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CLDN4 as Pan-Cancer Prognostic Marker and its Effects on Immune Infiltration in the Tumor Microenvironment

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ABSTRACT

Background: CLDN4, a member of tight junction protein, whose abnormal expression will lead to dysregulated differentiation and polarity in tumors. This research aims to explore the effect of CLDN4 expression on staging and prognosis in pan-cancer, and the relationship between CLDN4 expression in immune infiltration microenvironment and immunomodulatory genes.

Methods: We downloaded RNA-seq expression datasets from TCGA and GTEx databases to analyze the datasets of CLDN4. HPA, gene card, and string database were used to investigate the degree of CLDN4 protein expression and subcellular localization. The clinical survival data of TCGA were used to explore the staging relationship and influence on prognosis. CLDN4 was enriched and analysed. Finally, the correlation between CLDN4 and cell infiltration in tumor immune microenvironment in TCGA pan-cancer samples was analyzed, and the expression of CLDN4 was scored with stromal cells and immune cell infiltration degrees. The relationship between CLDN4 and chemokine, receptor, MHC, immune inhibitor, and immune stimulator was further evaluated.

Results: We found that CLDN4 is at a high level of expression in most cancer types, and it is highly expressed in the advanced stage of most cancer types, which is different from the prognosis of different cancer types and mostly negatively correlated. The expression of CLDN4 is obviously related to TMB and MSI, and further research shows that it is negatively related to most tumor-infiltrating immune cells. For example, all cancer types are negatively related to native CD8+T cells, and CLDN4 is related chiefly to the immune regulator gene.

Conclusion: The final conclusion is that the expression of the CLDN4 gene plays a role in the occurrence, invasion, and tumor immune cell infiltration of most tumors, which can not only be used as a marker of prognosis but also provide a target for tumor immunotherapy.

INTRODUCTION

Disordered transcription programs lead to gene changes expressed by cells, which may lead to cancer Bradner et al. (2017). A variety of mutated and overexpressed genes can promote metastasis. The causes of tumor proliferation and metastasis caused by altered genes include: evading tumor immune microenvironment, sustaining, promoting proliferative cell signals, evading growth inhibition signals, inhibiting cell death, and promoting precancerous angiogenesis, thereby activating invasion and metastasis Hanahan et al. (2011). In recent years, immunotherapy for cancer has become an indispensable treatment Riley et al.(2019). We analyze the gene expression of pan-cancer and evaluate their correlation with clinical prognosis and immune infiltration, hoping to find new immunotherapy targets. CLDN4 has been found to be highly expressed in various types of cancer compared to normal tissues Morin et al. (2005). Upregulated claudins may have an impact on motility, invasion, and survival Agarwal et al. (2005). Claudin protein (CLDN), a member of tight junction protein, plays a major function in the formation and function of tight junction (TJ) Günzel et al. (2013). It plays a special epithelial or endothelial function, including maintaining cell polarity and paracellular barrier function, just like other functional structures, such as occludin and the junctional adhesion molecule 1 (JAM1) Pinto da Silva et al.(1982), Matter et al.(2003), Harhaj et al.(2004).

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Tumor cells often have abnormal tight junction function and dysregulated differentiation and polarity Nichols et al. (2004), Soler et al. (1999). CLDN4 proteins are overexpressed in BRCA, OV, PAAD, and PRAD Swisshelm et al. (2005). Recently, The research data of Sata et al. show that the expression level of CLDN4 mRNA and protein in advanced pancreatic cancer is significantly higher than that in pancreatic cancer in situ Sato et al.(2004). Recent work indicated that expression of CLDN4 may lead to an increase in invasion, motility, and cell survival in ovarian cancer. Nichols et al. have developed an alternative therapy for CLDN4-expressing cancer Nichols et al.(2004). Tight junctions can regulate the cellular movement of water, solutes, and immune cells by forming barriers between cells. Hammad et al.(2015) TJ dysregulation can lead to several autoimmune diseases Asmar et al.(2002), including celiac disease (CD) and type 1 diabetes (T1D) Clemente et al.(2003), Drago et al.(2006), Fasano et al.(2000), Sapone et al.(2006) .TJ dysfunction would trigger immunoregulation Turner et al.(2009). These researches show that CLDN4 may play a significant role in tumor microenvironment of tumor immune escape. However, there is no comprehensive pan-cancer study on CLDN4.

In this research, we evaluated the relationship between the expression of CLDN4 and the prognosis and staging of cancer patients. We further investigated the relationship between CLDN4 and tumor immune cell infiltration and its relation with immunoregulatory genes. Our results provide a potential mechanism through which CLDN4 affects tumor immune microenvironment and cancer immunotherapy.

MATERIALS AND METHODS

Data Collection and DEGs

RNA expression and clinical data were downloaded from The Cancer Genome Atlas (TCGA)database. String (https://string-db.org/)database was used to construct the protein-protein interaction network (PPI) of CLDN4. HPA database (https://www.proteinatlas.org/) is used to explore the detailed intracellular localization of CLDN4 and its expression in tumors and normal tissues. Log2 transformation was performed on the expression data, and two groups of T-tests were performed on these tumor types. $P < 0.05$ is considered a significant difference.

Immunohistochemistry (IHC) Staining

We explored the different expressions of CLDN4 at the protein level. We downloaded IHC images of CLDN4 protein expression in normal tissues and six tumor tissues from HPA. (http://www.proteinatlas.org/), including Ovarian serous cystadenocarcinoma(OV), Thyroid carcinoma(THCA), Stomach adenocarcinoma(STAD), Pancreatic adenocarcinoma(PAAD, Colon adenocarcinoma(COAD), and Kidney renal papillary cell carcinoma(KIRP).

Staged correlation analysis

Comprehensive and standardized datasets: TCGA Pan-Cancer (PANCAN, n = 10535, g = 60499) were downloaded from the UCSC (https://xenaburowser.net/) database. Screening of peripheral blood and tumor samples from patients with primary tumors and converting each expression value to log2 (x+0.001). Finally, we excluded cancer types with less than three samples in a single cancer type and obtained expression data for 30 cancer types. R software (version 3.6.4) was used to calculate the difference of gene expression in samples of different clinical stages in each tumor, unpaired Student's t-Test was used to analyze the difference between pairs, and ANOVA was used to test the difference of multiple groups of samples.

Prognostic Analysis

Evaluate the overall survival rate (OS) of patients with each type of cancer using Kaplan Meier analysis. The HR value of CLDN4 in predicting overall survival OS, disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) of pancreatic cancer was calculated by univariate Cox regression.

Gene Set Variation Analysis

The correlation between CLDN4 and all genes was analyzed in TCGA datasets. Pearson's correlation coefficients were calculated. Genes related to CLDN4 were used in gene set enrichment analysis (GSVA) ($P< 0.05$). For Gene Set Variation Analysis (GSVA), we calculated the enrichment score of each sample from GSVA (DOI: 10.18129/B9. Bioc. GSVA, version 1.40.1) by using the R software package. We predefined the gene rank, set the minimum gene set to 5 and the maximum gene set to 5000, and calculated the enrichment score of each sample in each gene set.

Tumor Mutation Burden of CLDN4

We downloaded a comprehensive and standardized pan-cancer data set: TCGA pan-cancer (PANCAN, n = 10535, g = 60499) from the UCSC (https://xenaburowser.net/) database, calculated the TMB and MSI scores of each tumor and made log2(x+0.001) for each expression value.

Immune Cell Infiltration

Unified and standardized pan-cancer datasets were downloaded: TCGA target GTEx (PANCAN, n = 19131, g = 60499) from the UCSC (https://xenaburowser.net/) database, and the expression data of CLDN4 was extracted in each sample. Log2(x+0.001) for each expression value was transformed. In addition, the expression profile was mapped to gene symbol,

the R software package IOBR (version 0.99.9, Https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8283787/'s Timer method was used. Patients were divided into two groups (high and low CLDN4 expression based on the median CLDN4 expression level) to compare the extent of immune cell infiltration in each tumor type. According to the gene expression, the infiltration scores of B cell, CD4+T cell, CD8+T cell, Neutrophil, Macrophage ,and DC of each patient in pan-cancer were re-evaluated.

Statistical Analyses

Differences between groups were analyzed using a paired t-test. Data are presented as means ± standard error (SD). Statistical analyses were performed using R (version 3.6.4) .P-value < 0.05 (two-tailed) was considered statistically significant.

RESULTS

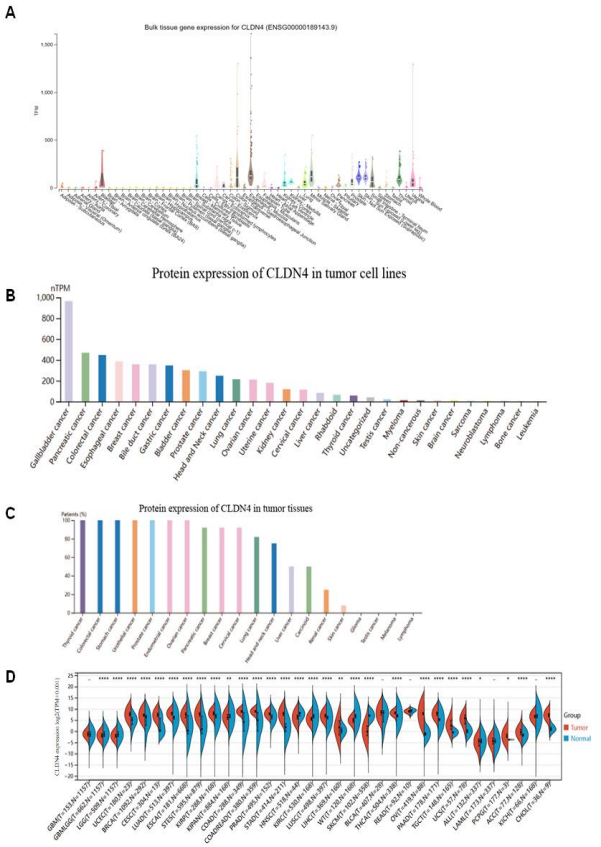
CLDN4 Expression Analysis in Pan-Cancer

The CLDN4 gene expression map in normal tissues was downloaded from the GTEx database (Figure 1A). Degrees of expression were highest in the duodenum of normal tissues, and expressed lowest in adipose tissues. The relative expression levels of CLDN4 in different tumor cell lines were downloaded from the HPA database (Figure 1B). In addition, we analyzed CLDN4 expression in various tumor tissues (Figure 1C). Finally, the expression levels of CLDN4 between 34 types of cancer and their matched normal samples were evaluated from TCGA data (Figure 1D). We observed significant up-regulation in 21 kinds of tumors, such as UCEC、BRC、CES、LUAD、ESCA、STES、KIRP、COAD、COAD、READ、PRAD、STAD、LUSC、LIHC、THC、OV、PAAD、TGCT、UCS、PCPG、ACC、CHOL. In comparison, low CLDN4 expression was observed in 8 tumors: GBMLGG、LGG、KIPAN、HNSC、KIRC、WT、SKCM、ALL.

Figure 1: Differential expression of CLDN4.

(A)CLDN4 expression in normal tissues. (B) CLDN4 expression in tumor cell lines. (C) CLDN4 expression in 20 types of cancer. (D) Comparison of CLDN4 expression between tumor and normal samples. *P < 0.05, **P < 0.01, ***P < 0.001.

Furthermore, we evaluated the IHC maps of normal tissues and corresponding cancer tissues from the HPA database and compared CLDN4 gene expression from the TCGA database (Figures 2A–F). The results of the IHC plot are basically consistent with the results of the TCGA data analysis. Among the six tissues extracted, the staining of tumor tissue was significantly more substantial than that of normal tissue with CLDN4-IHC staining.



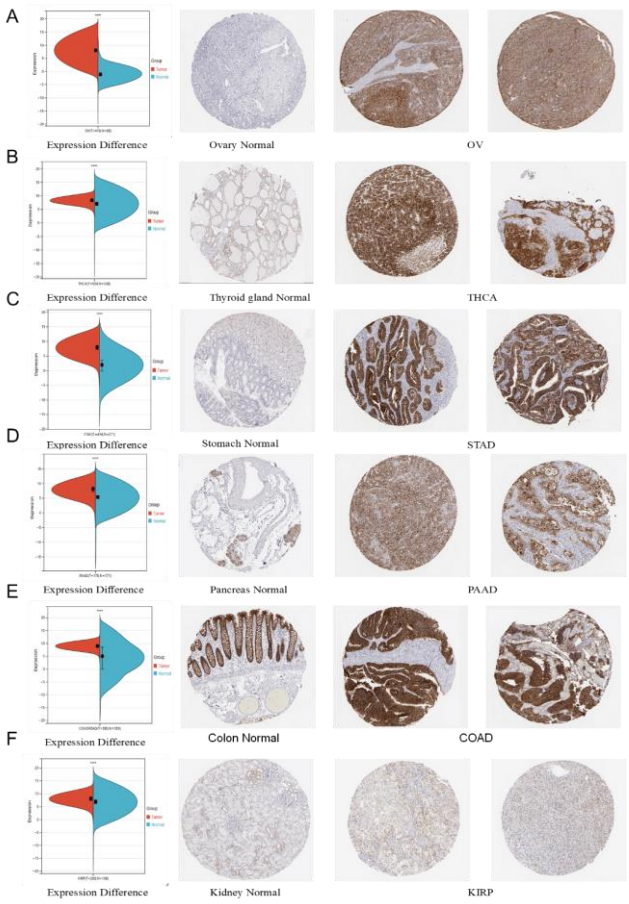
We found from the HPA database that CLDN4 is mainly localized on the plasma membrane of cells, which is the most important biological feature as a target (Supplementary Figure 1A). In addition, we searched the protein network interacting with CLDN4 protein from the string database and found that it closely interacts with OCLN, TJP1, CLDN34, EPHA2, TJP3, CLDN7, TJP2, CDH1, CLDN12, and GRHL2 proteins (Supplementary Figure 1B).

Figure 2: IHC images of CLDN4 gene expression between normal and tumor tissues from HPA database.

(A)Ovary normal tissues and OV. (B)Thyroid normal tissues and THCA. (C) Stomach normal tissues and STAD. (D)Pancreas normal tissues and PAAD. (E) Colon normal tissues and COAD. (F) Kidney normal tissues and KIRP.

Correlation of CLDN4 Expression With cancer stages of World Health Organization

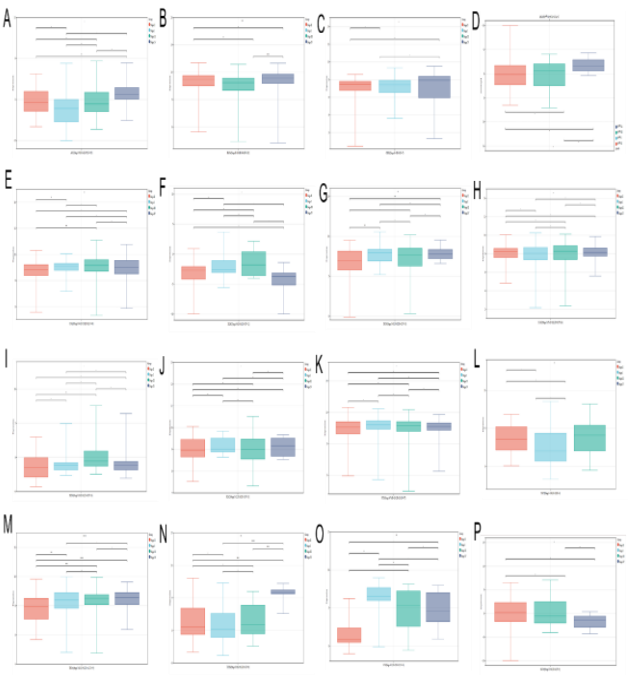
CDN4 expression in different cancer stages of pan-cancer was further assessed. The results showed that in most cancer types, such as ACC, BLCA, CHOL, COAD, DLBC, ESCA, MESO, TGCH, THCA, THYM, UCS, and SKCM, the higher the tumor stage, the higher the expression of CLDN4. (Figures 3A–O) There are also examples of the opposite situation, such as SKCM (Figure 2P). Similarly, the results of pairing tumor tissue with normal tissue in pan-cancer are



basically consistent with the expression of DEGs (Figures 4A-L).

Figure 3: Pan-cancer CLDN4 expression in clinical stages.

(A–P) Expression of CLDN4 in different stages in pan-cancer. *p < 0.05; **p < 0.01; ***p < 0.001 and ****p < 0.0001; -, not significant.



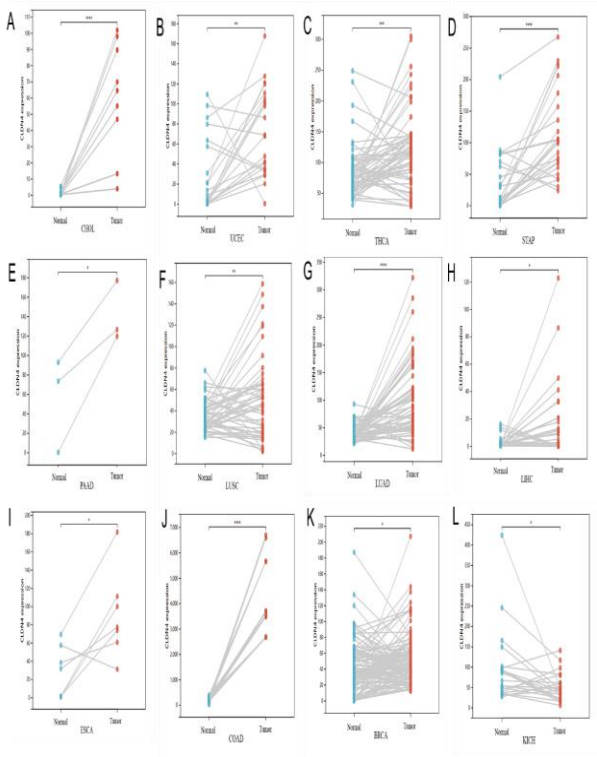


Figure 4: Comparison of paired samples CLDN4.

(A–L) Expression of CLDN4 in paired tumor samples and adjacent normal tissue samples of pan-cancer. **p < 0.01; ***p < 0.001 and ****p < 0.0001.

Prognostic Value of CLDN4 Across Cancers

The prognostic significance of CLDN4 in pan-cancer was further evaluated. The results of Kaplan-Meier OS analysis indicated that CLDN4 is a risk factor for patients with ACC, ALL, PAAD, ESCA, GBM, UVM, LAML, LGG, LIHC, LUAD, UCEC, MESO, and OV (Figures 5A–M); On the contrary, for SARC, KIRC, BLCA, it is a protective factor (Figures 5N-M). The OS results revealed that CLDN4 acts as a risk factor for patients with GBMLGG, LGG, PAAD, LAML, GBM, and LIHC; In contrast, it is a protective factor for patients with KIPAN, KIRC, and KICH (Figure 6A). CLDN4 acts as a risk factor for patients with UCEC and TGCT in DFS results(Figure 6B). CLDN4 acts as a risk factor for patients with GBMLGG, LGG, PAAD, UVM, and ACC in PFI analysis; A protective factor for patients with KIPAN, KIRC, and KICH (Figure 6C). Finally, CLDN4 is a risk factor for patients with GBMLGG, LGG, PAAD, and THYM in DFI analysis; A protective factor for patients with KIPAN, KIRC, KICH, and BLCA on the contrary (Figure 6D).

Figure 5: Kaplan-Meier analysis of CLDN4.

(A–P) Kaplan Meier overall survival analysis based on the expression of CLDN4 in various cancer types.

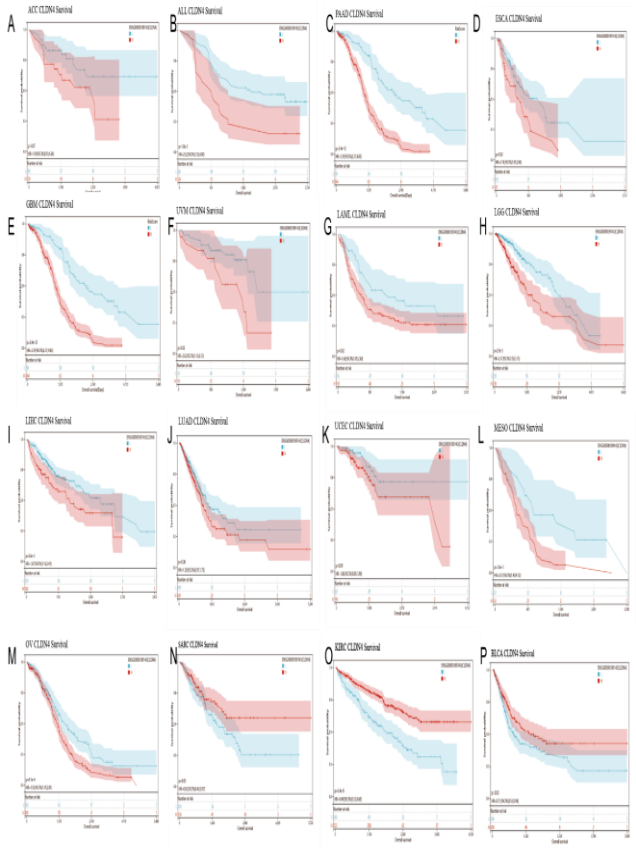
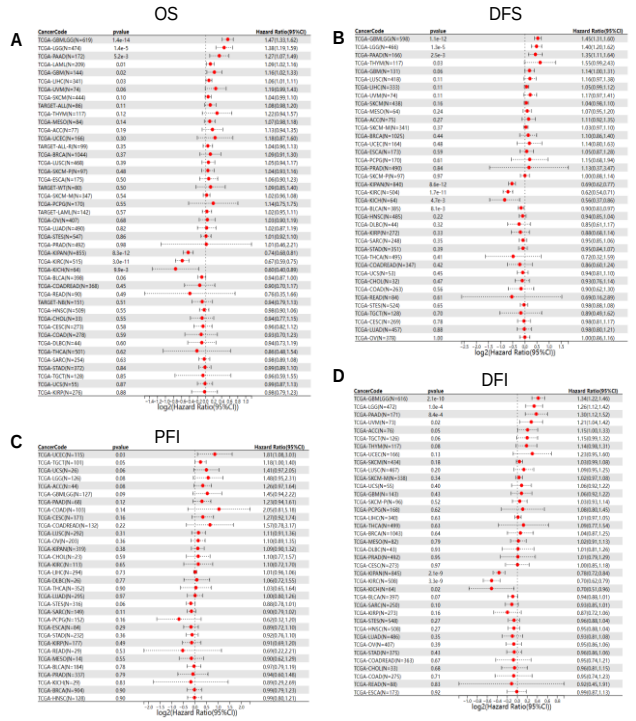


Figure 6: Forest map analysis of OS, DFS, DFI, and PFI for CLDN4.



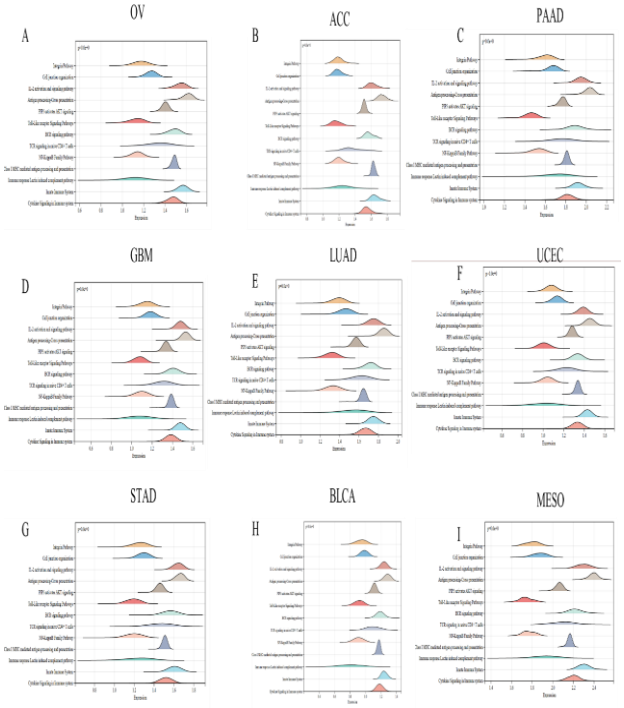
(A) Regression analysis of OS in patients with pan-cancer. (B) Regression analysis of DFS in patients with pan-cancer. (C) Regression analysis of PFI in patients with pan-cancer. (D) Regression analysis of DFI in patients with pan-cancer.

GSEA of CLDN4

The GSEA pathway that CLDN4 may be involved in the pan-cancer of TCGA was studied. The results showed that CLDN4 was significantly correlated with immune-related pathways, including cytokine signaling in immune system pathways, TCR signaling in naive CD4+ T cell pathways, etc.(Figures 7A–I). These results show that CLDN4 is closely related to regulating the tumor immune microenvironment and inhibiting the immune system to eliminate tumors.

Figure 7: GSEA of CLDN4 in pan-cancer.

(A–I) TOP13 GSEA terms in indicated tumor types



Genetic Alterations of CLDN4

Epigenetic changes will cause gene expression changes, leading to pathological changes. We used cBioPortal to explore the genetic alterations of CLDN4, and observed that patients with Melanoma or Esophagogastric Cancer had a high frequency of Alteration (Figure 8A). Diploid is the most common type of CLDN4 mutation (Figure 8B). Colorectal cancer has the most significant number of mutations (Figure 8C). The expression DEGs of CLDN4 mutation in different clinical stages of pan-cancer was further calculated. We observed significant DEGs in 8 tumors, including LUAD, BRCA, STES, STAD, PRAD, HNSC, LUSC, and OV (Figure 8D).

Figure 8: Relationship between CLDN4 mutation burden and pan-cancer

(A) The proportion of CLDN4 mutation copy number in pan-cancer. (B) Types of CLDN4 mutation. (C) Copy number of CLDN4 mutations in pan-cancer. (D)

Mutation differences in different tumor stages.

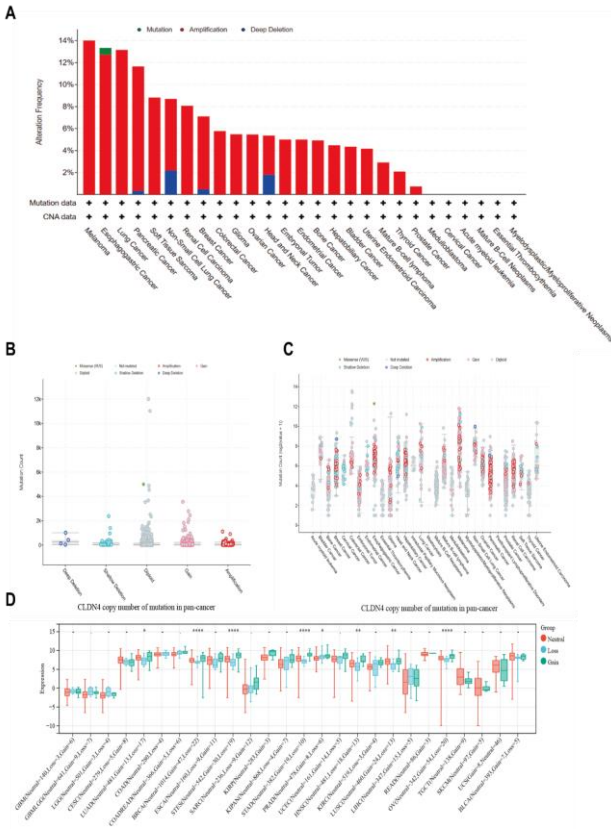
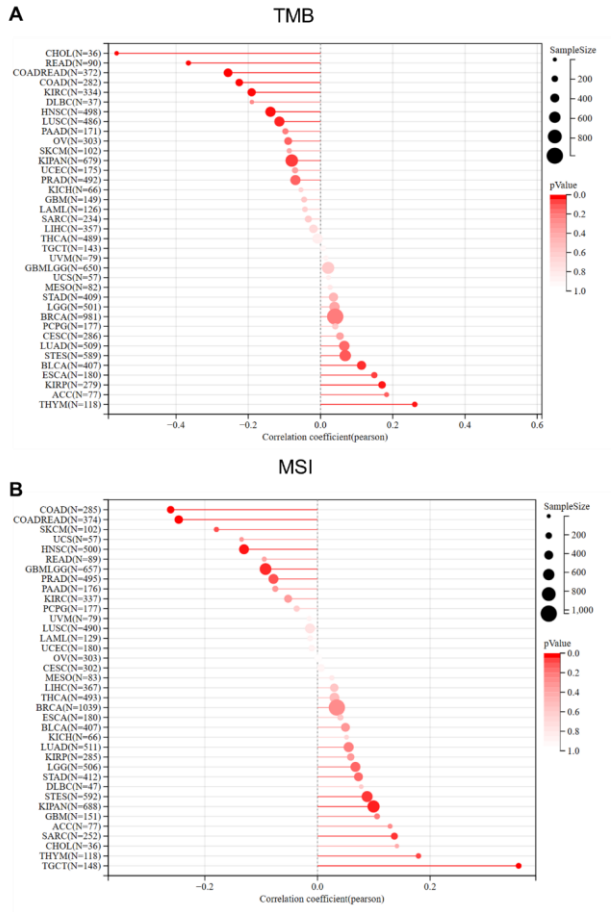


Figure 9: Expression tumor mutational burden (TMB) and microsatellite instability (MSI) of CLDN4.



Lollipop map illustrating the relationship between CLDN4 and TMB. (B) Lollipop map illustrating the relationship between CLDN4 and MSI.

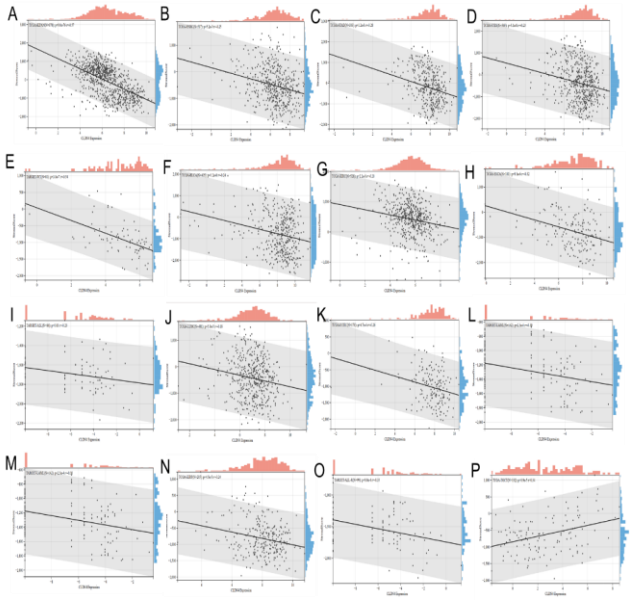
TMB and MSI are well-recognized biomarkers for predicting pan-cancer, which can guide accurate tumor immunotherapy. Then we investigated whether there is an association between CLDN4 and TMB, MSI. We observed a significant correlation among 12 tumors in TMB, among which there was a significant positive correlation among four tumors, including ESCA, KIRP, THYM, and BLCA, and a significant negative correlation among eight tumors, including COAD, COADREAD, KIPAN, HNSC, KIRC, LUSC, READ and CHOL (Figure 9A). A significant positive correlation was observed in MSI among four kinds of tumors, including STES, SARC, KIPAN, and TGCT, and a significant negative correlation was observed in four kinds of tumors, including GBMLGG, COAD, COADREAD and HNSC (Figure 9B).

Immune Cell Infiltration Analysis

It is acknowledged that the tumor immune microenvironment takes an indispensable impact on the occurrence and development of tumors. Next, we further explored the relationship between the expression of TME and CLDN4 in pan-cancer. We use the ESTIMATE algorithm to calculate the scores of stromal cells and immune cells in pan-cancer, and analyze the relationship between the expression level of CLDN4 and these two scores, respectively. We obtained immune infiltration scores for 10180 tumor samples out of 44 tumor types. In the end, we observed a significant correlation between the expression of this gene and stromal score in 26 cancer types, with four being significantly positively correlated and 22 being significantly negatively correlated (Figure 10A-P). Immune scores shows 25 cancer types were negatively correlated, and 5 types were positively correlated with immune cells (Figure 11A-P). Moreover, the rest results are shown in Supplementary Figures 2,3.

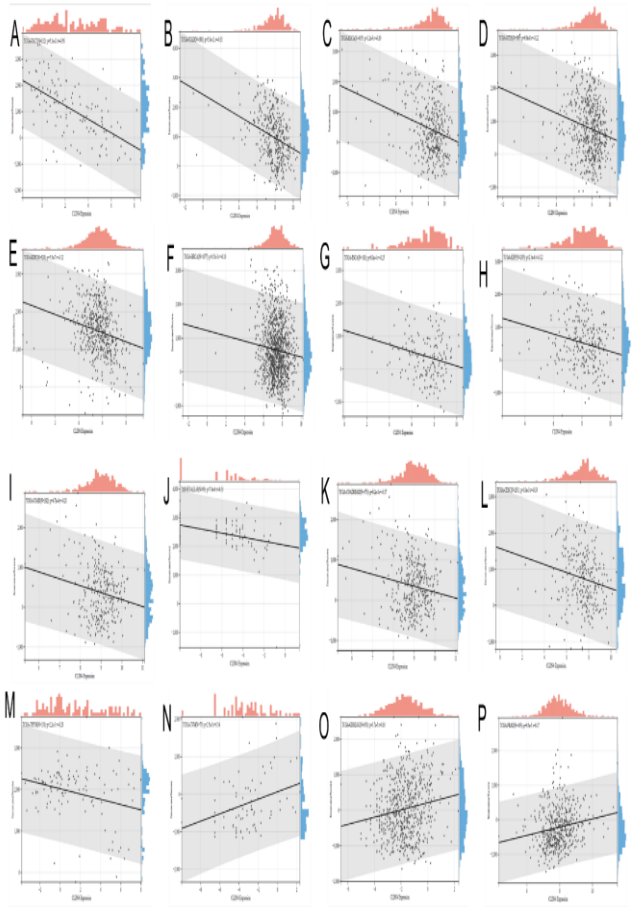
Subsequently, we use xCell algorithms to calculate the immune cell infiltration abundance. We obtained 67 types of immune cell infiltration scores for 10180 tumor samples from 44 tumor types. We used the Corr. test function of the R software package Psych (version 2.1.6) to calculate Pearson's correlation coefficient between genes and immune cell infiltration scores in each tumor to determine significantly correlated immune infiltration scores. The results demonstrate that CLDN4 is associated with immune infiltration in pan-cancer (Supplementary Figure 4). The expression of CLDN4 is negatively correlated with most immune cell infiltration can also be found. For example, in almost all types of cancer, the expression of CLDN4 is negatively correlated with the level of infiltrating natural CD8+T cells (Figure 12A-P).

Figure 10: Stromal score of CLDN4 in pan-cancer.



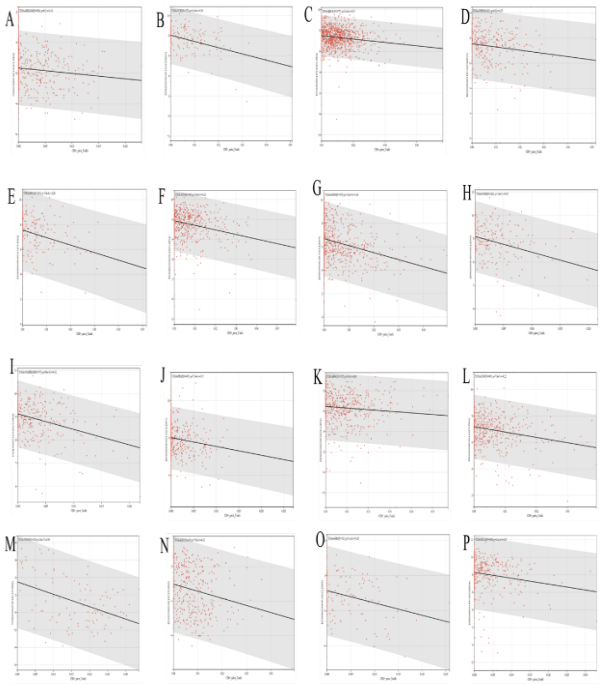
(A-O) Correlation is negative. (P) Correlation is positive.

Figure 11: Immune score of CLDN4 in pan-cancer.



(A-M) Correlation is negative. (N-P) Correlation is positive.

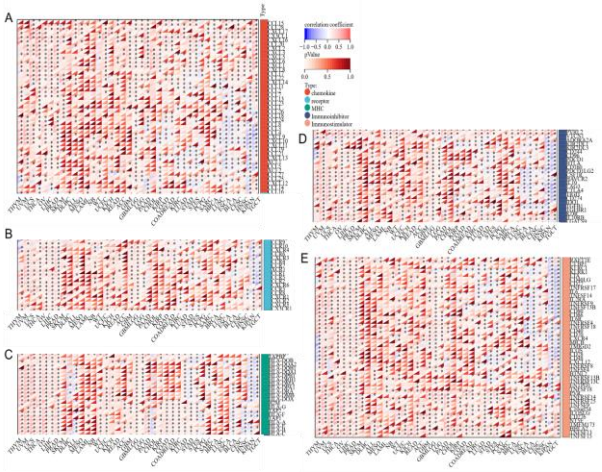
Figure 12: Correlation between CLDN4 and native CD8+ T cell in various cancer types.



(A-P) Correlation is positive.

In addition, we discussed the relationship between the expression of CLDN4 and immune-related genes in various tumors by gene co-expression analysis. Chemokine, receptor, MHC, immune inhibitor, and immune stimulator were analyzed. The heatmap showed that almost all genes related to immune regulation were co-expressed with CLDN4, and most genes were positively correlated with CLDN4 (Figure 13A-E).

Figure 13: Co-expression of CLDN4 and immune-related genes. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.



(A)Co-expression of CLDN4 and chemokine. (B) Co-expression of CLDN4 and receptor. (C) Co-expression of CLDN4 and MHC. (D) Co-expression of CLDN4 and immunoinhibitor. (E) Co-expression of CLDN4 and immunostimulatory.

DISCUSSIONS

In recent years, immunotherapy has become one of the most essential and an indispensable method to treat cancer Bagchi et al. (2021), Vesely et al. (2022). Many immune checkpoint inhibitors have entered the clinical treatment stage Vesely et al. (2022). TJ dysregulation can lead to many AIDs Asmar et al. (2002), Clemente et al. (2003), Drago et al. (2006), Fasano et al. (2000), Sapone et al. (2006).TJ dysfunction would trigger immunoregulation Turner et al. (2009). CLDN is an indispensable structure of TJ, and we used the TCGA database to evaluate the role of CLDN4 in pan-cancer.

In this research, we compared the differential expression of CLDN4 in various tumors and its impact on prognosis, and DEGs analysis displays that it is overexpressed in most cancer types, such as UCEC, LUAD, BRCA, and CESC. Correspondingly, significant downregulation was observed in 8 types of tumors, including SKCM, HNSC, and ALL. Subsequently, The analysis of differential expression between cancer tissue and normal tissue in IHC confirms this conclusion. However, the expression of cancer is lower in a few types of cancer. It may be because a few samples analyzed in our study are advanced tumor samples. We found that the expression of CLDN4 protein was the highest in thyroid cancer and the lowest in lymphoma. CLDN4 was mainly expressed in cell membranes from the gene-card database. Further, the PPI network diagram shows that CLDN4 is closely related to cell tight junction components.

The correlation between the expression level of CLDN4 and various tumor stages was further analyzed, and it was found to be significant. It is highly expressed in the later stage of most tumors, including ACC, BLCA, CHOL, COAD, DLBC, ESCA, MESO, TGCH, THCA, thymus, UCS, SKCM, etc. In contrast, a higher expression of CLDN4 was observed at the lower stage in SKCM.

Kaplan-Meier analysis showed that CLDN4 was a risk factor for survival in most cancer types, including COAD, MESO, CHOL, etc., from Kaplan-Meier analysis. CLDN4 is a protective factor for patients with SARC, KIRC, and BLCA. However, deaths caused by non-cancer do not necessarily reflect tumor progression and response to treatment. Therefore, we further use DFI and PFI methods, which can more accurately reflect the influence of various factors on the survival of patients. CLDN4 acts as a risk factor for patients with GBMLGG, LGG, PAAD, UVM, and ACC from PFI analysis; A protective factor for patients with KIPAN, and KIRC (Figure 6C). Finally, The DFI analysis revealed that CLDN4 acts as a risk factor for patients with GBMLGG, LGG, PAAD, and THYM; For KIPAN, KIRC, and KICH patients, the opposite effect is observed. The above results indicate that high expression of CLDN4 leads to a decrease in survival

GSEA analysis of CLDN4 shows that it is significantly related to immune-related pathways, especially: cytokine signaling in immune system pathways and TCR signaling in naive CD4+ T cells pathways. It can be concluded from these results that CLDN4 is closely correlated to the interaction between Tumor immunosuppression and regulating tumor immune microenvironment.

Melanoma or Esophagogastric Cancer had a high frequency of Alteration. Diploid is the most common type of CLDN4 mutation. Colorectal cancer has the largest number of mutations. Significant differences in mutations between stages were observed in eight cancers, including LUAD, BRCA, STES, STAD, PRAD, HNSC, LUSC, and OV. There are 12 kinds of tumors in TMB, among which four kinds of tumors (including ESCA, KIRP, thymus, and BLCA) have a significant positive correlation, and eight kinds of tumors (including COAD, COADREAD, KIPAN, HNSC, KIRC, LUSC, READ and CHOL) have a significant negative correlation. MSI was positively correlated in four kinds of tumors, including STES, SARC, KIPAN, and TGCT, and negatively correlated in four kinds of tumors, including GBMLGG, COAD, COADREAD, and HNSC. We conclude that tumors with TMB and MSI positively related to CLDN4 may have a better therapeutic effect on ICI.

Our results show that CLDN4 plays a significant role in the tumor immune microenvironment. We found that the scores of stromal and immune cells in most cancer types were negatively correlated. CD8+ T cells mediate anti-tumor response. The function of immune cells in tumors will be impaired Philip et al. (2022), Srihari et al. (2021), McLane et al. (2019). In most cancer types, the higher the expression level of CLDN4, the lower the expression of B cells, CD4+T cells, CD8+T cells, and adipocytes. The expression of CLDN4 is negatively correlated with the level of infiltrating native CD8+ T cells in almost cancer types. In addition, we also explored the trend between the expression of CLDN4 and immune-related genes in various tumors by gene co-expression analysis. Chemokine, receptor, MHC, immune inhibitor, and immune stimulator were analyzed. The heatmap showed that almost all genes related to immune regulation were co-expressed with CLDN4, and most genes were positively correlated with CLDN4.

In conclusion, the first analysis of CLDN4 in pan-cancer demonstrated that the gene differentially expressed between tumor and normal tissues exists significance and revealed its correlation with clinical prognosis and immune infiltration. We conclude that the expression degrees of different tumor types affect different prognoses. Moreover, the expression of CLDN4 is related to the infiltration degree of Chemokine, receptor, MHC, immune inhibitor, and immune stimulator.

Our conclusion not only elucidates the role of CLDN4 in tumor development but also provides a reference for more personalized immunotherapy in tumors in the future.

DECLARATIONS

Conflict of Interest

There are no conflicts of interest regarding the publication of this article.

Authors' contributions

Yi Xu conceived and designed the study, drafted the article, revised the article critically, and be responsible for materials.

Si Chen had final approval of the submitted versions.

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Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The following information was supplied regarding data availability: Data is available at the TCGA database (The Cancer Genome Atlas Program (TCGA) - NCI).

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Consent for publication

Not applicable

REFERENCES

1.Bradner J.E, Hnisz D, Young R.A, et al. 2017 Feb 9. Transcriptional Addiction in Cancer. Cell. 168(4):629-643.

2.Hanahan D, Weinberg R.A. 2011 Mar 4. Hallmarks of cancer: the next generation. Cell. 144(5):646-74.

3.Riley R.S, June C.H, langer R, et al. 2019 Mar. Delivery technologies for cancer immunotherapy. Nat Rev Drug Discov. 18(3):175-196.

4.Morin P.J. 2005 Nov 1. Claudin proteins in human cancer: promising new targets for diagnosis and therapy. Cancer Res. 65(21):9603-6.

5.Agarwal R, D'Souza T, Morin P. J, et al. 2005 Aug 15. Claudin-3 and claudin-4 expression in ovarian epithelial cells enhances invasion and is associated with increased matrix metalloproteinase-2 activity. Cancer Res. 65(16):7378-85.

6.Günzel D, Yu A.S. 2013 Apr. Claudins and the modulation of tight junction permeability. Physiol Rev. 93(2):525-69.

7.Pinto da Silva P, Kachar B. 1982. On tight-junction structure. Cell. 28(3): p. 441-50.

8.Matter K, Balda M.S. 2003 Mar. Signalling to and from tight junctions. Nat Rev Mol Cell Biol. 4(3):225-36.

9.Harhaj N.S, Antonetti D.A. 2004 Jul. Regulation of tight junctions and loss of barrier function in pathophysiology. Int J Biochem Cell Biol. 36(7):1206-37.

10.Nichols L.S, Ashfaq R, Iacobuzio-Donahue C.A, et al. 2004 Feb. Claudin 4 protein expression in primary and metastatic pancreatic cancer: support for use as a therapeutic target. Am J Clin Pathol.121(2):226-30.

11.Soler A.P, Miller R.D, Laughlin K.V, et al. 1999 Aug. Increased tight junctional permeability is associated with the development of colon cancer. Carcinogenesis. 20(8):1425-31.

12.Swisshelm K, Macek R, Kubbies M, et al. 2005 Apr 25. Role of claudins in tumorigenesis. Adv Drug Deliv Rev. 57(6):919-28.

13.Sato N, Fukushima N, Maitra A, et al. 2004 Mar. Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. Am J Pathol.164(3):903-14.

14.Hammad H, Lambrecht B.N. 2015 Jul 21. Barrier Epithelial Cells and the Control of Type 2 Immunity. Immunity. 43(1):29-40.

15.Asmar R. El, Panigrahi P, Bamford P, et al. 2002 Nov. Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure. Gastroenterology. 123(5):1607-15.

16.Clemente M.G, Virgiliis S.De, Kang Js, et al. 2003 Feb. Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. Gut. 52(2):218-23.

17.Drago S, Asmar R.Ei, Pierro M.Di, et al. 2006 Apr .Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol.41(4):408-19.

18.Fasano A, Not T, Wang W, et al. 2000 Apr 29. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet. 355(9214):1518-9.

19.Sapone A, Magistris L de, Pietzak M, et al. 2006 May. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes. 55(5):1443-9.

20.Turner J.R. 2009 Nov. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 9(11):799-809.

21.Bagchi S, Yuan R, Engleman E.G, et al. 2021 Jan 24.Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. Annu Rev Pathol. 16:223-249.

22.Vesely M.D, Zhang T, Chen L, et al. 2022 Apr .Resistance Mechanisms to Anti-PD Cancer Immunotherapy. Annu Rev Immunol. 40:45-74.

23.Philip M, Schietinger A. 2022 Apr. CD8(+) T cell differentiation and dysfunction in cancer. Nat Rev Immunol. 22(4):209-223.

24.Srihari T G. 2021 Jan. Innate and adaptive immune cells in the tumor microenvironment. Gulf J Oncolog. 1(35):77-81.

25.McLane L.M, Abdel-Hakeem M.S, Wherry E.J, et al. 2019 Apr 26. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annu Rev Immunol.37:457-495.

