Good prognosis in patients with gastric adenocarcinoma correlates with IgA+ Tertiary Lymphoid Structures

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Research

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Abstract

The roles of tumor infiltrating B lymphocytes (TIBs) and tertiary lymphoid structures (TLSs) in solid tumor genesis and anti-tumor therapy have been recognized by researchers, but the specific formation and effect of TLSs have not been fully understood. In this study, single-cell RNA sequencing, multiple immunofluorescence assays, and cellular spatial organization mapper analysis were used to explore the structures of TLSs in gastric adenocarcinoma (GADC). Furthermore, the relationships between TLSs and clinicopathological characteristics and prognosis of GADC patients were analyzed. The results identified the phenotype of immunoglobulin A (IgA)⁺-TLSs which contained higher level of IgA⁺ plasma cells in GADC, and several types of immune cells in IgA⁺-TLSs had higher levels of cellular interactions and migration ability. Furthermore, IgA⁺-TLSs correlated with age, differentiation, distant metastasis, TNM stage, expression of programmed death-ligand 1, J-chain, SSR4, and good overall survival. In conclusion, IgA⁺ plasma cells higher expressed in IgA⁺-TLSs, and the formation of IgA⁺-TLSs promotes a better prognosis for GADC patients.

Introduction

Gastric adenocarcinoma (GADC) is a kind of gastric cancer (GC), which is caused by malignant changes of gastric gland cells, and the incidence rate accounts for 90% of gastric malignant tumors [1]. The growth, invasion and metastasis of GADC are complex and dynamic process, which are involved in the genetic abnormalities inherent in the tumor tissue and tumor-immune cell interactions in the local microenvironment [2-4]. Moreover, B cell mediated mucosal immunity and humoral immunity also plays an important role in gastrointestinal tumors [2,5]. Immunoglobulin A (IgA) is the main force of mucosal immunity against infection in the body. IgA induces B and T cell responses and recruits them to various effector sites, induces the differentiation of B cells into plasma cells that can continue to secrete IgA and play a mucosal protective role [6, 7].

The tumor microenvironment (TME) of GADC is a dynamic network and is a key factor affecting the metastasis and promotion or inhibition of immune response against the tumor cells [8]. Infiltrating immune cells in the TME, also known as tumor-infiltrating lymphocytes (TILs), mediated the anti-tumor response [9, 10]. In the gastric TME, there is bidirectional regulation between tumor cells and immune cells. The interaction between the two types of cells can disrupt homeostasis in the body, mobilize internal and external resources of cells, produce TMEs suitable for tumor cell growth, and affect the response of GC cells to GC immunotherapy [2].

The TME contains not only TILs but also tertiary lymphoid structures (TLSs) [11, 12]. TLSs, also known as ectopic lymphoid tissue, is a new type of lymphoid tissue found in recent years in areas that are stimulated by certain autoimmune diseases, graft rejection, and chronic inflammatory responses. Its structure and function are very similar to secondary lymphatic organs such as lymph nodes and spleen. TLSs can be seen in both primary and metastatic tumors [13], and mainly composed of T and B lymphocytes, follicular dendritic cells (DC), and mature DC [14], which can penetrate into the “hinterland

of the enemy” much like a “Trojan horse” and effectively inhibit tumor growth and inflammation [15]. Furthermore, the density of TLSs and the ratio of TLSs to tumor area, as well as the number of tumor-infiltrating B lymphocytes (TIBs) were reported to be higher in patients who were sensitive to immune check point (ICI) treatment than in ICI-insensitive patients [15].

In our previous study, we collected 4 cases of fresh GADC tissue samples and found the potential importance of immunoglobulin A (IgA) - mediated humoral immunity in GADC patients with TLSs formation through single-cell RNA sequencing (scRNA-SEQ) [15]. To further explore the formation of IgA⁺-TLSs in GADC, we conducted gene ontology (GO) and cellular spatial organization mapper (CSOmap) analysis on the CD79A⁺/J-chain⁺ cell cluster. Next, we collected 24 cases of GADC with TLSs to compare structures and densities of tumor-associated TLSs using multiple immunofluorescence assays. We also analyzed the relationships between IgA⁺-TLSs formation and clinicopathological characteristics and prognosis in patients with GADC through 165 cases of TMA analysis. Our research provided the information about CD79A⁺/J-chain⁺ B cells in GADC and indicated that IgA⁺-TLSs was associated with good prognosis for GADC patients.

Materials And Methods

2.1 Sample Collection

All sample collection was approved by the Human Research Ethic Committee of Bayannur Hospital and Nanjing First Hospital. scRNA-SEQ data came from the previous research data of our team [16]. All paraffin tissue blocks (including 24 cases samples in multiple immunofluorescence and 165 cases samples in immunohistochemistry assay) were surgical specimens, and received from the department of pathology at the Bayannur Hospital and Nanjing First Hospital between Jan. 2018 to Dec. 2020, and preparation onto tissue microarray (all samples from 120 GADC patients). The formation of germinal center structures is determined by two experienced pathologists. An effective TLS is defined as an area of ectopic lymphocyte aggregation greater than a 200 × visual field (1 mm in diameter). Two distinct regions of TLSs are observed under the microscope: the bright region and dark region. Clinical pathology information includes gender, age, differentiation, Tumor Node Metastasis (TNM) stage, chemotherapy effect and TLSs location. TNM stage standard according to American Joint Committen on Cancer (AJCC) Cancer Staging Manual Eighth Edition [17]. We divided the locations of TLSs into tumor center, para-cancerous tissues and the junction between tumor (T) and normal (N) tissues (T/N junction). Overall survival (OS) period was defined from initial diagnosis to death. Matched normal tissue was obtained from sites displaced at least 5 centimeters from the tumor and was confirmed to lack tumor cells on histopathology review. All patients did not receive any other therapy before surgery, more details about samples please see Table 1.

2.2 Analysis of CD79A⁺/J-chain⁺ B Cell Cluster Subgrouping in 10 × Single Cell Transcriptome sequencing

High-throughput sequencing of the library was performed using Illumina sequencing platform's double-terminal sequencing mode. Cell Ranger, 10×Genomics' official analysis software (<https://support.10xgenomics.com/single-cell-gene-expression/software/overview/welcome>), was used to filter, compare, quantify, and identify the recovered cells. Finally, the gene expression matrix of each cell was obtained. Using the Seurat (<https://satijalab.org/seurat/>) for cell filtering, standardization, cell subgroup classification, differentially gene analysis and marker gene screening. Cell Ranger software was used to conduct data quality statistics for the original data and compare the reference genome.

For clustering and dimension reduction analysis, t-distributed stochastic neighbor embedding (t-SNE) was used in the algorithm, resolution was set at 0.5, and harmony was used to remove the batch effect. A total of 14,660 cells were retained from 54,167 cells after screened through CD79A and J-chain marker (Supplementary Table 1-2). Bimod algorithm was adopted in the difference analysis between different clusters, and p value is set as < 0.01 , the filter condition $\log_2FC \geq 0.26$ [17].

2.3 TISCH database and UCSC Xena database

Tumor Immune Single-cell Hub (TISCH) (<http://tisch.compgenomics.org/home/>) is a scRNA-sequencing database focused on the TME. One scRNA-sequencing data from GADC patients (STAD_GSE134520) in this study was selected from the database [18].

University of California Santa Cruz (UCSC) Genome Browser (<https://xenabrowser.net/datapages/>) is a landmark work of genome sequencing data visualization. It is a new generation of data analysis and visualization platform integrating analysis, visualization and Galaxy. 1098 common datasets with 91 queues including TCGA, ICGC, TARGET, GTEx and CCLE have been standardized [19].

2.4 GO Analysis

The GO is an internationally standardized functional classification system for genes. It provides a dynamically updated standard vocabulary to comprehensively describe the properties of genes and gene products in an organism. The basic unit of GO is "term", and each term corresponds to an attribute. Firstly, all the significantly differential expressed genes were mapped to the terms of the GO database, and the number of genes in each term was calculated. Then, hypergeometric test was applied to find out the GO items that were significantly enriched in the significantly differential expressed genes compared with the whole genome background [20, 21]. GGPLOT2 was used to carry out enrichment analysis on the GO results and scatter plot display: Rich factor represents the number of differential genes located in the GO/the total number of genes located in the GO. The greater the Rich factor, the higher the GO enrichment degree [22].

2.5 Cellular Spatial Organization Mapper (CSOmap)

We analyzed 38,283 cells from 4 cases tumor samples in our previous study to reconstruct the cell spatial organization. The ligand-receptor interaction database in CSOmap is based on FANTOM5 (<https://>

phantom. gsc. riken. Jp / 5 / suppl / Ramilowski_et_al_2015/) for estimating the cell-cell affinity matrix [23,24]. Function Annotation Of The Mammalian Genome (FANTOM) 5 compiled putative ligands from known interacting ligands, orphan Human Plasma Membrane Receptome (HPMR) ligands and from a set of secreted proteins that were not found in the set of known receptors. FANTOM5 compiled putative receptors from known interacting receptors, orphan HPMR receptors and from a set of PM proteins that were not found in the set of known ligands. And then FANTOM5 obtained predicted ligand–receptor pairs by searching for validated protein-protein interactions (Human Protein Reference Database and STRING32 databases) between putative ligands and putative receptors.

2.6 Immunohistochemistry, Multiple Immunofluorescence Assay

Tissue paraffin sections were incubated in the oven at 65°C for 1h, dewaxed by xylene for 3 times, gradient alcohol hydration was performed, and antigens were retrieved by microwave. Sections were pretreated and stained with monoclonal antibodies directed against CD79A (SP18, Abcam), J-chain (OTI2B1, Invitrogen), CD4 (EPR6855, Abcam), SSR4 (11655-2-AP, Proteintech), CD8 (SP16, Abcam), CD14 (SP192, Abcam) and PD-L1 (28-8, Dako), DAPI (D3571, Thermo Fisher Scientific) on a Leica-Bond III/max autostainer platform (Leica Biosystems), and used PBS to replace the primary antibodies as negative control (Supplementary Fig. S1). Incubated overnight at room temperature (RT), added biotin-labeled secondary antibody at RT for 1h, and adding horseradish peroxidase labeled streptomycin opaletin working solution. Incubated at 37°C for 30min, and using anti-immunoglobulin coupled horseradish peroxidase with 3,3'-diaminobenzidine (DAB, OptiView Kit, Roche Diagnostics) as substrate. Nuclear counterstaining was performed with Mayer hematoxylin. The degree of J-chain expression in tissues were scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. The frequency of positive cells was defined as follows: 0 < 5%, 1, 5% - 25%; 2, 26% - 50%; 3, 51% - 75%; and 4, greater than 75%. Multiply the two scores, and for statistical analysis, score of 0 - 7 were considered low or no expression and score of 8 to 12 considered high expression. PD-L1 positive defined as $\geq 5\%$.

Lymphocyte regions on tissue images were manually circled, the number and proportion of positive cells labeled by antibodies in the whole image and lymphocyte cells region were quantitatively analyzed [25].

2.7 Statistical Analysis

All data were analyzed by SPSS 23.0 software. Survival curves were generated by the Kaplan-Meier method. The results of Kaplan-Meier method is displayed with HR and *P* or Cox *P*-values from a log-rank test. Pearson χ^2 test was used to compare the relationship between the expression levels of gene and clinicopathological parameters. The independent paired *t* test was used to compare the mean values of the two groups. *P* < 0.05 is considered a significant difference.

Results

3.1 The information of CD79A⁺/J-chain⁺ B cells in GADC Tissue Samples

A larger number of B cells, in which CD79A and J-chain were highly expressed, were been observed in four GADC patients though scRNA-SEQ. After clustering and dimension reduction analysis, 14,660 CD79A⁺/J-chain⁺ cells were re-clustered into 27 separate subsets. These cells were analyzed through t-SNE algorithm (Fig. 1A). The distribution of CD79A⁺/J-chain⁺ B cells in tumor tissue samples is shown in the Figure 1B. Therefore, we selected CD79A⁺/J-chain⁺ B cells cluster from tissue samples, and found the part of B cells higher expressed immunoglobulin heavy chain alpha (IGH) A1, IGHA2 and signal receptor subunit 4 (SSR4) genes (Fig. 1C); meanwhile, we also found that SSR4 is expressed in several types of immune cells, especially plasma cells through TISCH database (STAD_GSE134520) (Supplementary Fig. S2). We further analyzed the correlation between CD79A, JCHAIN, and SSR4 through transcriptome data of gastric adenocarcinoma from TCGA database (UCSC Xena, [HTSeq - Counts](#) GDC Hub, n=407), and found that SSR4 was highly correlated with JCHAIN and CD79A (Fig. 1D). The study performed GO enrichment analysis, and found that the main functions of these cells were related to secretory immunoglobulin complex and monomeric IgA immunoglobulin complexes, and positive regulation of B cell activation, and antibacterial humoral response (Fig. 1E).

3.2 Different Structures and Densities of Tumor Associated TLSs

To reconstruct the cell spatial organization, CSOmap was used to analyze the dominant ligand-receptor pairs that contributed to the distribution of immune cells in the tumor tissue. The results showed that T1 and T2 samples, which contained higher number of CD79A⁺/J-chain⁺ B cells, had a greater number of cells and different types of cellular interactions and migration. The dominant ligand-receptor pairs ($P < 0.01$) included CXCL13-CXCR3 for follicular DC and B cell interactions, CCL4-CCR8 for T regulatory cells and mononuclear macrophages, and HLA-E-KLRC1 for B cells and natural killer T cells (Fig. 2A).

The study further collected 24 cases of paraffin tissue blocks, and each samples with TLSs. Twelve of these tissues contained TLSs that formed germinal centers (bright region and dark region). Multiple immunofluorescence assays detected the morphology and structure of the TLSs in these tissues. We selected anti-CD79A antibody as a marker for B cells, anti-CD4 antibody for helper T (Th) cells, anti-CD8 antibody for CD8⁺ T cells, anti-CD14 antibody for mononuclear macrophages. In addition, the expression of SSR4 in the immune cells was explored. The results indicated that there were different types of TLSs in GC tissues. The CD79A⁺B cells cluster together to form a region and are closely linked to the CD4⁺ Th cell region (Fig. 2B-C). We found that CD79A⁺B cells was concentrated in the center of TLSs containing germinal centers (mTLSs), and the cell density was much higher than that of TLSs without germinal centers (nTLSs) ($P < 0.001$) (Fig. 2D).

Through OSCmap analysis and multiple immunofluorescence assays, the results indicated that TLSs with germinal centers have more type of immune cells and higher density of cells than those in normal TLSs.

3.3 The information and the Correlation of IgA⁺-TLSs with Clinicopathological Characteristics in Patients with GADC

To acquire more information about IgA⁺-TLSs, the following studies were carried out to correlation between IgA⁺-TLSs in GADC and clinicopathological parameters. We collected 165 samples from 120 GADC patients that contained TLSs and analyzed protein expression using tissue microarray. The formation of TLSs was detected by determining the location of CD79A protein and the expression of J-chain at each sample point. We defined IgA⁺-TLSs as CD79A expressed in the core of the TLSs and simultaneously detected the expression of J-chain. We also detected the expression levels of PD-L1 and SSR4 proteins, and the relationship with clinicopathological information was performed (Fig. 3A).

By using IHC, we found that the formation of IgA⁺-TLSs, as determined by CD79A expression in the core of TLSs, was present in 58/165 (35.15%) of GADC tissue. Furthermore, we found the expression of IgA⁺-TLSs was correlated with age ($\chi^2 = 17.442$, $P < 0.001$), differentiation ($\chi^2 = 6.253$, $P = 0.014$), distant metastasis ($\chi^2 = 4.425$, $P = 0.049$), TNM stage ($\chi^2 = 9.359$, $P = 0.025$), PD-L1 expression ($\chi^2 = 4.953$, $P = 0.034$), and SSR4 expression ($\chi^2 = 65.653$, $P < 0.001$); however, no significant relationship was found for gender, tumor size, lymph nodes, or TLS location (Table 1).

3.4 IgA⁺-TLSs was Associated with Good Prognosis in GADC Patients

To determine the relationship between IgA⁺-TLSs formation and OS of GADC patients, Kaplan-Meier survival curve analyses were performed and it was suggested that IgA⁺-TLSs formation was significantly associated with a better prognosis for GADC patients (log rank = 13.604, $P < 0.001$) (Fig. 3B).

In univariate analysis, IgA⁺-TLSs formation was associated with a better OS period (HR, 2.065; 95% CI, 1.380–3.091; $P < 0.001$), and other prognostic markers, including distance metastasis (HR, 2.702; 95% CI, 1.181–6.182; $P = 0.019$), TNM stage (HR, 1.972; 95% CI, 1.316–2.955; $P = 0.001$), chemotherapy effect (HR, 4.166; 95% CI, 2.088–8.312; $P < 0.001$), TLSs location (HR, 1.57; 95% CI, 1.014–2.434; $P = 0.044$). In multivariate analysis, the formation of IgA⁺-TLSs (HR, 2.004; 95% CI, 1.261–3.186; $P = 0.003$), TNM stage (HR, 2.651; 95% CI, 1.614–4.356; $P < 0.001$), chemotherapy effect (HR, 4.325; 95% CI, 1.867–10.021; $P = 0.001$), and TLSs location (HR, 1.775; 95% CI, 1.060–2.973; $P = 0.029$) were associated with better OS period (Fig. 4). These results indicated that IgA⁺-TLSs predicts a better treatment outcome and a longer survival prognosis for GADC patients.

Discussion

In recent years, it has been suggested that the TIBs and TLSs may regulate the efficacy of immunotherapy in several types of tumors, and may closely related to the prognosis of immunotherapy [26-27]. Therefore, these possibilities open a new immunotherapy research direction. The roles of TIBs and TLSs in tumorigenesis and immunotherapy have been recognized by researchers, but the specific regulating effect of TIBs and TLSs are not fully understood [28, 29].

In our study, the results showed that a large number of B cells in GADC tumor tissues expressed CD79A/J-chain gene, and most of these cells were IgA⁺ plasma cells (high expression of IGHA1 and IGA2

genes). Through GO enrichment analysis, the study also found CD79A⁺/J-chain⁺ B cells have complement activation, B cells activation and antibacterial humoral response. These results confirmed that CD79A⁺/J-chain⁺ B cells were mainly IgA⁺ plasma cells, and were involved in mucosal and humoral immunity.

IgA produced in the gastrointestinal mucosa is secretory IgA (SIgA), which is mainly composed of dimers [30, 31]. The two monomers are linked by a J-chain protein, forming an important immune protective layer on the mucosal surface. The J-chain is a glycoprotein with a molecular weight of approximately 15 kD and is mainly synthesized by IgA or IgM plasma cells and connects two monomeric units of IgA or IgM [26]. Although J-chain can polymerize IgA and IgM, IgM mainly exists in blood, and SIgA plays an important role in mucosal immunity of the body. Based on this, it is reasonable to speculate that CD79A/J-chain B cells play a role in mucosal immunity.

In addition to IgA related genes, we also found SSR4 gene was also highly expressed in CD79A⁺/J-chain⁺ B cells, and SSR4 was highly correlated with JCHAIN and CD79A. The SSR4 gene encodes the translocon-associated protein δ (TRAP δ) subunit. The TRAP complex comprises four transmembrane subunits (α , β , γ and δ), which exist in the endoplasmic reticulum (ER) and are involved in protein transport across the ER membrane. Studies suggest that the TRAP complex is involved in the regulation of humoral immunity by guiding the secretion and transport of immunoglobulins [32]. It is reasonable to speculate that the immune cells with high expression of SSR4 may be related to the performance of humoral immunity in human body, and further studies are needed to confirm it.

Next, we determined the spatial structure of single cells through CSOmap, and found that T1 and T2 samples contained more cells and different types of cellular interactions and migration ability compared with cells in T3 and T4 tissues. Moreover, we observed increased expression of receptors and ligands associated with chemokines, and these data suggests that the immune cells in T1 and T2 samples may have a stronger ability to migrate. Denton et al., confirmed that CXCL13 can recruit immune cells to generate an environment permissive for germinal center formation in the lung [33]. It supports our results that the TLSs in T1-2 tumor tissue form germinal nodal structure.

The germinal center is located in the follicular region of secondary lymphatic organs. It is a special structure formed by the aggregation of B cells in the humoral immune response, and it is also a transient and dynamic micro-anatomical structure [33]. Antigen-specific B cells gather in this particular structure, and then they expand to produce somatic high frequency mutations, antibody type changes and eventually become high affinity B cells for specific antigens [34].

In order to obtain more information about TLSs, we continued to collect 24 cases of GADCs tumor samples with or without germinal centers. We compared the structural differences by multiple immunofluorescence and digital quantitative analysis techniques. And found that CD79A⁺B cells was concentrated in the center of TLSs containing germinal centers, and the cell density was much higher than that of TLSs without germinal centers ($P < 0.001$). These results also support the analysis results of

CSOmap prediction model and indicated that TLSs with germinal centers have more type of immune cells and higher density of cells than those in normal TLSs.

B cells are a type of lymphocytes, which enters the peripheral lymphoid tissue after maturation of the fetal liver and bone marrow mature [35]. Subsequently, B cells proliferate and mutate in germinal centers, differentiate into memory B cells and plasma cells, and secrete antibodies to induce humoral immunity and mediate cellular immunity. The germinal center is the site of clonal expansion and affinity maturation of B cells [34,36]. TIBs exists in some tumor tissues and are also an important part of TLSs. At present, there is still some controversy about the role of TIBs in anti-tumor immunity. Some studies showed that some immunosuppressive TIBs subtypes promoted tumor progression [35]; another study suggested that TIBs promotes tumor immunity and inhibits the growth of tumor cells by producing tumor-specific antibodies and presenting tumor antigens [27].

To understand more about the role of TIBs and TLSs in GADC, we collected 165 tissue samples from 120 patients with GADC who developed TLSs, and each case included complete clinicopathological information, treatment and prognosis information. IHC analyzed results indicated that the formation of IgA⁺-TLSs was correlated with age, differentiation, distant metastasis, TNM stage, PD-L1 expression, and SSR4 expression. Moreover, the presence of IgA⁺-TLS in GADC patients was associated with better OS period.

Conclusions

In conclusion, our work provided the newly insight about TLSs and found that IgA⁺-TLSs may indicate a good prognosis in GADC patients. Future studies will be required to confirm whether the presence of SSR4 in the TLS is predictive of an IgA-immune response and promotes an improved prognosis in patients with GADC.

Declarations

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Author contributions

WZ supervised the research, designed experiments, and revise the manuscript. LJ and TW performed experiments and wrote the paper; SZ and KF modified the article and performed molecular experiments; YZ contributed to clinical sample and data preparation. All authors contributed to the article and approved the submitted version.

Data accessibility

The raw single cell RNA-seq data analysed in this study is available in Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE188895; Other data that support the findings of this study are available from the corresponding author upon request.

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Tables

Table 1 Associations between IgA⁺-TLSs formation with clinicopathologic characteristics in GADC patients.

Characteristics	n	IgA ⁺ -TLSs n (%)	nTLSs n (%)	P	χ^2
Total	165	58 (35.15)	107 (64.85)		
Gender				0.108	0.853
male	122	42 (34.43)	80 (65.57)		
Female	43	16 (37.21)	27 (62.79)		
Age				17.442	< 0.001*
≤60	54	31 (57.41)	23 (42.59)		
≥60	111	27 (24.32)	84 (66.67)		
Differentiation				6.253	0.014*
well-middle	75	34 (45.33)	41 (54.67)		
middle-poor	90	24 (26.67)	66 (73.33)		
Tumor size				2.241	0.524
T ₁	23	8 (34.78)	15 (65.22)		
T ₂	4	0 (00.00)	4 (100.00)		
T ₃	113	41 (36.28)	72 (63.72)		
T ₄	25	9 (36.00)	16 (64.00)		
Lymph nodes				4.022	0.17
N ₀	33	10 (30.30)	23 (69.70)		
N ₁	43	11 (25.58)	32 (74.42)		
N ₂	35	12 (34.29)	23 (65.71)		
N ₃₋₄	54	25 (46.30)	29 (53.70)		
Distant metastasis				4.425	0.049*
M ₀	137	53 (38.69)	84 (61.31)		
M ₁	28	5 (17.86)	23 (82.14)		
TNM stage				9.359	0.025*
I	17	7 (41.18)	10 (58.82)		
II	50	13 (26.00)	37 (74.00)		
III	68	32 (47.06)	36 (52.94)		
IV	30	6 (20.00)	24 (80.00)		
TLSs location					
tumor center	98	34 (34.69)	64 (65.31)	0.031	0.985
para-carcinoma tissue	34	12 (35.29)	22 (64.71)		
T/N junction	33	12 (36.36)	21 (63.64)		
PD-L1 expression				4.953	0.034*
≥5%	83	36 (43.37)	47 (56.63)		
< 5%	82	22 (26.83)	60 (73.17)		

SSR4 expression	65.653 < 0.001*		
high	90	56 (62.22)	34 (37.78)
Low or no	75	2 (2.67)	73 (97.33)

* means $P < 0.05$.

Abbreviations

CSOmap: cellular spatial organization mapper; IGH: immunoglobulin heavy chain alpha; GADC: gastric adenocarcinoma; GEPIA: gene expression profiling interactive analysis; GO: gene ontology; HPMR: human plasma membrane receptome; ICI: immune checkpoint inhibitor; scRNA-SEQ: single-cell sequencing; SSR4: signal sequence receptor subunit 4; t-SNE: t-distributed stochastic neighbor embedding; TIBs: infiltrating B lymphocytes; TLSs: tertiary lymphoid structures; TAM: tumor-associated macrophage; TIMER: tumor immune estimation resource; TME: tumor microenvironment.

Figures

Figure 1

The information of CD79A/J-chain B cells in GADC tissue sample. (A) t-distributed stochastic neighbor embedding (tSNE) of the 14,660 CD79A/J-chain-B cells profiled here, with each cell color-coded for the associated cell subsets. (B) The distribution of 14,660 CD79A/J-chain-B cells in tumor tissue samples. (C) Ridge plot of the expression level of IgA1, IgA2 and SSR4 genes in CD79A/J chain-B cell cluster. (D) Correlation analysis between CD79A, JCHAIN, and SSR4 through TCGA database (UCSC Xena, [HTSeq - Counts](#) GDC Hub, n=407). (E) GGLOT2 of differential gene expression in CD79A/J chain-B cell cluster

through GO enrichment analysis. The Rich Factor represents the number of differential genes/the total number of genes.

Figure 2

Different structures and densities of tumor associated TLSs. (A) Parts of whole showing the dominant ligand-receptor pairs contributing to distribution of single cells in tumor tissue through CSOmap analysis ($P < 0.01$). Representative multiple immunofluorescence assay images of CD4, CD8, CD14, CD79A, and SSR4 in TLSs with germinal center (B) or not (C). 100 × magnification. (D) Analyzed the number of CD79A⁺, CD4⁺, SSR4⁺, CD14⁺, CD8⁺, and total cells in mature TLSs with germinal center (mTLSs) and normal TLSs (nTLSs). The blue circles represent the manually analysis areas, ** $P < 0.001$.

Figure 3

Representative images of difference types of TLSs and survival analysis for GADC patients. (A) IgA⁺-TLSs and normal TLSs (nTLSs) with positive CD79A expression. IgA⁺-TLSs with positive SSR4 expression and nTLSs with low SSR4 expression. IgA⁺ TLSs with high J-chain expression and nTLSs with low or no J-chain expression. IgA⁺-TLSs with negative PD-L1 expression and nTLSs with positive PD-L1 expression. Line a is staining with×20 (bar = 200 μm), line b is staining with×200 (bar = 50 μm). (B) Overall survival (OS) curves for patients with the IgA⁺-TLS (black line) or the nTLSs (red line); Log Rank = 13.604, $P < 0.001$.

Figure 4

Univariate and multivariate analysis of prognostic markers for overall survival in GADC. The forest plot displays the hazard ratios (HR) and 95% confidence intervals (CI) of the characteristics by univariate and multivariate Cox regression analysis.