Down-regulated CLDN10 Predicts Favorable Prognosis and Correlates With Immune Infiltration in Gastric Cancer

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Abstract

Background: CLDN10, an important component of the tight junctions of epithelial cells, plays a crucial role in a variety of tumors. The effect of CLDN10 expression in gastric cancer, however, has yet to be elucidated.

Methods: Differential expression of CLDN10 was evaluated using Oncomine, ULCAN, and TIMER2.0 databases. Correlations between CLDN10 expression and clinical outcomes of gastric cancer were explored by Kaplan-Meier Plotter. The correlations between CLDN10 expression and immune cell infiltration and somatic copy number mutation (SCNA) in gastric cancer were explored by TIMER2.0 and GEPIA2.0.

Results: CLDN10 expression was lower in gastric cancer compared to adjacent normal tissues, and associated with better prognosis. CLDN10 also showed significant differences at different T stages, Lauren classification, treatments and HER2 status. Low expression of CLDN10 was associated with increased infiltration levels of certain immune cells in the tumor microenvironment. In addition, it was found that different somatic copy number changes (SCNA) in CLDN10 might affect the level of immune cell infiltration. Furthermore, the expression of CLDN10 was significantly associated with the expression of several immune cell markers, especially B cell markers, follicular helper T cell (Tfh) markers and T cell exhaustion markers.

Conclusion: CLDN10 may act as a potential prognostic biomarker and correlate to immune infiltration levels in gastric cancer.

Background

Gastric cancer is the fifth most common malignant tumor in humans. One million new cases of GC are diagnosed yearly and it is the third leading cause of cancer-related deaths [1]. Due to the lack of reliable biomarkers and early screening methods, gastric cancer is usually first diagnosed at an advanced stage. As a result patients remain at a high risk for metastasis and death despite the continuous advancements that have been achieved in surgery, perioperative chemotherapy, and targeted therapy [2, 3]. In recent years, immunotherapy has become a hot topic for tumor treatment. Immune checkpoint inhibitors targeting PD1, CTLA4 and other targets have shown remarkable achievements in combatting malignant melanoma, non-small cell lung cancer, renal cell carcinoma, gastrointestinal tumors and other tumors [4–9]. Thus, identification of new biomarkers and therapeutic targets is crucially important for developing novel treatments of gastric cancer.

Tight junctions, also known as occluding junctions, are one of the important connection forms of cell adhesion structures[10]. Tight junctions have two main functions (i) they act as a barrier and regulate molecular transport, and (ii) they play a role in maintaining cell polarity. Such a loss of cell polarity is a biological characteristic of cancer cells. When the tight junction function is impaired, its barrier function decreases, leading to increased tissue permeability causing the polarity of endothelial cells to disappear, eventually leading to genetic diseases, immune diseases and even tumors[11, 12]. Claudins are key components of tight junctions, and abnormal expression could promote or inhibit the occurrence and development of tumors, and play an important role in the process of tumor proliferation, invasion, migration and metastasis. Accumulated evidence has proven that claudins could be used as prognostic biomarkers or to predict targets of immunotherapy[13]. CLDN10, a member of the claudin family, maintains cell-cell adhesion, the loss of which has been considered to be the initial stage of tumor cell migration [14]. Abnormal expression of CLDN10 may cause dysfunction of tight junctions, thereby affecting tumor progression, although this effect is complex. Previous reports have shown that CLDN10 is highly expressed in hepatocellular carcinoma, papillary thyroid carcinoma and lung adenocarcinoma. Its expression is associated with poor prognosis.
of hepatocellular carcinoma, but with better survival of thyroid papillary carcinoma and lung adenocarcinoma [15–17]. In addition, down-regulated CLDN10 is correlated with poor prognosis of ovarian cancer and clear cell renal cell carcinoma [18, 19]. Currently, there is a lack of systematic studies to identify the underlying value of CLDN10 in the prognosis and clinicopathological characteristics of gastric cancer.

Here Oncomine, ULCAN, Kaplan–Meier plotter and GEPIA 2.0 were used to analyze CLDN10 expression and its correlation with the prognosis and clinicopathological features of gastric cancer. Subsequently the association between CLDN10 and the level of immune cell infiltration in the tumor microenvironment was investigated using TIMER 2.0. Our data revealed an important role of CLDN10 in gastric cancer, and to some extent explains the potential relationship and mechanism of the interaction between CLDN10 and the immune system.

Materials And Methods

Oncomine and ULCAN database

The expression levels of CLDN10 in gastric cancer were identified in the Oncomine database (https://www.oncomine.org) [20]. In this study, a p-value of 0.05, a fold change of 1.5, and a gene rank in the top 10% were set as the significance thresholds. UALCAN (http://ualcan.path.uab.edu/analysis.html) [21] was used to verify the differential expression of CLDN10. This is a cancer data online analysis, mainly based on the TCGA RNA-seq and clinical data of 31 types of cancer.

GEPIA 2.0 database

Gene Expression Profiling Interactive Analysis 2.0 (GEPIA 2.0) is a publicly accessible, online cancer database designed to analyze various cancer data based on TCGA data (http://gepia2.cancer-pku.cn/) [22]. The correlation between the expression levels of CLDN10, disease-free survival (DFS) and overall survival (OS) of the patients with gastric cancer was analyzed through GEPIA 2.0. The results are shown through survival curves. In addition, the GEPIA 2.0 database was used to confirm the correlation between CLDN10 expression in gastric cancer and a number of immune cell gene markers. The correlation analysis of CLDN10 expression and signatures was determined by Spearman's correlation with statistical significance (p < 0.05 was considered significant).

Kaplan–Meier plotter database

Kaplan-Meier Plotter (http://kmplot.com/analysis/) [23] is an online comprehensive database that contains gene expression and clinical data for 21 types of tumors with large sample sizes for the breast (n = 6,234), ovarian (n = 2,190), lung (n = 3,452) and gastric (n = 1,440) cancer cohorts. This database was used to investigate the correlation between CDLN10 expression and the prognosis and clinicopathological features of gastric cancer through Hazard ratios (HRs) of 95%, confidence intervals (CIs), and log-rank p-value.

TIMER 2.0 database

The Tumor Immune Estimation Resource 2.0 (TIMER2.0) database [24] is a publicly available immunity and gene expression repository. A variety of computational methods were undertaken (including deconvolution) to analyze and visualize the expression profile data from the TCGA to show the characteristics of tumor immune infiltration and gene expression of various cancers. Firstly, the Gene DE module was used to study the differential expression of CLDN10 in various tumors. Secondly, CLDN10 expression and its correlation with the level of immune cell infiltration in STAD were evaluated by the Gene module. Additionally, the SCNA module was used to analyze the relationship between somatic copy number alterations (SCNA) and immune infiltration level. Furthermore, the Gene_Corr module
was used to verify the correlation of CLDN10 expression with gene expression markers of tumor infiltrating immune cells by Spearman analysis. The levels of gene expression were demonstrated by log2 TPM.

**Results**

**Expression analysis**

To determine the differential expression of CLDN10 in both gastric cancers and various other cancer types, we used the TIMER 2.0 database to investigate the CLDN10 expression in human cancers. To more accurately assess CLDN10 expression in gastric cancer, we used the Oncomine and UALCAN database to verify CLDN10 expression levels. As shown in Fig.1A, CLDN10 expression was significantly down-regulated in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), glioblastoma multiforme (GBM), head and neck cancer (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KRIP), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD) and uterine corpus endometrial carcinoma (UCEC) compared to adjacent normal tissues. However, CLDN10 expression was higher in cholangiocarcinoma (CHOL), adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and thyroid carcinoma (THCA) than in the adjacent normal tissues. Furthermore, data from Oncomine (Fig.1B) and UALCAN (Fig.1C) confirmed that CLDN10 was down-expressed in gastric cancer.

**Prognostic and clinicopathological significance of CLDN10 expression in gastric cancer**

In order to better understand potential correlations of CLDN10 expression in gastric cancer, we used Kaplan-Meier Plotter and GEPIA2.0 database to investigate the prognosis and clinicopathological significance of CLDN10. From the Kaplan-Meier Plotter, the results showed that overexpression of CLDN10 corresponded with an unfavourable prognosis for gastric cancer patients (Fig.2A, OS HR=1.62, 95% CI=1.36–1.92, \( p=3.7\times10^{-8} \); Fig.2C, PFS HR= 1.48, 95% CI=1.21–1.81, \( p= 0.00011 \)). Moreover, we used GEPIA2.0 to verify this association and got similar results (Fig.2B, OS HR=1.3, \( p=0.064 \); Fig.2D, DFS HR= 1.7, \( p= 0.0055 \)).

To further understand the in-depth significance and underlying mechanism of the differential expression of CLDN10 in gastric cancer tissues and adjacent normal tissues, we explored the interrelation between CLDN10 expression levels and the clinicopathological characteristics of gastric cancer using the Kaplan-Meier Plotter database. High CLDN10 expression was associated with poor OS and PFS in both male and female gastric cancer patients (Table 1; \( p<0.05 \)). TNM staging is a widely accepted standard for cancer classification, which can predict the prognosis of gastric cancer. It means the invasive depth of primary tumor (T), regional lymph node (N), and distant metastasis (M) [25, 26]. Regardless of the clinical stage, high CLDN10 expression was related to poorer OS, but only stage 2 was related to PFS. The expression of CLDN10 in T2 gastric cancer patients was associated with OS and PFS (OS \( p=0.03 \); PFS \( p=0.027 \)), and the value of HR has a decreasing trend with increasing tumour development. A statistically significant correlation between CLDN10 expression and gastric cancer prognosis was observed in stage N0\N1\N3\N1+2+3 patients but not N2. The expression of CLDN10 was statistically correlated with OS and PFS in stage M0 gastric cancer, while only OS in stage M1. According to the Lauren classification, it was observed that the expression of CLDN10 in patients with intestinal-type gastric cancer was related to OS and PFS. In mixed-type group, it was associated with OS but not with PFS, while in diffuse-type gastric cancer, no statistically significant association was observed. In terms of treatment, the expression of CLDN10 in gastric cancer patients who have only
undergone surgery was related to OS and PFS (OS \( p=0.017 \); PFS \( p=0.044 \)). However, it was not significantly related to the prognosis of patients who had received 5-FU-based adjuvant chemotherapy. In addition, CLDN10 expression was associated with OS and PFS in HER2-negative gastric cancer patients (OS \( p=2.1e-07 \); PFS \( p=0.00033 \)), but not with HER2-positive patients.

**CLDN10 expression and somatic CNV is correlated with immune cell infiltration in gastric cancer**

The gene module in the TIMER 2.0 database was used to investigate the relationship between CLDN10 expression and immune cell infiltration in STAD (Fig. 3). It was seen that CLDN10 expression level was negatively related to the infiltrating levels of T cell CD8+ central memory, T cell CD8+ effector memory, T cell CD4+ memory, T cell CD4+ Th1, T cell CD4+ Th2, T cell regulatory (Tregs), B cell plasma, macrophage M1, macrophage M2, plasmacytoid dendritic cell, and common lymphoid progenitor in STAD, while positively correlated with cancer associated fibroblast (CAF), endothelial cell, eosinophil, granulocyte–monocyte progenitor and hematopoietic stem cell (\( P<0.05 \)). Most of the immune cells mentioned above were negatively associated with CLDN10 level, which meant that there were more infiltrating immune cells in the tumor microenvironment in gastric cancer patients with low expression of CLDN10. This could partly explain why a decline in CLDN10 was associated with better outcomes. Moreover, we analyzed and compared the immune infiltration levels among gastric cancer patients with the presence of different somatic copy number alterations (SCNA) for CLDN10 (Fig. 4). It was observed that high amplification in STAD was negatively associated with the infiltration of CD8+ T cells, CD4+ T cells, B cells, neutrophil, macrophages M1, neutrophils, myeloid dendritic cells, NK cells and T cell follicular helper (\( P<0.05 \)). Furthermore, arm-level gain was also inversely correlated with the above immune cell infiltration (\( P<0.05 \)), except for macrophage M1 and T cell follicular helper. However, only the level of NK cell infiltration was significantly correlated with the arm-level deletion (\( P<0.05 \)). These results showed us that SCNA of CLDN10 may affect the level of immune cell infiltration.

**Correlation analyses between immune marker genes and CLDN10 expression**

The TIMER 2.0 database interrogation concentrated on the correlations between CLDN10 in STAD and related marker genes of infiltrating immune cells (Table 2). After purity-related adjustments, it was shown that CLDN10 levels were notably related to some marker genes in a variety of immune cells, including T cells (general), M1 macrophages, neutrophils, natural killer cells, dendritic cells (DCs) and different functional T cells, such as Tregs, Th1, Tfh, Th17 and exhausted T cells. It was of note that the levels of the main markers of B cell (CD19, CD79A), Tfh (BCL6, IL21) and exhausted T cells (PD1, CTLA4, LAG3, TIM3, GZMB) were all significantly correlated with CLDN10 levels in STAD. Tfh could promote the differentiation of B cells into memory B cells and the survival of plasma cells by providing signals to B cells, which played an important role in humoral immunity [27, 28]. In exhausted T cells, these gene markers (PD1, CTLA4, LAG3, TIM3 and GZMB) were consistently significantly correlated with the expression of CLDN10, which further suggested that the expression level of CLDN10 in gastric cancer is related to immune infiltration.

**Discussion**
It has been reported that abnormal claudin expression relates to the occurrence, development and metastasis of malignant tumors. This has potential clinical value in the diagnosis and treatment of tumors and could therefore be used as a diagnostic marker or a target for immunotherapy [13, 29, 30]. The low expression of CLDN1 in lung adenocarcinoma promoted the migration, invasion and metastasis of cancer cells, and was associated with a shorter OS [31]. CLDN3 and CLDN4 is overexpressed in ovarian cancer and increases cell invasion and motility, thereby promoting tumor occurrence and metastasis [32]. The low expression of CLDN18 in gastric cancer could be used as a marker of adverse outcome [33]. Furthermore, claudiximab, a monoclonal antibody targeting CLDN18.2, has been used in the treatment of advanced gastric cancer, showing the broad prospects of claudins in tumor treatment[34]. However, to date, the clinical significance of CLDN10 expression in gastric cancer has remained unclear.

In this study, we evaluated the level of CLDN10 mRNA in gastric cancer using Oncomine, UALCAN and TIMER2.0 databases. The results from these three databases mutually confirmed that the expression level of CLDN10 in gastric cancer tissues was lower than that in adjacent normal tissues. Furthermore, it was seen that low CLDN10 expression was associated with better OS, PFS, and DFS in gastric cancer patients, suggesting that CLDN10 expression is a potential prognostic marker for gastric cancer. In order to explore the underlying reasons, the relationship between CLDN10 and various clinicopathological features was assessed. It was found that the high expression of CLDN10 had the highest HR value in stage 1, stage T2, stage N0, intestinal type, surgery only and HER2-negative subgroups, and the correlation was statistically significant. These results supported the hypothesis that CLDN10 might be a potential prognostic marker for gastric cancer.

A previous study has confirmed that CLDN10 was an immune-related gene and could predict the prognosis of papillary thyroid carcinoma [16]. In our analysis, the decreased expression of CLDN10 was associated with increased infiltration of several major immune cells, potentially enhancing the body's anti-tumor immune response, which partly explains why low expression of CLDN10 was associated with better prognosis in gastric cancer. Somatic copy number alterations are one of the driving factors for tumorigenesis and have been used in tumor prognosis prediction and treatment [35, 36]. The relationship between the infiltration level of immune cells and SCNA was then assessed. It could be found from the violin diagram that the SCNA types of high amplification and arm-level gain mainly affected the level of immune infiltration (Fig. 4). High amplification of CLDN10 correlated with a decrease in the infiltration level of some immune cells and the same situation occurred in the arm-level gain group, with the exception of macrophage M1 and T cell follicular helper. In addition, arm-level deletion was only found to be statistically associated with the changes in NK cell infiltration level.

To provide a more comprehensive insight into the relationship between CLDN10 expression and immune infiltration, the relationship between CLDN10 expression and gene markers of immune cells was further analyzed. The expression level of CLDN10 was seen to have a statistically significant positive or negative correlation with the gene markers of many immune cells (Table 2). Tfh could promote B cell differentiation, antibody class switching and germinal center formation [37]. Our results showed that CLDN10 expression significantly correlated to the expression of B cells (CD19, CD79A) and Tfh cells (BCL6, IL21) markers, which indicated a potential mechanism for CLDN10 to regulate B cell function in gastric cancer. In addition, PD1, CTLA4, LAG3, TIM3 and GZMB were common inhibitory receptors (IRs) in the tumor microenvironment, which could inhibit immune responses and even cause immune escape or tolerance after binding with corresponding ligands. Immune checkpoint inhibitors that targeted and antagonized these IRs have been developed to treat a variety of tumors with remarkable success and are promising therapies for the future [38–41]. The results shown here demonstrate that CLDN10 expression negatively correlates to these gene markers. These correlations suggest that CLDN10 might participate in immune escape by regulating T cell exhaustion. Furthermore, significant correlations could be found between CLDN10 expression and the regulation
of some markers of T cell (CD3D), M1 Macrophage (NOS2), Neutrophils (CCR7), Natural killer cell (KIR2DL4), Dendritic cell (HLA-DQB1, HLA-DRA and CD1C), Th1 (NRP1, STAT1 and IFGN), Th17 (STAT3), Treg (FOXP3 and STAT5B) in gastric cancer. Based on the above results, CLDN10 might play an important role in the regulation of immune cell infiltration in gastric cancer.

In summary, CLDN10 is down-regulated in gastric cancer and associated with a better prognosis. The regulation of the level of immune cell infiltration by CLDN10 may be a potential mechanism for the improvement of prognosis. However, there are limitations in this research, and further experiments are needed to verify the mechanism of CLDN10 regulating immune infiltration in the gastric cancer tumor microenvironment.

**Conclusions**

CLDN10 may act as a potential prognostic biomarker and correlate to immune infiltration levels in gastric cancer.

**Abbreviations**

SCNV
somatic copy number alterations; TCGA:The Cancer Genome Atlas; STAD:Stomach adenocarcinoma; OS:Overall survival; PFS:Progression-Free Survival; DFS:Disease-free survival; HR:hazard ratio; Tfh:follicular helper T cell.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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Authors' contributions

XHR, NQW and XBW designed the study. XHR, JLJ, ZHL, JBZ, ZHZ, RH and HYQ collected the data. XHR, JLJ, ZHL and NQW analyzed the data. XHR, JLJ, ZHL and NQW organized the manuscript. XHR, NQW and XBW reviewed the papers and revised the manuscript. All the authors have read and approved the final manuscript. All authors contributed to data analysis, drafting of the paper and manuscript revisions and agree to be accountable for all aspects of the work.

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Tables

Table 1 Correlations of CLDN10 expression and clinicopathological significance in gastric cancer.
| Sex          | OS   | PFS  |   |   |   |   |   |   |   |   |   |   |
|--------------|------|------|---|---|---|---|---|---|---|---|---|---|
| Female       | 236  | 2.18(1.46-3.28) | 0.0001 | 201 | 1.98(1.31-2.99) | 0.00095 |
| male         | 544  | 1.64(1.32-2.04) | 5.354e-6 | 437 | 1.61(1.21-2.15) | 0.00092 |
| Stage        |      |      |   |   |   |   |   |   |   |   |   |   |
| 1            | 67   | 3.22(1.11-9.32) | 0.023 | 60 | 2.37(0.77-7.3) | 0.12 |
| 2            | 140  | 2.55(1.33-4.88) | 0.0034 | 131 | 2.42(1.32-4.42) | 0.0031 |
| 3            | 305  | 1.7(1.27-2.27) | 3e-04 | 186 | 0.8(0.53-1.23) | 0.31 |
| 4            | 148  | 1.66(1.07-2.59) | 0.023 | 141 | 1.54(0.96-2.46) | 0.073 |
| Stage T      |      |      |   |   |   |   |   |   |   |   |   |   |
| 2            | 241  | 1.56(1.02-2.39) | 0.037 | 239 | 1.59(1.05-2.41) | 0.027 |
| 3            | 204  | 0.83(0.58-1.19) | 0.31 | 204 | 0.77(0.55-1.1) | 0.15 |
| 4            | 38   | 0.58(0.23-1.5) | 0.26 | 39 | 0.61(0.24-1.51) | 0.28 |
| Stage N      |      |      |   |   |   |   |   |   |   |   |   |   |
| 0            | 74   | 3.06(1.25-7.48) | 0.01 | 72 | 2.96(1.21-7.22) | 0.013 |
| 1            | 225  | 2.01(1.26-3.2) | 0.0028 | 222 | 1.84(1.18-2.88) | 0.0063 |
| 2            | 121  | 0.88(0.54-1.43) | 0.6 | 125 | 0.74(0.48-1.13) | 0.16 |
| 3            | 76   | 1.82(1.03-3.22) | 0.036 | 76 | 1.92(1.05-3.52) | 0.032 |
| 1+2+3        | 422  | 1.33(1.02-1.74) | 0.034 | 423 | 1.28(0.99-1.65) | 0.059 |
| Lauren class|      |      |   |   |   |   |   |   |   |   |   |   |
| intestinal   | 320  | 2.15(1.55-2.98) | 2.3e-06 | 263 | 1.6(1.13-2.27) | 0.0079 |
| diffuse      | 241  | 1.28(0.89-1.83) | 0.1 | 231 | 1.18(0.82-1.69) | 0.37 |
| mixed        | 32   | 1.71(0.54-5.38) | 0.36 | 28 | 0.43(0.15-1.21) | 0.099 |
| Treatment    |      |      |   |   |   |   |   |   |   |   |   |   |
| surgery alone| 380  | 1.54(1.08-2.19) | 0.017 | 375 | 1.43(1.01-2.02) | 0.044 |
| 5 FU based adjuvant | 152  | 0.74(0.5-1.08) | 0.12 | 152 | 0.73(0.49-1.08) | 0.12 |
| other adjuvant | 86  | 0.37(0.15-0.89) | 0.021 | 80 | 0.42(0.19-0.93) | 0.028 |
| HER2         |      |      |   |   |   |   |   |   |   |   |   |   |
| negative     | 532  | 1.8(1.44-2.26) | 2.1e-07 | 408 | 1.62(1.24-2.12) | 0.00033 |
| positive     | 343  | 1.25(0.95-1.64) | 0.11 | 232 | 1.31(0.9-1.92) | 0.16 |
| Stage M      |      |      |   |   |   |   |   |   |   |   |   |   |
Table 2 Correlation analysis between CLDN10 and relate genes and markers of immune cells in TIMER2.0.

|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
|   |   |   |   |   |   |   |
| 0 | 444 | 1.42 | 1.07-1.87 | **0.013** | 443 | 1.35 | 1.03-1.76 | **0.027** |
| 1 | 56 | 2 | 1.08-3.71 | **0.024** | 56 | 1.82 | 0.97-3.39 | 0.058 |

**Table 2** Correlation analysis between CLDN10 and relate genes and markers of immune cells in TIMER2.0.
| Description          | Gene markers | STAD | Purity |
|----------------------|--------------|------|--------|
|                      |              | None |        |
|                      |              | Cor  | P      | Cor  | P    |
| CD8 + T cell         | CD8A         | -0.063 | 0.198 | -0.077 | 0.133 |
|                      | CD8B         | 0.054  | 0.274 | 0.058  | 0.262 |
| T cell (general)     | CD3D         | -0.083 | 0.0899 | -0.116 | **0.0245** |
|                      | CD3E         | -0.045 | 0.356 | -0.079 | 0.126 |
|                      | CD2          | -0.066 | 0.18  | -0.091 | 0.0781 |
| B cell               | CD19         | 0.203  | **3.21E-05** | 0.174 | **6.80E-04** |
|                      | CD79A        | 0.153  | **1.76E-03** | 0.127 | **1.34E-02** |
| Monocyte             | CD86         | -0.059 | 2.32E-01 | -0.081 | 0.115 |
|                      | CSF1R        | 0.027  | 0.589 | -0.003 | 0.954 |
| TAM                  | CCL2         | 0.106  | **0.0305** | 0.098 | 0.0569 |
|                      | CD68         | -0.065 | 0.19  | -0.068 | 0.189 |
|                      | IL10         | 0.049  | 0.32  | 0.02   | 0.698 |
| M1 Macrophage        | NOS2         | -0.169 | **5.54E-04** | -0.19 | **2.04E-04** |
|                      | IRF5         | 0.083  | **9.18E-02** | 0.072 | 0.162 |
|                      | PTGS2        | 0.111  | **2.34E-02** | 0.099 | 5.40E-02 |
| M2 Macrophage        | CD163        | -0.032 | 0.519 | -0.047 | 0.362 |
|                      | VSIG4        | -0.01  | 0.843 | -0.015 | 0.768 |
|                      | MS4A4A       | 0.007  | 0.887 | -0.007 | 0.888 |
| Neutrophils          | CEACAM8      | 0.08   | 0.106 | 0.078  | 0.127 |
|                      | ITGAM        | 0.019  | 0.695 | 0.001  | 0.214 |
|                      | CCR7         | 0.146  | **2.93E-03** | 0.12 | **1.97E-02** |
| Natural killer cell  | KIR2DL1      | 0.019  | 0.698 | 0.013  | 0.795 |
|                      | KIR2DL3      | -0.052 | 0.289 | -0.063 | 0.224 |
|                      | KIR2DL4      | -0.129 | **8.65E-03** | -0.132 | **1.01E-02** |
|                      | KIR3DL1      | -0.047 | 0.344 | -0.048 | 0.349 |
| Gene       | KIR3DL2  | Dendritic cell | HLA-DPB1  | HLA-DQB1  | HLA-DRA  | HLA-DPA1  | CD1C      | Th1       | NRP1      | ITGAX     | TBX21     | Th2       | GATA3     | Stat6     | Stat5a    | IL13      | Tfh       | BCL6      | IL21      | Th17      | STAT3     | IL17A     | Treg      | FOXP3     | CCR8      | Stat5b    | TGFB1     |
|------------|----------|----------------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|            | -0.062   | -0.038         | 0.437     | 0.11      | -0.08    | -0.052    | 0.238     | 0.124     | 0.117     | -0.008    | -0.056    | 0.047     | 0.023     | -0.003    | -0.037    | 0.299     | 0.29      | 0.021     | -0.09     | 0.138     | -0.052    | -0.11     | 0.104     |
|            | 0.206    | 0.437          | 0.104     | 0.256     | 0.26     | 0.29      | 9.35E-07  | 1.17E-02  | 0.878     | 0.785     | 0.34      | 0.633     | 0.947     | 0.455     | 4.83E-10  | 6.64E-02  | 0.677     | 6.69E-03  | 7.81E-03  | 2.47E-06  | 2.51E-02  | 5.01E-03  |
|            | -0.071   | -0.064         | 0.019     | 0.219     | -0.103   | -0.076    | 9.35E-07  | 1.17E-02  | -0.031    | -0.031    | 0.032     | 0.036     | 0.022     | 0.058     | 4.83E-10  | 6.64E-02  | 0.01      | 6.69E-03  | 7.81E-03  | 2.47E-06  | 2.51E-02  | 5.01E-03  |
|            | 0.17     | 0.21           | 0.019     | 0.165     | 4.49E-02 | 4.05E-02  | 1.65E-05  | 4.66E-02 |
|            | 0.274    | 0.274          | 4.49E-02  | 4.05E-02  |
|            | 6.71E-02 | 7.65E-02       | 4.05E-02  | 4.05E-02  |
|            | 1.05E-02 | 1.05E-02       | 1.05E-02  | 1.05E-02  |
|            | 0.121    | 0.121          | 0.121     | 0.121     | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    | 0.121    |
| T cell exhaustion | PD1(PDCD1) | -0.088 | 7.17E-02 | -0.102 | 4.62E-02 |
|-------------------|------------|--------|----------|--------|----------|
| CTLA4             | -0.106     | 3.02E-02 | -0.122 | 1.74E-02 |
| LAG3              | -0.151     | 2.09E-03 | -0.161 | 1.69E-03 |
| TIM3(HAVCR2)      | -0.093     | 5.84E-02 | -0.109 | 3.37E-02 |
| GZMB              | -0.228     | 2.57E-06 | -0.244 | 1.57E-06 |