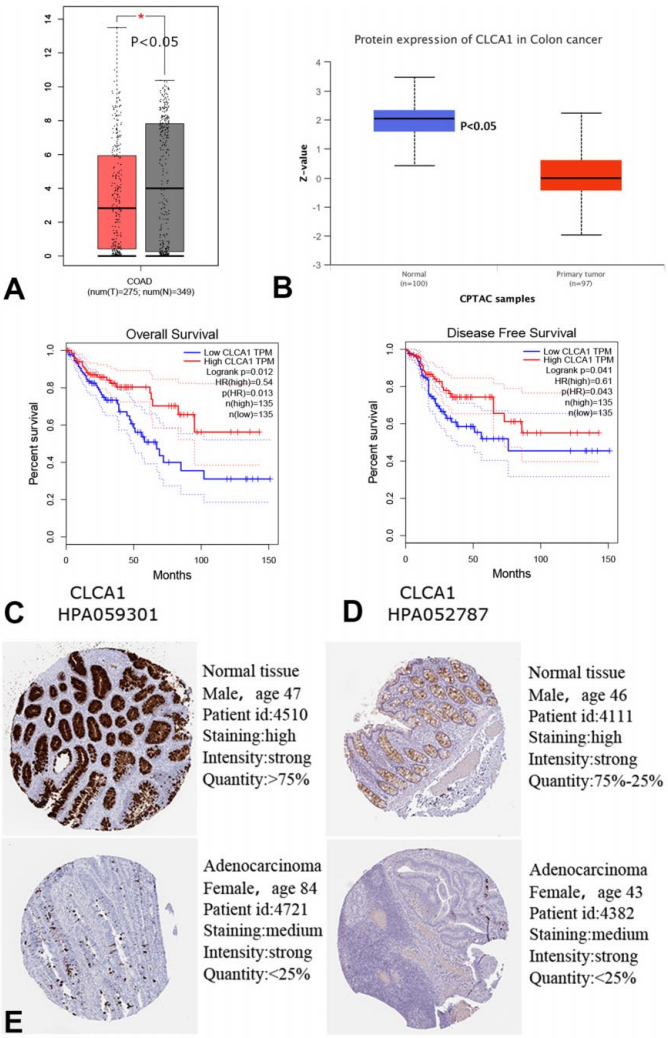


Fig. 2. CLCA1 expression in various cancer kinds in TIMER. Note: Red is the cancer group, and the healthy control group is blue. ***p<0.001; **p<0.01; *p<0.05.



(P=0.013) (Fig. 3C), and the DFS of individuals with COAD who had raised CLCA1 expression was greater than that of those with reduced CLCA1 expression (p=0.043) (Fig. 3D); thus CLCA1 can be regarded as a tumor suppressor gene of COAD.

By utilizing the Human Protein Atlas database, we validated the presence of CLCA1 protein expression and further confirmed this result by clinicopathologic analysis. The immunohistochemical findings indicated that human CLCA1 was expressed mainly in the mucosa of the small intestine and colon, with strong staining in glandular cells by the HPA052787 antibody and in goblet cells by the HPA059301 antibody, but not in endocrine cells, endothelial cells, intestinal epithelial cells, fibroblasts, mucosal lymphoid cells, and peripheral nerves/ganglia. In colorectal malignant tissues, CLCA1 protein expression was significantly lesser than that in healthy colon tissues, and the representative immunohistochemical diagram is presented in Figure 3E. From the above analysis, the analysis outcomes of GEPIA and ULCAN datasets matched those of immunohistochemical analysis at the protein level.

Fig. 3. COAD Survival Analysis and CLCA1 Expression. (A–B) Analysis of differential CLCA1 expression in COAD tissues and healthy colon tissues. Correlation between OS (C) and DFS (D) in COAD and CLCA1. (E) CLCA1 expression (immunohistochemistry) in normal intestinal and COAD tissues from the HPA database.

Correlation of CLCA1 mRNA expression with COAD clinicopathological characteristics. We further confirmed the relationship between clinicopathologic features of COAD and CLCA1 mRNA expression. According to the findings, there exists no significant difference between CLCA1 expression and cancer stage; Nevertheless, a dissimilarity was observed between stage II and stage III ($P<0.05$). In addition, there was no relationship between the CLCA1 gene and *sex*, race, age, pT stage, and pN stage. However, it was strictly associated with lymph node metastasis. In comparison to the group of colon cancer with metastasis (M1), the expression of CLCA1 was noticeably elevated in the group without metastasis (M0) ($p=0.035$) (Fig. 4A). By further comparing the prognosis of patients in the M0 and M1 groups, it was found that patients with decreased expression of CLCA1 in the M1 group exhibited a considerably reduced overall survival rate (Fig. 4B). This indicates that the down-regulation of CLCA1 expression enhances the invasion and metastasis of colon cancer.

Each variable effect on OS in colon cancer. According to the above results, the CLCA1 expression and other medical characteristics (like race, age, *sex*, and pTNM stage) impact on OS were assessed using multivariate and univariate Cox regression analyses. The outcomes indicated that CLCA1, age, and TNM stage ($p<0.05$ for all), in addition to race and *sex*, were all vital factors affecting the prognosis of individuals with colorectal cancer. We used these factors to create a nomogram analysis of risk prediction (Figs. 5A–B). The "rms" package can be utilized to generate a nomogram that can predict the likelihood of patient survival at different time intervals, such as 1, 3, and 5 years. The consistency index (C-index) was 0.717 in the verification

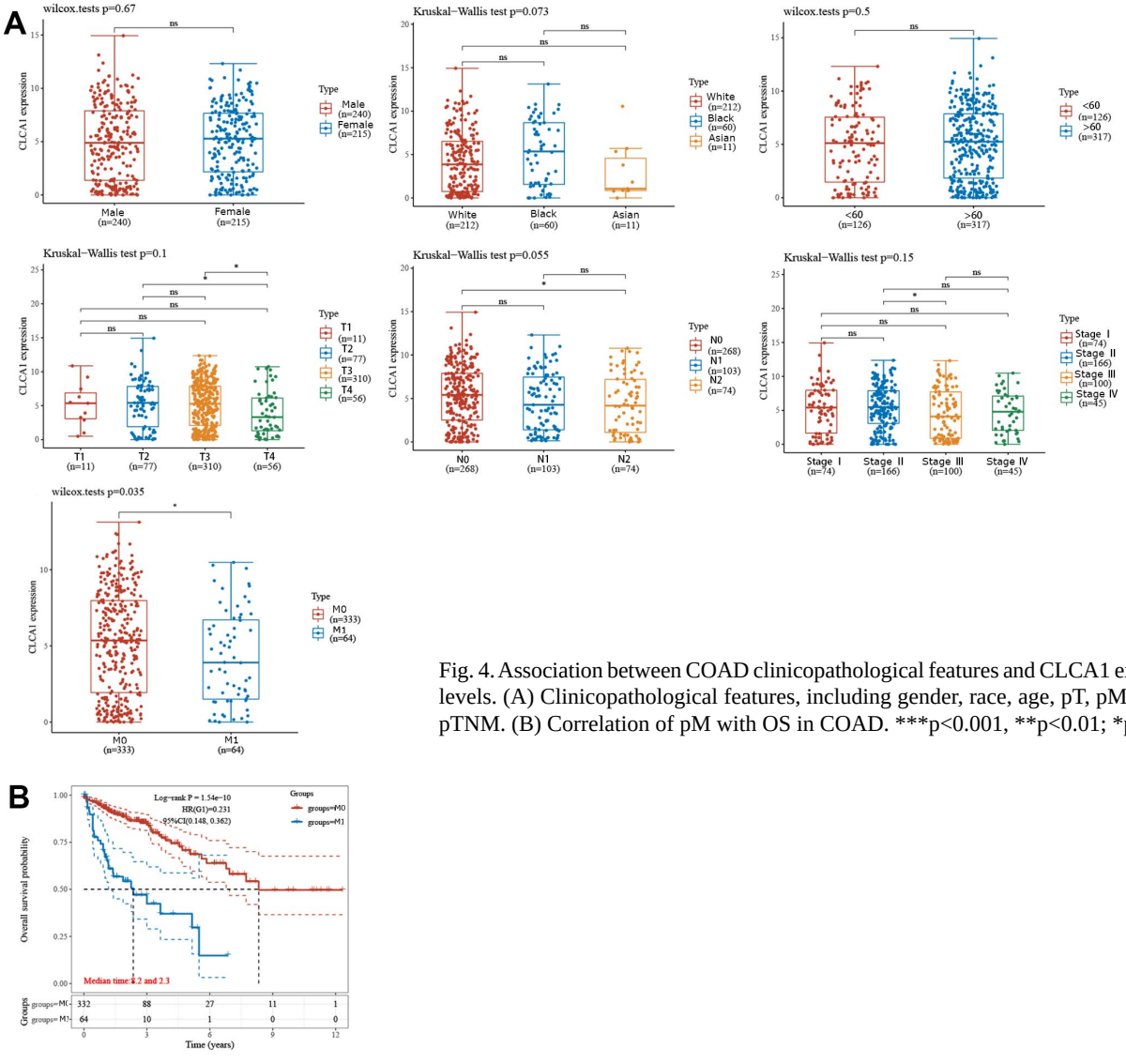


Fig. 4. Association between COAD clinicopathological features and CLCA1 expression levels. (A) Clinicopathological features, including gender, race, age, pT, pM, pN, and pTNM. (B) Correlation of pM with OS in COAD. ***p<0.001, **p<0.01; *p<0.05.

outcomes of the risk prediction nomogram (Fig. 5C), and the 1-, 3-, and 5-year survival correction curves were all close to the optimum 45°dotted line (Fig. 5D), showing high agreement between anticipated and actual survival. Thus, the CLCA1 gene and prognosis indicated significant variability in both multivariate and univariate factors, suggesting that the CLCA1 gene is an independent variable of other clinical factors.

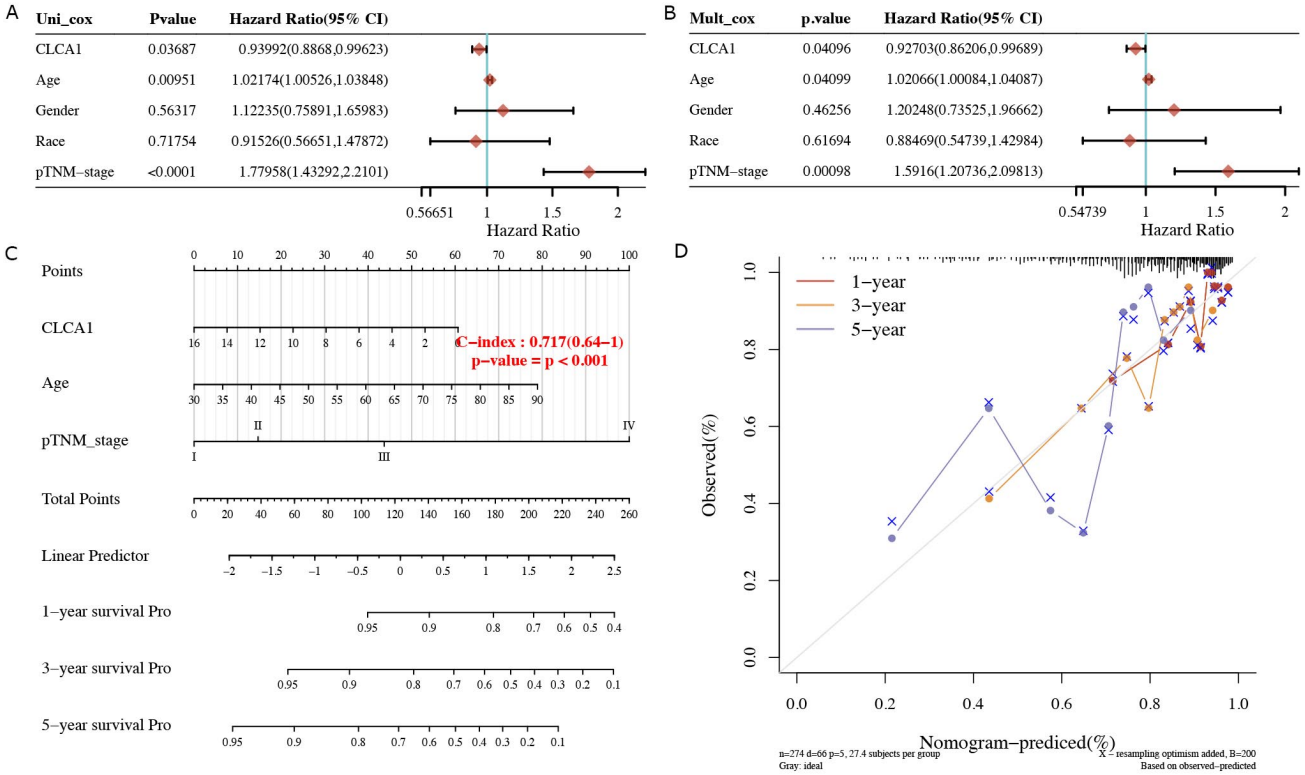


Fig. 5. Evaluating the predictive value of CLCA1 in COAD. (A–B) Univariate and multivariate Cox analyses of P-values, hazard ratios (HR), and 95 % confidence intervals (CI) for CLCA1 gene expression and clinical characteristics. (C) Constructing a nomogram to forecast the survival rates at 1, 3, and 5 years for patients diagnosed with colon cancer. (D) Assessing the accuracy of the nomogram model for predicting overall survival (OS) through a calibration curve. Note: The diagonal dashed line illustrates the ideal nomogram, while the blue, red, and orange lines show the observed 1-, 3-, and 5-year nomogram.

Correlation between CLCA1 expression and immune cells infiltration levels in COAD tissues. In the study of COAD, we aimed to explore the influence of the CLCA1 gene on the development of cancer. To gain deeper insights into this, we analyzed the relationship between the expression of CLCA1 and the infiltration levels of various immune cells using the TIMER dataset. The outcomes exhibited that CLCA1 gene expression connected to CD4+T cell infiltration (Cor=0.099, p=4.83e-02) and B cell infiltration (Cor=0.207, p=3.95e-03) (Fig. 6A). Furthermore, we employed the CIBERSORT algorithm to assess the degree of immune infiltration of COAD tissues by other immune cells. We discovered that CLCA1 was connected to T cell CD4+ memory resting (Cor = 0.425, p = 1.68e-13), activated NK cells (Cor = -0.16, p = 7.73e-03), M0 Macrophages (Cor = -0.263, p = 1.00e-05), activated myeloid dendritic cells (Cor = 0.124, p = 3.98e-02), activated mast cells (Cor= 0.132, p = 2.90e-02) (Fig. 6B). These results suggest that CLCA1 has differential regulatory effects on different immune-infiltrating cells.

Immune checkpoint analysis. We investigated the expression of immune checkpoints, including TIGIT, HAVCR2, SIGLEC15, CD274, CTLA4, PDCD1, LAG3, and PDCD1LG2, in COAD. We analyzed their expression in the high- and low-expression groups of CLCA1. Interestingly, we found that the high CLCA1 group showed significantly lower levels of SIGLEC15 expression compared to the low CLCA1 group (P=4.56e-02) (Figs. 7A–B). This observation suggests a potential association between CLCA1 expression and the regulation of SIGLEC15 levels in COAD (Cor = -0.1) (Fig. 7C). In addition, the TIDE score of individuals with COAD who had low CLCA1 expression was increased in comparison to the CLCA1 high expression group (Fig. 7D), indicating that individuals who belong to the CLCA1 low expression group might exhibit a favorable response to ICB treatment.

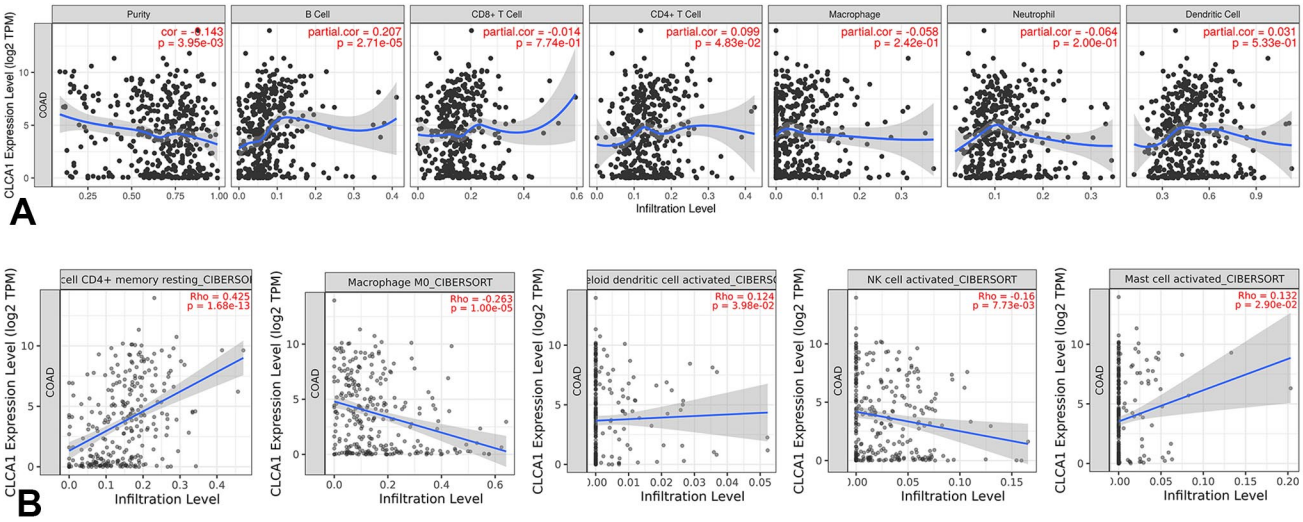


Fig. 6. Investigation into the correlation between infiltration of immune cells and the expression of the CLCA1 gene in COAD. (A) The association of CLCA1 with several immune infiltrations. (B) CLCA1 correlates with the presence of activated myeloid dendritic cells, M0 Macrophages, activated NK cells, memory-resting CD4+ T cells, and activated mast cells.

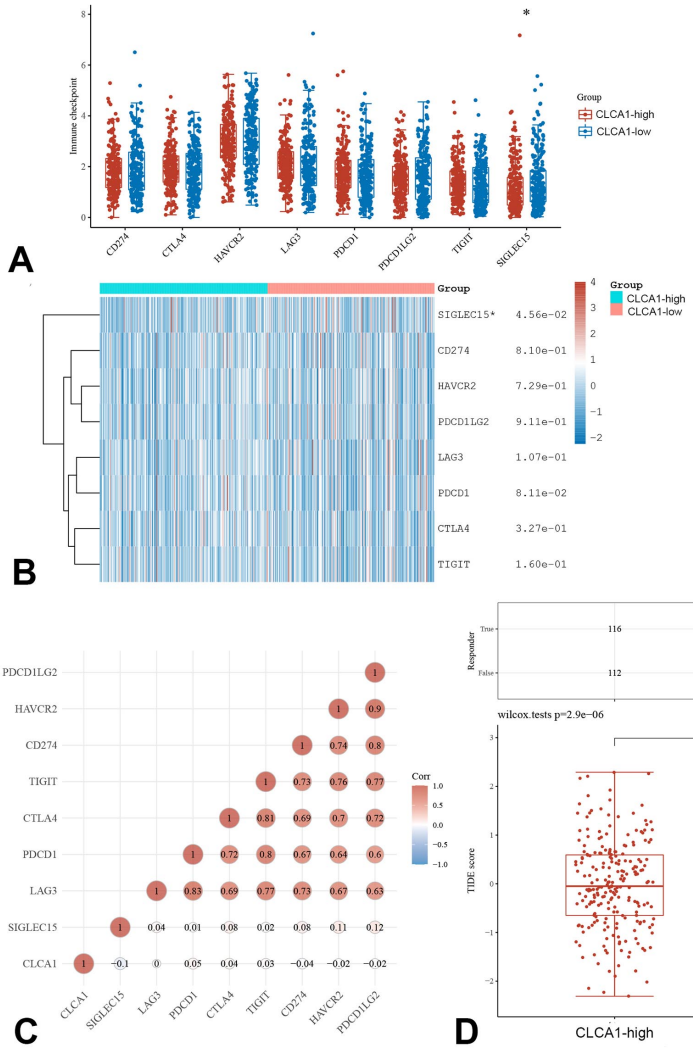


Fig. 7. Analysis of gene expression and response to ICB. (A) The expression of immune checkpoint genes in CLCA1 high and CLCA1 low groups was investigated. (B) The distribution of immune scores among groups with low and high expression was analyzed. (C) Association between immune checkpoints and CLCA1 in COAD. (D) TIDE scores in groups of COAD with CLCA1 high and CLCA1 low expression. *p<0.05; **p<0.01; ***p<0.001.

Construction and analysis of CLCA1-related gene protein interaction network. We logged into GENEMANIA (enemania.org), entered "CLCA1", selected "Homo sapiens", and plotted the network. The network revealed 20 CLCA1-related genes, 21 of which (plus CLCA1) were shown in Figure 8A. The CLCA1 PPI network was created using the dataset String and the maximum number of interactors displayed was limited to 20 (Fig. 8B).

GO and KEGG enrichment analysis of genes related to CLCA1. We used the Metscape online tool to assess the biological functions of genes linked to CLCA1 expression. GO functional annotation analysis exhibited that the genes were mostly enriched in the basal plasma membrane, isomerase activity, carbohydrate binding, digestion, brush margin, organic acid transport and intracellular ligand-gated ion channel activity, mucus layer, inorganic molecular entity transmembrane transporter activity and metal ion transport (Fig. 8C). KEGG pathway enrichment analysis of related genes connected to CLCA1 exhibited that they were mainly involved in pancreatic secretion and estrogen and cAMP signaling mechanisms (Fig. 8D).

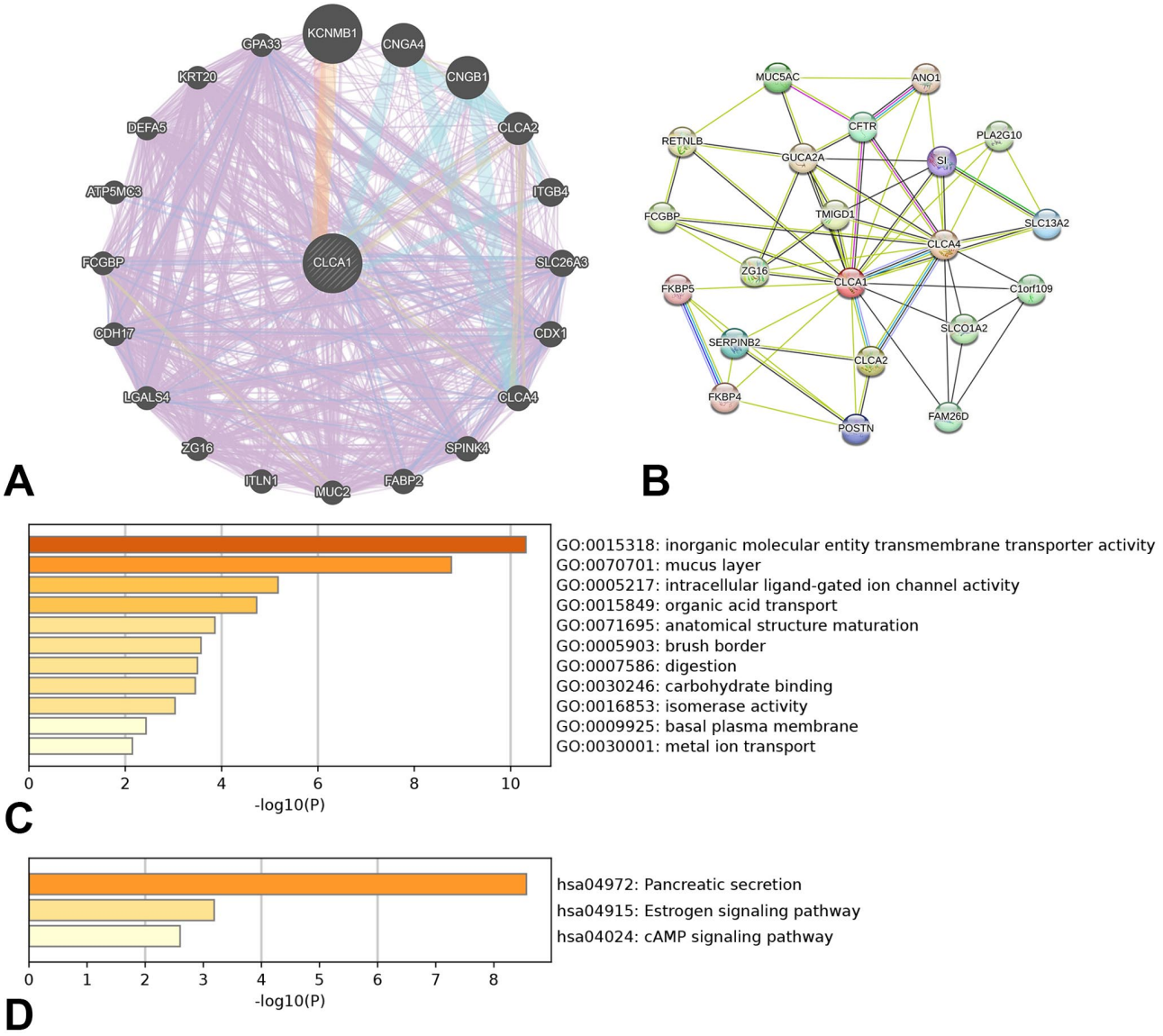


Fig. 8. Possible role of CLCA1 in COAD. (A) GeneMANIA constructed a network map of 20 genes related to CLCA1. (B) PPI network diagram of CLCA1 in COAD; (C) GO enrichment analysis; (D) KEGG enrichment analysis.

DISCUSSION

Colorectal cancer is a common and serious health risk digestive cancer (Torre *et al*., 2015). Timely recognition and personalized intervention play a pivotal role in improving the likelihood of survival among individuals who receive a colorectal cancer diagnosis. However, there is currently a lack of a highly sensitive and specific diagnostic or monitoring marker for colon cancer. The growth of colon cancer is influenced by the activation or inactivation of various oncogenes (Ling *et al*., 2015). Therefore, the identification of possible indicators is crucial in timely identification and targeting of therapeutic objectives. Through TMEM16A, the released protein CLCA1 modulates ion release and the formation of airway mucus. Goblet cell disease linked with several lung disorders has increased CLCA1 expression in particular (Centeio *et al*., 2021) and plays an oncogenic role in colon cancer (Li *et al*., 2017). Investigations showed that CLCA1 can enhance spontaneous malignant colon cell differentiation and decrease cancer cell proliferation (Yang *et al*., 2013). CLCA1 upregulation has been shown to inhibit colon cancer aggressiveness. This inhibition is associated with the suppression of the Wnt signaling pathway (Li *et al*., 2017). CLCA1 can also inhibit tumor development by inhibiting the human serine/threonine kinase 33 (STK33)-mediated signal transduction (Luo *et al*., 2021). In colorectal cancer tissues, the expression level of CLCA1 was greatly reduced when compared to the surrounding normal tissues (Yu *et al*., 2016), and low CLCA1 expression predicted poor prognosis (Chen *et al*., 2018; Liu & Shi, 2019). These diverse CLCA1 biological roles indicate that this protein has a complex function in the clinical significance of tumors.

In this investigation, we discovered that in COAD tissues, the CLCA1 expression was significantly lower than in healthy colon tissues. After that, we examined the correlation between CLCA1 and COAD prognosis and found that COAD with reduced CLCA1 expression had worse OS, suggesting that decreased CLCA1 expression predicted poor prognosis in COAD. At the same time, we found that the DFS of individuals with COAD who had elevated CLCA1 expression was better than that of those with COAD who had decreased CLCA1 expression, and prolonging DFS means preventing or delaying recurrence or metastasis. Furthermore, an association between COAD clinicopathological characteristics and CLCA1 was demonstrated. In contrast to the metastatic colon cancer group (M1), the non-metastatic colon cancer group (M0) had substantially elevated CLCA1 expression, and individuals in the M1 group with low CLCA1 expression had significantly shorter OS. The causal relationship between this prognostic result and this gene is not rigorous, and wet experiments are needed to verify whether the prognosis is reversed after the knockdown/low of this gene. In order to reinforce the verification of CLCA1's prognostic and diagnostic significance in the clinical setting, independent prognostic factor analysis utilizing multivariate and univariate Cox regression was conducted, which confirmed the considerable prognostic role of CLCA1 in COAD. Furthermore, we built nomograms to anticipate 1-, 3-, and 5-year OS in subjects with COAD. This result suggests that CLCA1 may be a beneficial predictive COAD marker.

The infiltration of cancer immune cells shows a correlation with the growth of malignancies and their response to immunotherapy (Zhu *et al*., 2022). The research of Wei FZ's team demonstrated that the CLCA1 expression in colon cancer is strictly linked to the Th17 level (Wei *et al*., 2020a,b). Our analysis demonstrated that CLCA1 expression was positively connected to B, CD4+ T, and other immune cells, revealing that COAD individuals with increased CLCA1 expression had elevated tumor immune cell infiltration levels. Investigations on T cell infiltration in cancer tissue have demonstrated the inhibition of tumor metastasis and invasion. The occurrence of enhanced infiltration of T cells in patients has repeatedly been noted to correlate with a positive prognosis (Ogden *et al*., 2017). Moreover, we assessed the correlation between CLCA1 and immune checkpoints and found that CLCA1 was only negatively associated with SIGLEC15. In contrast, high expression of SIGLEC15 in individuals diagnosed with colorectal cancer was associated with a decreased survival period and an unfavorable reaction to neoadjuvant radiotherapy (Wang *et al*., 2021b). This partly explains the tumor suppressor effect of CLCA1, as increased immune checkpoint expression is related to decreased T cells and poorer prognosis. More importantly, the CLCA1 low expression group had a higher TIDE score than that of the CLCA1 high expression group, suggesting that subjects with COAD who had low CLCA1 expression may benefit more from ICB treatment. In addition, tumor heterogeneity, patient health status, and changes in the microenvironment of immune system can all cause immune checkpoints to fail to respond and reduce therapeutic efficacy, which is the main reason why many modern immunotherapies are ineffective (Hu *et al*., 2022, 2023). Finally, to discover the potential CLCA1 biological function, we examined the genes and signaling pathways associated with CLCA1. Biological functional analysis of genes related to CLCA1 expression revealed that they were mainly concentrated in functional activities such as matrix membrane, intracellular ligand-gated ion channel activity, mucosal layer, carbohydrate binding, digestion, and pancreatic secretion. It is speculated that CLCA1 may be related to the goblet cell mucus

production or secretion modulation and have a vital function in the carbohydrate digestion phase. Research studies reported that CLCA1 is connected to the incidence of respiratory and gastrointestinal diseases caused by hypersecretion of mucus (Nyström *et al*., 2018). The function of CLCA1 in the regulation of tissue inflammation and airway mucus release is crucial for maintaining proper immune responses. It achieves this by controlling the synthesis of cytokines and chemokines, which play essential roles in inflammation and immune cell recruitment (Liu & Shi, 2019). Based on the findings of Erickson *et al*. (2016), the important role of CLCA1 in mouse colitis induced by dextrose sodium sulfate highlights its crucial participation in the control of cytokine release during the initial immune reaction. In a mouse model of acute lung inflammation, a lack of CLCA1 resulted in leukocyte recruitment and reduced cytokine expression (Erickson *et al*., 2018). Macrophages were activated in vitro (Mishina *et al*., 2015) and are highly associated with IL-13 expression in childhood asthma (Xu *et al*., 2022). In ovarian cancer, the existence of intracellular chloride channels, volume regulation, and voltage-gating has been observed through experiments conducted both in vitro and in vivo. These findings suggest a possible association between these factors and adhesion, growth, and invasion (Frede *et al*., 2013). Our study also suggests a potential role of CLCA1 in COAD. However, It is crucial to mention that our research has certain constraints. Despite the utilization of accessible databases, the explicit molecular mechanisms related to the involvement of CLCA1 in immune infiltration within COAD remains unexplored. To gain a deeper understanding of tumor initiation and progression, we intend to conduct in vivo trials to validate and elucidate the detailed action pathway of CLCA1 in COAD.

CONCLUSIONS

In conclusion, our previous research results indicated that increased CLCA1 expression was positively related to good COAD prognosis. CLCA1 expression could influence the immune microenvironment and affect the COAD prognosis indirectly, which may become a new independent prognostic factor for COAD.

ZHAO, X.; CHEN, Y.; SUI, D.; WANG, L. & LU, J. Análisis completo de la expresión de CLCA1, infiltración inmune y puntos de control inmunológico en colonadenocarcinoma. *Int. J. Morphol., 41(6)*:1764-1774, 2023.

RESUMEN: El adenocarcinoma de colon (COAD) es una enfermedad prevalente a nivel mundial, conocida por sus altas tasas de mortalidad y morbilidad. Sin embargo, el alcance de la investigación sobre la correlación entre la expresión de CLCA1 de COAD y la infiltración de células inmunes sigue siendo insuficiente. Este estudio busca examinar la expresión y el pronóstico de CLCA1 en COAD, junto con su relación con el microambiente inmunológico del tumor. Estos hallazgos ofrecerán conocimientos valiosos para los profesionales clínicos y contribuirán al conocimiento existente en el campo. Para evaluar la importancia de pronóstico de CLCA1 en personas diagnosticadas con cáncer colorrectal, realizamos un análisis exhaustivo utilizando modelos de regresión de Cox univariados y multivariados junto con un análisis de la curva característica operativa del receptor (ROC). Este estudio se realizó con los datos de pacientes de COAD obtenidos de la base de datos The Cancer Genome Atlas (TCGA). Se desarrollaron nomogramas para anticipar la influencia pronóstica de CLCA1. Además, se analizó la asociación de CLCA1 con la infiltración inmunitaria tumoral, los puntos de control inmunitarios, la respuesta de bloqueo de los puntos de control inmunitarios (ICB), la red de interacción y el análisis funcional de genes relacionados con CLCA1. Descubrimos que los tejidos de adenocarcinoma de colon tenían una expresión significativamente menor de CLCA1 en comparación con los tejidos sanos. Además, el estudio reveló que el grupo con alta expresión de CLCA1 demostró una tasa de supervivencia general (SG) significativamente mayor en comparación con el grupo con baja expresión. El análisis de regresión de Cox multivariado y univariado reveló el potencial de CLCA1 como factor de riesgo independiente de COAD. Estos resultados se confirmaron mediante nomogramas y curvas ROC. Además, el análisis de la red de interacción proteína-proteína (PPI) y el enriquecimiento de genes funcionales mostraron que CLCA1 puede estar asociado con actividades funcionales como la secreción pancreática, la señalización de estrógenos y la señalización de AMPc, así como con la infiltración de células inmunes específicas. Por lo tanto, como nuevo predictor independiente y biomarcador potencial de COAD, CLCA1 desempeña un papel crucial en el avance del cáncer de colon.

PALABRAS CLAVE: Adenocarcinoma de colon; CLCA1; Análisis bioinformático; Infiltración inmune; Puntos de control inmunológico; Análisis de supervivencia.

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