CLDN6 and CLDN10 are Associated with Immune Infiltration of Ovarian Cancer: A Study of Claudin Family

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Primary research

Keywords: Ovarian cancer, CLDN6, CLDN10, Prognosis, Immune Infiltration

Posted Date: July 6th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-40048/v1>

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Abstract

Background: Claudin family is a group of membrane proteins related to tight junction. There are many studies about them in cancer, but few studies pay attention to the relationship between them and the tumor microenvironment. In our research, we mainly focused on the genes related to the prognosis of ovarian cancer, and explored the relationship between them and the tumor microenvironment of ovarian cancer.

Methods: The cBioPortal provided the genetic variation pattern of claudin gene family in ovarian cancer. The ONCOMINE database and Gene Expression Profiling Interactive Analysis (GEPIA) were used to exploring the mRNA expression of claudins in cancers. The prognostic potential of these genes was examined via Kaplan-Meier plotter. Immunologic signatures were enriched by gene set enrichment analysis (GSEA). The correlations between claudins and the tumor microenvironment of ovarian cancer were investigated via Tumor Immune Estimation Resource (TIMER).

Results: In our research, claudin genes were altered in 363 (62%) of queried patients/samples. Abnormal expression levels of claudins were observed in various cancers. Among them, we found that CLDN3, CLDN4, CLDN6, CLDN10, CLDN15 and CLDN16 were significantly correlated with overall survival of patients with ovarian cancer. GSEA revealed that CLDN6 and CLDN10 were significantly enriched in immunologic signatures about B cell, CD4 T cell and CD8 T cell. What makes more sense is that CLDN6 and CLDN10 were found related to the tumor microenvironment. CLDN6 expression was negatively correlated with immune infiltration level in ovarian cancer, and CLDN10 expression was positively correlated with immune infiltration level in ovarian cancer. Further study revealed the CLDN6 expression level was negatively correlated with gene markers of various immune cells in ovarian cancer. And, the expression of CLDN10 was positive correlated with gene markers of immune cells in ovarian cancer.

Conclusions: CLDN6 and CLDN10 were prognostic biomarkers, and correlated with immune infiltration in ovarian cancer. Our results revealed new roles for CLDN6 and CLDN10, and they were potential therapeutic targets in the treatment of ovarian cancer.

Background

Ovarian cancer is the most lethal gynecologic oncology of genital system^[1]. Although the advancement of surgical techniques and the combined application of chemotherapy drugs since the 1970s, the five-year survival rate of advanced ovarian cancer is just about 40%-45%^[2]. Therefore, it is still urgent to improve treatment for ovarian cancer. Immunotherapy is an emerging treatment method for several solid tumors, because it would improve outcomes of patients. With the application of numerous immune-based interventions in ovarian cancer, immunotherapy has been proven to be useful in advanced ovarian cancer^[3].

Claudins are major components of tight junction and consist of more than 20 claudin proteins. They serve as a physical barrier to prevent molecules from passing freely through the paracellular space between epithelial or endothelial cell sheets, and also play critical roles in maintaining cell polarity and signal transductions^[4]. Previous research confirmed known claudins gene expression patterns and identified several genes dysregulated in cancers^[5]. They may play roles in the tumorigenesis of solid tumors^[6, 7], and represent promising targets for cancer detection, prognosis and therapy^[8]. However, the relationship between claudins and the tumor microenvironment has not been investigated. Here, we comprehensively analyzed claudins expression in ovarian cancer, and further explored the relationship between claudins and immune cell infiltration.

Methods

cBioProtal

The cBioProtal (<https://www.cbioportal.org/>)^[9, 10] is an open platform for cancer genomics analyses. 585 samples of ovarian serous cystadenocarcinoma (TCGA, PanCancer Atlas) were used for genetic variation analyses through the cBioProtal.

ONCOMINE Database Analysis

The expression of claudins in various cancers were analyzed via the ONCOMINE database (<https://www.ONCOMINE.org/resource/login.html>)^[11]. The ONCOMINE database includes more than 35 types of cancer and normal samples.

Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA version2 (<http://gepia2.cancer-pku.cn/>)^[12] is a web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from The Cancer Genome Atlas (TCGA) and the GTEx projects, using a standard processing pipeline. The expression profile of claudins in ovarian cancer were explored according to GEPIA2. The p-value cutoff was 0.05, and $|\log_2FC|$ cutoff was 1.

Kaplan-Meier Plotter Database Analysis

The Kaplan-Meier plotter (<http://kmplot.com/analysis/index.php?p=background>)^[13] is capable to assess the effect of 54 k genes on survival in 21 cancer types. The largest datasets include breast (n = 6,234), ovarian (n = 2,190), lung (n = 3,452), and gastric (n = 1,440) cancer. The system includes gene chip and RNA-seq data-sources for the databases include Gene Expression Omnibus (GEO), European Genome-Phenome Archive (EGA), and TCGA. Prognostic significance of claudins in ovarian cancer were analyzed via the online datasets.

Tumor Immune Estimation Resource (TIMER)

TIMER (<https://cistrome.shinyapps.io/timer/>)^[14] is a web server for comprehensive analysis of tumor-infiltrating immune cells. The correlation between claudins expression and immune cell infiltration were analyzed from this database. TIMER2.0, an updated and enhanced version of TIMER, can be used to systematically analyze immune infiltration across diverse cancer types.

Statistical Analyses

The expression of claudins were presented as mean. Kaplan–Meier survival curves were based on the log-rank test. The HR was performed using the Cox model. Spearman correlation test was used for correlation analysis. P-value < 0.05 was considered to be significant.

Results

1. Gene variation of claudins in ovarian cancer

Twenty-four reviewed proteins of claudin family were obtained from the UniProt Knowledgebase (UniProtKB) (<https://www.uniprot.org/>) (Table 1) (An additional file shows this in more detail [see Table 1]). Firstly, we investigated the genetic variation of claudin family in ovarian cancer through the cBioPortal for Cancer Genomics (<https://www.cbioportal.org/>). Twenty-four genes were queried in 585 samples of ovarian serous cystadenocarcinoma (TCGA, PanCancer Atlas). Figure 1A showed the alteration frequency of genetic variation in serous ovarian cancer. Figure 1B showed that queried genes were altered in 363 (62%) of queried patients/samples. Among them, the top three gene variation were CLDN11 (24%), CLDN16 (22%) and CLDN1 (16%). Then, overall survival differences between altered group and unaltered group were compared by Kruskal Wallis test. We found that overall survival is reduced in altered group compared to unaltered group ($p = 7.981e-3$) (Fig. 1C). Previous studies have shown that claudin gene family dysregulated in a variety of tumors and involved in diagnostic, tumorigenesis, and prognosis^[15–17]. Thus, this gene family is worthy of further research in ovarian cancer.

2. The expression of claudin family is dysregulated in various cancers

To explore the mRNA expression of claudin gene family, we investigated the expression profile of claudin genes in various cancer via the ONCOMINE. The thresholds were set: p-value of 0.05, fold change of 1.5, and gene rank of all. The significant unique analyses were shown in supplementary Fig. 1 (Those with less than 3 meaningful analyses were not considered). Most of claudins were dysregulated in various cancers. In order to further verify the expression of claudins in ovarian cancer, GEPIA2 were used to analyze the mRNA expression in TCGA samples and the GTEx data. The $|\log_2FC|$ cutoff was set 1, and p-value cutoff was set 0.01. As shown in Fig. 2, 8 genes were overexpression between ovarian cancer and normal samples, including CLDN1, CLDN3, CLDN4, CLDN6, CLDN7, CLDN9, CLDN10 and CLDN16; and 3 genes were low expression including CLDN5, CLDN11 and CLDN15.

3. Claudins expression were correlated with the prognosis of ovarian cancer

To identify which of these genes have clinical significance, we studied the relationship between these differentially expressed genes and the prognosis of patients with ovarian cancer using Kaplan-Meier plotter. As shown in Fig. 3, genes overexpression including CLDN3, CLDN4, CLDN6, and CLDN16 were found to be significantly correlated with poor overall survival (OS) (Fig. 3A) and progression free survival (PFS) (Fig. 3B) of patients with ovarian cancer. Besides, high expression of CLDN10 and CLDN15 predicted good prognosis among ovarian cancer (Fig. 3C-D). Surprisingly, CLDN10 is overexpression in cancer, but patients with high expression of CLDN10 predicted good overall survival (OS, HR = 0.73, logrank P = 1.6e-06), progression free survival (PFS, HR = 0.83, logrank P = 0.0067), and post progression survival (PPS, HR = 0.73, logrank P = 0.00029). These results are somewhat counterintuitive. Why does this happen? Further mechanism has to be explored.

4. GSEA of immunologic signature gene sets

To characterize the potential function of claudins, GSEA was performed using the gene expression data of ovarian cancer patients in TCGA. Immunologic signature gene sets were used. As shown in Fig. 4, we found that CLDN6 and

CLDN10 were related to effector differentiation of B cell, CD4 T cell, and CD8 T cell.

5. Correlation Analysis between claudins and the tumor microenvironment

To understand the role of claudins in immunity, we downloaded 379 RNA-seq FPKM (Fragments per kilobase per million) data of ovarian cancer from TCGA. Subsequently, the FPKM were converted to TPM (transcripts per million) [18]. ESTIMATE algorithm [19] was used to predict tumor purity based on TCGA ovarian cancer samples. Then, the relationship between claudins expression and tumor microenvironment was explored. As shown in Fig. 5A, a meaningful negative correlation between CLDN6 expression and immune score was observed (spearman correlation = -0.23, $p < 0.001$). And, there was a positive correlation between CLDN10 expression and immune score (spearman correlation = 0.21, $p < 0.001$) (Fig. 5B). Neither CLDN6 expression nor CLDN10 expression was correlation with stromal score. Immune score represents the infiltration of immune cells in tumor tissue.

Then, we examined the relationship between immune infiltration and claudins expression. RNA-seq TPM data ($n = 379$) from TCGA ovarian cancer were used to assess 22 immune cells subtypes concentrations through the CIBERSORT algorithm [20]. They were grouped by the median value of CLDN6 and CLDN10, respectively. Dendritic cells activated were found to be statistically significant different between CLDN6_high and CLDN6_low group. Several cell types were significantly different between the CLDN10_high and CLDN10_low group, including B cells naïve, B cells memory, T cells CD4 naïve, T cells CD4 memory activated, monocytes, M1 macrophage and dendritic cells activated (Fig. 5C).

Besides, the microarray expression values of ovarian cancer were used for calculation the abundances of six immune infiltrates (B cells, CD4 + T cells, CD8 + T cells, Neutrophils, Macrophages, and Dendritic cells) via TIMER algorithm [19]. The gene expression levels correlated with tumor purity were displayed on the left-most panel (Fig. 6A-B). Our results showed the CLDN6 expression was negatively related to B cell infiltration (partial correlation = -0.284, $p = 2.21\text{e-}10$), CD8 + T cell (partial correlation = -0.254, $p = 1.64\text{e-}08$), neutrophil (partial correlation = -0.152, $p = 8.29\text{e-}04$), and dendritic cell (partial correlation = -0.182, $p = 6.31\text{e-}05$) (Fig. 6A). In contrast, there is a small but significant positive correlation between CLDN10 expression and neutrophil (partial correlation = 0.185, $p = 4.66\text{e-}05$), and dendritic cell (partial correlation = 0.153, $p = 7.74\text{e-}04$) (Fig. 6B).

In order to more accurately describe the relationship of gene expression and immune cell infiltration, several methods including TIMER, CIBERSORT, quanTIseq, xCell, MCP-counter and EPIC algorithms were used to assess the immune infiltration of tumor tissue [21]. TIMER2.0 provides a platform for analysis immune infiltrates across diverse cancer types based on available TCGA RNA-seq data [22, 23]. The correlations between claudins (CLDN6 and CLDN10) expression and various immune cells infiltration of ovarian cancer were shown in Table 2. As shown in Fig. 6C, CLDN6 was negative correlated to immune cell infiltration including B cell, CD8 + T cell, CD4 + T cell effector memory, M1 macrophage and myeloid dendritic cell. By contrast, CLDN10 was positive correlated to immune cell infiltration including B cell, CD8 + T cell, CD4 + T cell effector memory, M1 macrophage and myeloid dendritic cell (Fig. 6D). Relevant evidences reported that cancer associated fibroblast (CAF) plays an important role in the progression of ovarian cancer [24, 25]. Interestingly, we also found that CAF has a positive correlation with CLDN6 expression, but a negative correlation with CLDN10 expression. In ovarian cancer, increased infiltration of tumor-infiltrating lymphocytes (TILs) and more specifically CD8 + T cells, has been proven to be associated with improved clinical outcome [26–28]. These results suggest that CLDN6 and CLDN10 may participate in the immune cells infiltration of ovarian cancer, and these mechanisms may be the reasons for poor prognosis of ovarian cancer.

6. Relationship between claudins expression and gene markers of immune cells

To further illustrate the correlations between claudins (CLDN6 and CLDN10) and immune infiltration, we focused on the relationship between claudins (CLDN6 and CLDN10) and gene markers of various immune cells in ovarian cancer through the TIMER 2.0 databases. We analyzed the correlations between claudins (CLDN6 and CLDN10) expression and gene markers of different immune cells, including B cells, T cells (general), CD8 + T cells, macrophages, dendritic cells, neutrophils, monocytes, NK cells and Tregs in ovarian cancer (Table 3). The purity-adjusted correlation heatmaps were shown on supplementary Fig. 2. After the correlation adjustment by purity, the results revealed the CLDN6 expression level was negatively correlated with most gene markers of dendritic cells, M1 macrophages, monocyte, NK cells, and tumor-associated macrophages (TAMs) in ovarian cancer. By contrast, the expression of CLDN10 was positive correlated with gene markers of dendritic cells, T cell (general) and TAMs in ovarian cancer.

Studies have shown that the infiltration of these immune cells in the tumor microenvironment is related to the tumor immunotherapy response^[29]. Immune cell-based immunotherapy^[30] including NK Cells^[31] and dendritic cells^[32] play important roles in the treatment of ovarian cancer. Taken these analyses together, our research showed that CLDN6 and CLDN10 may play important roles in immunotherapy in the future.

Discussion

Claudin-6 and claudin-10 are important components of the claudin family. Claudin-6 had been demonstrated overexpression in ovarian papillary serous carcinomas by immunohistochemistry^[33], and may be a novel targeted therapeutic for ovarian cancer as a receptor for clostridium perfringens enterotoxin (CPE)^[34]. Previous research observed that claudin-10 is a glandular epithelial marker in epithelial ovarian cancer^[35]. Studies have revealed that CLDN10 is not only related to OS of ovarian cancer, but also to the chemoresistance of ovarian cancer^[36]. Recent evidences suggested that claudin-10 in an immune-related key gene and play a key role in the progression of papillary thyroid cancer^[17, 37]. Our research innovatively found that the prognostic potential of CLDN6 and CLDN10 were related to the tumor immune microenvironment in ovarian cancer.

In this study, we found that both CLDN6 and CLDN10 were high expression in ovarian cancer. Prognostic analysis showed that overexpression of CLDN6 is related to poor prognosis of patients with ovarian cancer. However, CLDN10 overexpression predicted better prognosis compared to CLDN10 low expression group. Then, our research found that CLDN6 overexpression was negatively related to immune infiltration, and CLDN10 overexpression was positive correlated to immune cell infiltration. Moreover, we identified that CLDN6 and CLDN10 were related to gene markers of dendritic cells, NK Cells and TAMs. These results may explain why the overexpression of CLDN6 and the low expression of CLDN10 predict poor overall survival of ovarian cancer.

Relevant evidence has emerged that immune-related gene expressions and TILs were related to prognosis, recurrent^[38] and chemotherapeutic response^[39] of ovarian cancer. Growing evidence shown that the presence of TILs may improve clinical outcome of ovarian cancer patients^[40]. Immune cell-based immunotherapy^[30] including NK Cells^[31] and dendritic cells^[32] play important roles in the treatment of ovarian cancer. Our results suggested that CLDN6 may be involved in tumor immune evasion, and may represent ideal candidate for immunotherapy in ovarian cancer. Further studies on the combined application of molecular targeted therapy and immunotherapy may be meaningful.

Conclusions

CLDN6 and CLDN10 were prognostic biomarkers, and correlated with immune infiltration in ovarian cancer. Our results revealed new roles for CLDN6 and CLDN10 in ovarian cancer, and they were potential therapeutic targets in the treatment of ovarian cancer.

Abbreviations

GEPIA: Gene Expression Profiling Interactive Analysis; TIMER: Tumor Immune Estimation Resource; GSEA: gene set enrichment analyses; TCGA: The Cancer Genome Atlas; GEO: Gene Expression Omnibus; EGA: European Genome-Phenome Archive; FPKM: Fragments per kilobase per million; TPM: transcripts per million; TILs: tumor-infiltrating lymphocytes; CAF: cancer associated fibroblast; TAM: Tumor-associated macrophage; CPE: clostridium perfringens enterotoxin. OS: overall survival; PFS: progression free survival; PPS: post progression survival.

Declarations

Ethics approval and consent to participate:

Not applicable

Consent for publication:

Not applicable

Availability of data and materials:

Not applicable

Competing interests:

The authors declare that they have no competing interests

Funding:

This work was supported by Natural Science Foundation of China (81772775 to J.W.)

Authors' contributions:

PW was responsible for the study conception and design; PG, TP, CC, SL were involved in data acquisition, data analysis and interpretation; PG drafted the manuscript, and TP took charge of supervising the manuscript. All authors have read and approved the manuscript.

Acknowledgements:

Not applicable

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Tables

Table 1: Twenty-four reviewed proteins of claudin family from the UniProtKB

Entry	Status	Gene names	Protein names	Organism
O95832	reviewed	CLDN1	Claudin-1 (Senescence-associated epithelial membrane protein)	Homo sapiens
P78369	reviewed	CLDN10	Claudin-10 (Oligodendrocyte-specific protein-like) (OSP-like)	Homo sapiens
O75508	reviewed	CLDN11	Claudin-11 (Oligodendrocyte-specific protein)	Homo sapiens
P56749	reviewed	CLDN12	Claudin-12	Homo sapiens
O95500	reviewed	CLDN14	Claudin-14	Homo sapiens
P56746	reviewed	CLDN15	Claudin-15	Homo sapiens
Q9Y5I7	reviewed	CLDN16	Claudin-16 (Paracellin-1) (PCLN-1)	Homo sapiens
P56750	reviewed	CLDN17	Claudin-17	Homo sapiens
P56856	reviewed	CLDN18	Claudin-18	Homo sapiens
Q8N6F1	reviewed	CLDN19	Claudin-19	Homo sapiens
P57739	reviewed	CLDN2	Claudin-2 (SP82)	Homo sapiens
P56880	reviewed	CLDN20	Claudin-20	Homo sapiens
Q8N7P3	reviewed	CLDN22	Claudin-22	Homo sapiens
Q96B33	reviewed	CLDN23	Claudin-23	Homo sapiens
A6NM45	reviewed	CLDN24/CLDN21	Putative claudin-24 (Claudin-21)	Homo sapiens
C9JDP6	reviewed	CLDN25	Putative claudin-25	Homo sapiens
O15551	reviewed	CLDN3	Claudin-3 (CPE-receptor 2)	Homo sapiens
H7C241	reviewed	CLDN34	Claudin-34	Homo sapiens
O14493	reviewed	CLDN4	Claudin-4 (CPE-receptor)	Homo sapiens
O00501	reviewed	CLDN5	Claudin-5 (Transmembrane protein deleted in VCFS) (TMDVCF)	Homo sapiens
P56747	reviewed	CLDN6	Claudin-6 (Skullin)	Homo sapiens

O95471	reviewed	CLDN7	Claudin-7	Homo sapiens
P56748	reviewed	CLDN8	Claudin-8	Homo sapiens
O95484	reviewed	CLDN9	Claudin-9	Homo sapiens

Table 2: Correlation analysis between claudins and immune infiltration in ovarian cancer via TIMER2.0

cancer	infiltrates	CLDN6			CLDN10		
		rho	p	adj.p	rho	p	adj.p
OV (n=303)	B cell memory_CIBERSORT	-0.018	0.777	0.9214	-0.1938	**	*
OV (n=303)	B cell memory_CIBERSORT-ABS	-0.0185	0.7713	0.9214	-0.1795	**	*
OV (n=303)	B cell memory_XCELL	-0.0386	0.5446	0.7855	0.091	0.1521	0.3381
OV (n=303)	B cell naive_CIBERSORT	0.0053	0.9343	0.9895	0.255	***	***
OV (n=303)	B cell naive_CIBERSORT-ABS	-0.0058	0.9272	0.9895	0.2577	***	***
OV (n=303)	B cell naive_XCELL	0.0915	0.15	0.4803	-0.142	*	0.0952
OV (n=303)	B cell plasma_CIBERSORT	0.1164	0.0666	0.3075	-0.0337	0.5963	0.7755
OV (n=303)	B cell plasma_CIBERSORT-ABS	0.0741	0.2443	0.5768	0.0036	0.9552	0.9837
OV (n=303)	B cell plasma_XCELL	0.04	0.5302	0.7759	-0.12	0.0587	0.1821
OV (n=303)	B cell_EPIC	0.045	0.4801	0.7541	-0.149	*	0.0782
OV (n=303)	B cell_MCPCOUNTER	0.2482	***	**	-0.0836	0.1888	0.3814
OV (n=303)	B cell_QUANTISEQ	0.1153	0.0694	0.3139	-0.1177	0.0636	0.1866
OV (n=303)	B cell_TIMER	-0.3021	***	***	0.2164	***	**
OV (n=303)	B cell_XCELL	-0.1283	*	0.2616	0.0756	0.2345	0.4401
OV (n=303)	Cancer associated fibroblast_EPIC	0.1377	*	0.1353	-0.0907	0.1537	0.4081
OV (n=303)	Cancer associated fibroblast_MCPCOUNTER	0.1594	*	0.0746	-0.0955	0.133	0.3766
OV (n=303)	Cancer associated fibroblast_TIDE	0.197	**	*	-0.178	**	*
OV (n=303)	Cancer associated fibroblast_XCELL	0.1913	**	*	-0.1201	0.0585	0.2122
OV (n=303)	Class-switched memory B cell_XCELL	-0.1073	0.091	0.3747	0.1094	0.085	0.2267
OV (n=303)	Common lymphoid progenitor_XCELL	-0.0628	0.3235	0.6596	0.0795	0.2112	0.4607

OV (n=303)	Common myeloid progenitor_XCELL	-0.1444	*	0.139	0.0333	0.6009	0.8165
OV (n=303)	Endothelial cell_EPIC	0.092	0.1478	0.4554	-0.1135	0.0738	0.2627
OV (n=303)	Endothelial cell_MCPCOUNTER	0.15	*	0.1218	-0.1109	0.0807	0.2771
OV (n=303)	Endothelial cell_XCELL	0.0923	0.1466	0.4554	-0.0893	0.16	0.403
OV (n=303)	Eosinophil_CIBERSORT	0.1312	*	0.1921	-0.006	0.9255	0.9687
OV (n=303)	Eosinophil_CIBERSORT-ABS	0.1299	*	0.1983	-0.0054	0.9323	0.9707
OV (n=303)	Eosinophil_XCELL	0.0472	0.4588	0.7698	-0.0908	0.1531	0.3919
OV (n=303)	Granulocyte-monocyte progenitor_XCELL	0.0423	0.5061	0.7873	0.0061	0.9236	0.9687
OV (n=303)	Hematopoietic stem cell_XCELL	0.0704	0.2685	0.6192	-0.1648	**	0.0568
OV (n=303)	Macrophage M0_CIBERSORT	0.12	0.0586	0.2045	-0.1693	**	*
OV (n=303)	Macrophage M0_CIBERSORT-ABS	0.0854	0.1791	0.431	-0.1219	0.0546	0.168
OV (n=303)	Macrophage M1_CIBERSORT	-0.1565	*	0.0812	0.1868	**	*
OV (n=303)	Macrophage M1_CIBERSORT-ABS	-0.1201	0.0585	0.2045	0.1764	**	*
OV (n=303)	Macrophage M1_QUANTISEQ	-0.1115	0.0792	0.2541	0.1631	**	*
OV (n=303)	Macrophage M1_XCELL	-0.2436	***	**	0.2096	***	**
OV (n=303)	Macrophage M2_CIBERSORT	-0.1332	*	0.1481	0.0946	0.1366	0.3176
OV (n=303)	Macrophage M2_CIBERSORT-ABS	-0.1201	0.0585	0.2045	0.1292	*	0.1388
OV (n=303)	Macrophage M2_QUANTISEQ	-0.0632	0.3207	0.6029	0.1233	0.0521	0.1619
OV (n=303)	Macrophage M2_TIDE	0.3074	***	***	-0.2819	***	***
OV (n=303)	Macrophage M2_XCELL	-0.2827	***	***	0.0992	0.1183	0.2886
OV (n=303)	Macrophage/Monocyte_MCPCOUNTER	-0.1563	*	0.0812	0.0675	0.2884	0.5842
OV (n=303)	Macrophage/Monocyte_MCPCOUNTER	-0.1563	*	0.1115	0.0675	0.2884	0.5244

OV (n=303)	Macrophage_EPIC	-0.1983	**	*	0.1515	*	0.0698
OV (n=303)	Macrophage_TIMER	0.0371	0.5602	0.7984	-0.1785	**	*
OV (n=303)	Macrophage_XCELL	-0.2767	***	***	0.1879	**	*
OV (n=303)	Mast cell activated_CIBERSORT	0.0135	0.8325	0.9299	-0.0271	0.6699	0.8355
OV (n=303)	Mast cell activated_CIBERSORT-ABS	0.0118	0.8527	0.9352	-0.0284	0.6555	0.8323
OV (n=303)	Mast cell resting_CIBERSORT	-0.0645	0.3106	0.65	0.0765	0.2289	0.4775
OV (n=303)	Mast cell resting_CIBERSORT-ABS	-0.0775	0.223	0.5626	0.0979	0.1233	0.3433
OV (n=303)	Mast cell_XCELL	-0.1516	*	0.1157	-0.0698	0.2723	0.5282
OV (n=303)	MDSC_TIDE	0.3588	***	***	-0.1393	*	0.1339
OV (n=303)	Monocyte_CIBERSORT	0.0449	0.481	0.7776	0.0739	0.2454	0.5578
OV (n=303)	Monocyte_CIBERSORT-ABS	-0.0003	0.9966	0.9966	0.124	0.0507	0.2355
OV (n=303)	Monocyte_MCPCOUNTER	-0.1563	*	0.1115	0.0675	0.2884	0.5842
OV (n=303)	Monocyte_QUANTISEQ	-0.3974	***	***	0.1651	**	0.0626
OV (n=303)	Monocyte_XCELL	-0.1109	0.0807	0.3318	0.0824	0.195	0.5043
OV (n=303)	Myeloid dendritic cell activated_CIBERSORT	-0.1643	**	0.0559	0.1554	*	0.069
OV (n=303)	Myeloid dendritic cell activated_CIBERSORT-ABS	-0.1626	*	0.0573	0.1618	*	0.0564
OV (n=303)	Myeloid dendritic cell activated_XCELL	-0.2327	***	**	0.1691	**	*
OV (n=303)	Myeloid dendritic cell resting_CIBERSORT	-0.0371	0.5605	0.7955	-0.0546	0.3908	0.635
OV (n=303)	Myeloid dendritic cell resting_CIBERSORT-ABS	-0.0367	0.5642	0.7962	-0.0475	0.4551	0.6843
OV (n=303)	Myeloid dendritic cell_MCPCOUNTER	-0.1032	0.1044	0.2989	0.0276	0.6652	0.8057
OV (n=303)	Myeloid dendritic cell_QUANTISEQ	0.363	***	***	-0.1552	*	0.0693
OV (n=303)	Myeloid dendritic cell_TIMER	-0.3143	***	***	0.2908	***	***

OV (n=303)	Myeloid dendritic cell_XCELL	-0.1196	0.0595	0.2138	0.1565	*	0.0675
OV (n=303)	Neutrophil_CIBERSORT	-0.1029	0.1053	0.4127	0.1114	0.0793	0.2453
OV (n=303)	Neutrophil_CIBERSORT-ABS	-0.0951	0.1345	0.4605	0.1072	0.0913	0.2681
OV (n=303)	Neutrophil_MCPCOUNTER	-0.0017	0.9786	0.9929	-0.0367	0.5639	0.7514
OV (n=303)	Neutrophil_QUANTISEQ	0.1785	**	0.0595	-0.0207	0.7447	0.863
OV (n=303)	Neutrophil_TIMER	-0.0724	0.2552	0.61	0.0614	0.3348	0.5858
OV (n=303)	Neutrophil_XCELL	-0.0869	0.1714	0.5122	0.0842	0.1851	0.418
OV (n=303)	NK cell activated_CIBERSORT	-0.0263	0.6796	0.8663	0.0296	0.6423	0.8424
OV (n=303)	NK cell activated_CIBERSORT-ABS	-0.0404	0.5256	0.7786	0.12	0.0587	0.2122
OV (n=303)	NK cell resting_CIBERSORT	-0.1009	0.1124	0.3225	-0.0246	0.6989	0.8788
OV (n=303)	NK cell resting_CIBERSORT-ABS	-0.1109	0.0808	0.266	-0.0226	0.7224	0.8908
OV (n=303)	NK cell_EPIC	-0.1815	**	*	0.1149	0.0703	0.2474
OV (n=303)	NK cell_MCPCOUNTER	-0.1553	*	0.0848	0.1402	*	0.12
OV (n=303)	NK cell_QUANTISEQ	-0.0556	0.3821	0.6781	0.0411	0.519	0.7789
OV (n=303)	NK cell_XCELL	-0.0824	0.1951	0.4491	0.0799	0.2087	0.4765
OV (n=303)	Plasmacytoid dendritic cell_XCELL	-0.208	***	*	0.2213	***	**
OV (n=303)	T cell CD4+ (non-regulatory)_QUANTISEQ	-0.0536	0.3998	0.7259	-0.0638	0.3156	0.5912
OV (n=303)	T cell CD4+ (non-regulatory)_XCELL	0.0077	0.9032	0.9663	-0.0723	0.2555	0.5347
OV (n=303)	T cell CD4+ central memory_XCELL	0.0456	0.4736	0.7811	0.0344	0.5892	0.8122
OV (n=303)	T cell CD4+ effector memory_XCELL	-0.1513	*	0.1109	0.1302	*	0.1625
OV (n=303)	T cell CD4+ memory activated_CIBERSORT	-0.0047	0.9411	0.9798	0.0538	0.3982	0.6743
OV (n=303)	T cell CD4+ memory activated_CIBERSORT-ABS	-0.0041	0.9485	0.9798	0.0526	0.409	0.6835

OV (n=303)	T cell CD4+ memory resting_CIBERSORT	0.1047	0.0994	0.329	0.015	0.8141	0.9242
OV (n=303)	T cell CD4+ memory resting_CIBERSORT-ABS	0.0014	0.9827	0.992	0.0943	0.1378	0.3757
OV (n=303)	T cell CD4+ memory_XCELL	0.0253	0.6916	0.897	0.0693	0.2762	0.5595
OV (n=303)	T cell CD4+ naive_CIBERSORT	0.1349	*	0.1741	-0.1428	*	0.1147
OV (n=303)	T cell CD4+ naive_CIBERSORT-ABS	0.1349	*	0.1741	-0.1428	*	0.1147
OV (n=303)	T cell CD4+ naive_XCELL	-0.1611	*	0.0828	0.1101	0.083	0.2652
OV (n=303)	T cell CD4+ Th1_XCELL	-0.1385	*	0.1608	0.0499	0.4328	0.7009
OV (n=303)	T cell CD4+ Th2_XCELL	0.0625	0.3263	0.6506	0.0766	0.2287	0.522
OV (n=303)	T cell CD4+_EPIC	0.0428	0.5014	0.8099	-0.0148	0.8168	0.9242
OV (n=303)	T cell CD4+_TIMER	0.1149	0.0703	0.2735	-0.0058	0.9273	0.9753
OV (n=303)	T cell CD8+ central memory_XCELL	-0.1749	**	*	0.1568	*	0.0801
OV (n=303)	T cell CD8+ effector memory_XCELL	0.0858	0.177	0.4688	0.0796	0.2107	0.4441
OV (n=303)	T cell CD8+ naive_XCELL	0.1924	**	*	-0.1191	0.0606	0.2145
OV (n=303)	T cell CD8+_CIBERSORT	-0.0534	0.4012	0.6829	0.0301	0.6366	0.8318
OV (n=303)	T cell CD8+_CIBERSORT-ABS	-0.0453	0.4765	0.7086	0.0702	0.2695	0.5033
OV (n=303)	T cell CD8+_EPIC	0.0434	0.4951	0.7166	-0.0542	0.3944	0.6552
OV (n=303)	T cell CD8+_MCPCOUNTER	-0.0322	0.613	0.7909	0.0925	0.1455	0.3528
OV (n=303)	T cell CD8+_QUANTISEQ	-0.2023	**	*	0.1851	**	*
OV (n=303)	T cell CD8+_TIMER	-0.1707	**	*	0.1363	*	0.139
OV (n=303)	T cell CD8+_XCELL	-0.0544	0.3923	0.6765	-0.0078	0.9028	0.9629
OV (n=303)	T cell follicular helper_CIBERSORT	-0.036	0.5716	0.8255	0.0032	0.9605	0.9889
OV (n=303)	T cell follicular helper_CIBERSORT-ABS	-0.0618	0.3316	0.7046	0.058	0.3618	0.6466

OV (n=303)	T cell gamma delta_CIBERSORT	-0.0281	0.6591	0.8771	-0.0738	0.2458	0.5578
OV (n=303)	T cell gamma delta_CIBERSORT-ABS	-0.0276	0.6642	0.8771	-0.0735	0.2481	0.5578
OV (n=303)	T cell gamma delta_XCELL	-0.0545	0.3918	0.7431	0.03	0.6374	0.8533
OV (n=303)	T cell NK_XCELL	-0.1745	**	0.064	-0.001	0.9869	0.9937
OV (n=303)	T cell regulatory (Tregs)_CIBERSORT	-0.0056	0.9299	0.9886	-0.0417	0.5123	0.7769
OV (n=303)	T cell regulatory (Tregs)_CIBERSORT-ABS	-0.0278	0.6622	0.8546	-0.006	0.9248	0.971
OV (n=303)	T cell regulatory (Tregs)_QUANTISEQ	-0.001	0.9873	0.9998	0.1678	**	*
OV (n=303)	T cell regulatory (Tregs)_XCELL	0.0683	0.283	0.575	0.0402	0.5276	0.783
*P < 0.05;**P < 0.01;***P < 0.001							

Table 3: Correlation analysis between claudins and markers of immune cells in ovarian cancer via TIMER2.0

Cancer	Immune cells	Gene markers	CLDN6			CLDN10		
			rho	p	adj.p	rho	p	adj.p
OV (n=303)	B cell	CD19	0.1232	0.0522	0.1889	-0.0705	0.268	0.4797
OV (n=303)	B cell	CD79A	0.0252	0.692	0.8533	-0.0653	0.3047	0.5211
OV (n=303)	CD8+ T cell	CD8A	-0.1023	0.1073	0.3065	0.0977	0.1241	0.2928
OV (n=303)	CD8+ T cell	CD8B	-0.0322	0.613	0.7938	0.0925	0.1455	0.3306
OV (n=303)	DC	CD1C	-0.1568	*	0.0978	0.0864	0.1742	0.4657
OV (n=303)	DC	HLA-DPA1	-0.2513	***	**	0.2298	***	**
OV (n=303)	DC	HLA-DPB1	-0.3	***	***	0.2535	***	***
OV (n=303)	DC	HLA-DQB1	-0.2294	***	**	0.2259	***	**
OV (n=303)	DC	HLA-DRA	-0.3225	***	***	0.2428	***	**
OV (n=303)	DC	ITGAX	-0.1812	**	*	0.0859	0.1768	0.4689
OV (n=303)	DC	NRP1	0.1252	*	0.2315	-0.0004	0.9946	0.9947
OV (n=303)	M1 Macrophage	IRF5	-0.1856	**	*	0.0896	0.1587	0.3412
OV (n=303)	M1 Macrophage	NOS2	0.1436	*	0.1056	-0.0383	0.5475	0.7537
OV (n=303)	M1 Macrophage	PTGS2	0.0961	0.1305	0.3467	0.0093	0.8836	0.9482
OV (n=303)	M2 Macrophage	CD163	-0.1046	0.0996	0.2879	0.0646	0.31	0.5239
OV (n=303)	M2 Macrophage	MS4A4A	-0.1142	0.072	0.2358	0.1147	0.0707	0.2036
OV (n=303)	M2 Macrophage	VSIG4	-0.1522	*	0.0806	0.0768	0.2274	0.4343
OV (n=303)	Monocyte	CD86	-0.2212	***	**	0.1457	*	0.0884
OV (n=303)	Monocyte	CSF1R	-0.1906	**	*	0.0717	0.2596	0.4733
OV (n=303)	NK cell	KIR2DL1	-0.0061	0.924	0.9876	0.0991	0.1187	0.3858
OV (n=303)	NK cell	KIR2DL3	-0.2296	***	**	0.1527	*	0.0916
OV (n=303)	NK cell	KIR2DL4	-0.2568	***	**	0.1563	*	0.08
OV (n=303)	NK cell	KIR2DS4	-0.097	0.1271	0.3901	0.0847	0.1825	0.4785
OV (n=303)	NK cell	KIR3DL1	0.0189	0.7664	0.9376	0.1037	0.1025	0.3498
OV (n=303)	NK cell	KIR3DL2	-0.0633	0.3198	0.6371	0.1495	*	0.1017
OV (n=303)	NK cell	KIR3DL3	-0.0464	0.4656	0.7511	0.0571	0.3698	0.6882
OV (n=303)	Neutrophil	CCR7	-0.0628	0.3234	0.6383	0.0943	0.138	0.4201
OV (n=303)	Neutrophil	CEACAM8	-0.0605	0.3414	0.6588	-0.0324	0.6109	0.839
OV (n=303)	Neutrophil	ITGAM	-0.1805	**	*	0.0575	0.3667	0.6882
OV (n=303)	T cell (general)	CD2	-0.1567	*	0.0695	0.1651	**	*

OV (n=303)	T cell (general)	CD3D	-0.1452	*	0.1026	0.1524	*	0.0707
OV (n=303)	T cell (general)	CD3E	-0.1256	*	0.1795	0.1581	*	0.0591
OV (n=303)	TAM	CCL2	-0.1721	**	*	0.1709	**	*
OV (n=303)	TAM	CD68	-0.203	**	*	0.105	0.0983	0.2528
OV (n=303)	TAM	IL10	0.0496	0.4362	0.7057	-0.0047	0.9408	0.9719
OV (n=303)	Tfh	IL21	-0.1298	*	0.1634	-0.0126	0.8434	0.9304
OV (n=303)	Tfh	BCL6	-0.1985	**	*	0.1285	*	0.158
OV (n=303)	Th1	IFNG	-0.0838	0.1876	0.4398	0.1323	*	0.146
OV (n=303)	Th1	STAT1	-0.077	0.2259	0.4879	0.0894	0.1598	0.3844
OV (n=303)	Th1	STAT4	-0.0099	0.8763	0.9539	0.0768	0.2275	0.4688
OV (n=303)	Th1	TBX21	-0.1559	*	0.0798	0.1587	*	0.0613
OV (n=303)	Th1	TNF	-0.0368	0.5628	0.778	0.02	0.7529	0.8872
OV (n=303)	Th17	IL17A	-0.0713	0.2625	0.528	0.0043	0.9463	0.9841
OV (n=303)	Th17	STAT3	-0.0442	0.4878	0.7311	0.0117	0.8537	0.9368
OV (n=303)	Th2	GATA3	-0.0651	0.3065	0.5692	-0.0814	0.2006	0.4386
OV (n=303)	Th2	IL13	-0.0187	0.7691	0.8933	0.0647	0.3093	0.5745
OV (n=303)	Th2	STAT5A	-0.1185	0.062	0.2115	-0.051	0.423	0.6851
OV (n=303)	Th2	STAT6	-0.0476	0.4548	0.7101	0.0869	0.1716	0.396
OV (n=303)	Treg	CCR8	-0.0084	0.8953	0.9668	0.0211	0.7401	0.8872
OV (n=303)	Treg	FOXP3	-0.0519	0.4145	0.6735	0.0635	0.3187	0.5838
OV (n=303)	Treg	STAT5B	0.1542	*	0.0851	-0.1677	**	*
OV (n=303)	Treg	TGFB1	-0.1237	0.0512	0.1861	0.0153	0.8105	0.924
DC: Dendritic cell; NK cell: Natural killer cell; TAM: Tumor-associated macrophage; Tfh: Follicular helper T cell; Treg: Regulatory T cell; *P < 0.05; **P < 0.01; ***P < 0.001								

Figures

Figure 1

The genetic variation of claudin gene family in ovarian cancer through the cBioPortal. (A) The alteration frequency of claudin gene family in serous ovarian cancer. (B) The oncoprint of claudin gene family in serous ovarian cancer. (C) The overall survival difference of serous ovarian cancer between altered group and unaltered group (** $p < 0.01$).

Figure 2

The mRNA expression of claudins in TCGA samples and the corresponding GTEx normal samples via GEPIA2. (* p<0.01)

Figure 3

The relationship between claudins expression and the prognosis of ovarian cancer patients through Kaplan-Meier plotter. The overexpression of CLDN3, CLDN4, CLDN6, and CLDN16 were significantly correlated with poor OS (A) and PFS (B). (C) The overexpression of CLDN10 predicted good OS, PFS and PPS. (D) The low expression of CLDN15 predicted poor OS in ovarian cancer. OS: overall survival; PFS: progression free survival; PPS: post progression survival.

Figure 4

Gene set enrichment analysis (GSEA) of c7 (immunologic signatures) for CLDN6 and CLDN10.

Figure 5

Relationship between claudins expression and tumor microenvironment. (A) The expression of CLDN6 is negative correlation with immune score and ESTIMATE score. (B) The expression of CLDN10 is positive correlation with immune score and ESTIMATE score. (C) The difference of 22 immune cell between claudin-high group and claudin-low group (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Figure 6

The relationship between immune cells infiltration and claudins expression. Correlation analysis of immune cell infiltration and CLDN6 expression (A), and CLDN10 expression (B) based on the microarray expression values of ovarian cancer through TIMER. Correlation analysis of immune cell infiltration and CLDN6 expression (C), and CLDN10 expression (D) based on available TCGA RNA-seq data of ovarian cancer via TIMER2.

Supplementary Files

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- [Supplementaryfigure2.tif](#)
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