Infiltration Patterns of Immune Cells in Gastric Cancer and Their Clinical Correlation with Prognosis

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Research

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Abstract

Abstract: Objective Gastric cancer is a malignant tumour that severely affects the health of patients. This study analyses the correlation between gastric cancer-infiltrating immune cell patterns and clinical prognosis and provides a scientific basis for the development of comprehensive tumour prevention and treatment strategies. Method Transcripts and related clinical data from 9-2019 for gastric cancer were downloaded from the TCGA database. The proportions of 22 kinds of immune cells were calculated by CIBERSORT software, and the correlation of each immune cell component ratio with tumour grade, clinical stage and overall survival (OS) was evaluated. Results A total of 413 gene transcript data sets were obtained from the TCGA database, including 381 for gastric cancer and 32 for normal tissues. The expression of various macrophages in tumour tissues was abundant. The immune cell composition, which included resting dendritic cells (p=0.02), M1 macrophages (p=0.031), resting mast cells (p=0.02), CD8 T cells (p=2.445e-04), M0 macrophages (p=6.353e-04), activated mast cells (p=0.006), neutrophils (p=0.003), resting NK cells (p=0.014), and gamma delta T cells (p=0.033), is related to the pathological grade. As the tumour stage of gastric cancer patients progresses, the proportion of some immune cells, including eosinophils (p=0.013), activated mast cells (p=0.042), neutrophils (p=0.007), and resting NK cells (p=0.036) gradually increases, while the proportion of other immune cells, for example, CD8 T cells (p=0.018), Tregs (p=0.039), M1 macrophages (p=0.018), and activated NK cells (p=0.042) gradually decreases. Higher expression of CD8 T cells suggests a better prognosis. Conclusion The composition of tumour-infiltrating immune cells differed greatly in different pathological grades and stages of gastric cancer. CD8 T cells can be used as a prognostic factor for gastric cancer patients.

Introduction

Gastric cancer is the 5th most common cancer and the 3rd leading cause of tumour-related death worldwide. There are more than 1.03 million new cases of gastric cancer each year and more than 780000 deaths worldwide[1]. Despite the various treatment methods applied in recent years, such as surgery, chemotherapy, radiotherapy, targeted therapy and immunotherapy, the overall therapeutic effects are still not obvious[2, 3]. Biomarkers that assist in the selection of individualized treatment options are lacking. With the development of immunotherapy, an increasing number of researchers are focusing on the correlation between tumours and immunity. The distribution of tumour-infiltrating immune cells is associated with tumour growth, progression and prognosis. Immune cell infiltration in tumours may be used as a target for drug research[4].

Previous studies have mostly relied on flow cytometry or immunohistochemistry. However, these methods rely on the recognition of cell surface markers and may lead to cell loss or distortion of results. Researchers developed the new biological software CIBERSORT[5] in 2015; this software can calculate the immune cell composition of complex tissues based on gene expression profile data. This method enriched specific cell types and successfully evaluated the composition of immune cells in colorectal cancer, lung cancer, breast cancer and liver cancer tissues[6-8]. In our study, 413 samples of gastric cancer

patients were obtained from The Cancer Genome Atlas (TCGA). Perl, R software and CIBERSORT software were used to calculate the compositions of cells involved in the immune response in tumours. Then, we analysed the proportion of tumour-infiltrating immune cells between gastric cancer tissues and adjacent tissues, along with the accompanying clinical pathological data. We further analysed the correlation between these tumour-infiltrating immune cells and clinical factors, such as pathological grade, clinical TNM stage and overall survival (OS).

Materials And Methods

Database

The gene expression quantification files were obtained from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) in September 2019, including 381 files for gastric cancer tissues and 32 files for normal gastric tissues. The clinical data of 449 gastric cancer samples were also downloaded. The clinical characteristics included survival time and status, age, gender, tumour pathological grade, and clinical TNM stage. No special exclusion criteria were set for data download.

Assessment of the composition of immune cells in gastric cancer

In this study, RNA-seq transcription profiling of gastric cancer and normal stomach tissues was performed. The data were normalized by using the limma package in R software (version 3.6.1). The Perl programming language (https://www.perl.org/get.html) and CIBERSORT (https://cibersort.stanford.edu/) software were used to estimate the relative proportions of 22 tumour-infiltrating immune cells in each tissue. The twenty-two immune cell components included naive B cells, memory B cells, plasma cells, CD8 T cells, CD4 naive T cells, resting CD4 memory cells, activated CD4 memory T cells, follicular helper T cells, regulatory T cells (Tregs), gamma delta T cells, resting NK cells, activated NK cells, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, resting dendritic cells, activated dendritic cells, resting mast cells, activated eosinophils, and neutrophils. In the CIBERSORT calculation, samples were selected based on the filtering criterion P value<0.05 to ensure the accuracy of the inferred results. In each sample, the ratio of all tumour immune infiltration cells was equal to 1.

Association between tumour-infiltrating immune cells and clinical characteristics in gastric cancer patients.

Patients were divided into 2 groups according to the median level of tumour-infiltrating immune cells (high vs low). Their correlation with clinicopathological parameters was further explored.

Statistical analysis

All statistical analyses were performed using R software (version 3.6.1) and the Bioconductor software package (https://www.bioconductor.org/). The t-test was used to compare the different proportions of tumour-infiltrating immune cells between gastric cancer tissues and non-tumour tissues. The relationship between each tumour-infiltrating immune cell and clinicopathological parameter in gastric cancer

patients was evaluated by using one-way analysis of variance (ANOVA). Overall survival (OS) curves were tested by the K-M log-rank method. For all statistical analyses, $P<0.05$ was considered statistically significant. The K-M log-rank method was used to determine its correlation with overall survival, which was then graphed. For all statistical analyses, $P <0.05$ was considered statistically significant.

Results

Transcriptome profiling of the stomach

A total of 413 transcriptome profiling files were obtained from TCGA, including 381 gastric cancer tissue files and 32 non-tumour tissue files. When merging and normalizing all the transcription sample data, 60485 genes were detected in each sample, and 19660 mRNA expression profiles were extracted.

Distribution of tumour-infiltrating immune cells in gastric cancer

After applying the filter criterion $P<0.05$, the proportion data for 22 subtypes of tumour-infiltrating immune cells were obtained by using CIBERSORT software. A total of 179 patients (13 normal cases and 166 tumour cases) were included in the analysis. We drew a bar plot of the proportion of each immune cell in all samples (Figure 1). The types of immune cells that were most abundant in all the samples were resting CD4 memory T cells (15.5%), CD8 T cells (13.0%), M0 macrophages (11.6%), M2 macrophages M2 (11.4%), M1 macrophages (7.2%), and plasma cells (6.2%). The immune cells with low infiltration levels (<1%) were gamma delta T cells, monocytes, eosinophils, and naive CD4 T cells. The percentages of 22 tumour-infiltrating immune cells in gastric cancer and non-tumour tissues are shown using heatmaps (Figure 2) and violin plots (Figure 3). The distribution ratios of M0 macrophages, M1 macrophages and M2 macrophages were significantly higher in gastric cancer tissues than in the non-tumour tissues, while the proportion of plasma cells and monocytes was significantly lower. The correlation of 22 immune cells was further calculated (Figure 4). There were positive correlations between the following types of immune cells: activated mast cells vs activated CD4 memory T cells (0.49), M1 macrophages vs activated CD4 memory T cells (0.44), CD8 T cells vs activated CD4 memory T cells (0.42). In contrast, there were negative correlations between the following types of immune cells: CD8 T cells vs resting CD4 memory T cells (-0.62), activated CD4 memory T cells vs resting CD4 memory T cells (-0.6), CD8 T cells vs M0 macrophages (-0.48).

Clinical characteristics of gastric cancer patients

Similarly, we downloaded clinical data for 449 gastric cancer patients from TCGA. The clinicopathological characteristics, including age, gender, grade, and clinical stage, are shown in Table 1. Among the patients, 290 (64.6%) were male, and 159 (35.4%) were female. The age at the initial pathological diagnosis ranged from 30 years to 90 years, and 9 patients were of unknown age. The diagnosis of four pathological stages in these patients included stage I (59, 13.1%), stage II (131, 29.2%), stage III (184, 41%), and stage IV (44, 9.8%); 31 (6.9%) patients had an unknown stage. The diagnosis of

3 grades in these patients included G1 (12,2.7%), G2 (159,35.4%), and G3 (263,58.6%); 15 (3.3%) patients had an unknown grade.

Correlation between tumour-infiltrating immune cells and clinicopathological parameters in gastric cancer patients

The resting dendritic cell (p=0.02), M1 macrophage (p=0.031), resting mast cell (p=0.02), and CD8 T cells (p=2.445e-04) fractions gradually increased with cancer pathological grade (G1→G3), while the M0 macrophage (p=6.353e-04), activated mast cell (p=0.006), neutrophil (p=0.003), resting NK cell (p=0.014), and gamma delta T cell (p=0.033) fractions were significantly associated with the most advanced pathological grade (G3) (Figure 5).

There is also a certain correlation between the immunocyte fraction and clinical stage in gastric cancer patients. Cells with statistically significant differences (P <0.05) include CD8 T cells (p=0.018), Tregs (p=0.039), eosinophils (p=0.013), M1 macrophage (p=0.018), activated mast cells (p=0.042), neutrophils (p=0.007), activated NK cells (p=0.028), and resting NK cells (p=0.036). The activated mast cells, neutrophils, and eosinophils were present at significantly high levels in the T4 stage. Moreover, M1 macrophages, activated NK cells, CD8 T cells, and Tregs were present at significantly low levels in the T4 stage (Figure 6).

Correlation between tumour-infiltrating immune cells and overall survival in gastric cancer patients

We further explored the correlation between tumour-infiltrating immune cells and overall survival by K-M analysis. We found that a higher CD8 T cell fraction was associated with better OS in gastric cancer patients (Figure 7). There was no significant difference between the rest of the immune cells and the prognosis (P values were all >0.05) (Supplementary survival.xls).

Discussion

In this study, the Perl programming language, R and CIBERSORT software were used to calculate the distribution of tumour-infiltrating immune cells in gastric cancer. We found that there are some differences in tumour-infiltrating immune cells between gastric cancer tissues and non-tumour tissues. We further identified that some tumour-infiltrating immune cells were related to clinicopathological characteristics.

Cancer is a multi-factor, multi-step disease that slowly develops over a long period of time. The prognostic role of immune infiltration in the tumour microenvironment is associated with a landscape of spatial organization. Macrophages are located at the centre of the tumour. Other components of the cellular immune system, such as NK cells, mast cells and neutrophils, are mostly on the edge of the invasion. However, other types of immune cells, such as B-lymphocytes and dendritic cells, are mainly distributed in tumour-adjacent lymphoid tissues[9]. The location of these cells in the tumour

microenvironment (TME) is guided by vascular and lymphatic vessels and fibroblast matrix support. Therefore, the prognostic characteristics of the major immune cell types in the TME are different[10].

Tumour-associated macrophages (TAMs) are a type of plastic and heterogeneous cell population in the TME, accounting for the largest proportion of immune-infiltrating cells in most human solid tumour neoplasms (macrophages and metabolism in the tumour microenvironment). Generally, TAMs are classified into M1 macrophages (classic) and M2 macrophages (alternative)[11]. M1 macrophages express high levels of the pro-inflammatory cytokines IL-1, IL-6, IL-12, TNF and LPS. These cells have a high level of antigen presentation, thereby stimulating the Th1 response. Therefore, M1 macrophages are a key component of the antibacterial defence and are involved in acute tumour inflammation and antitumour immune function. Conversely, IL-4, IL-13, IL-10 and M-CSF/CSF-1 stimulate macrophage polarization towards an anti-inflammatory M2 state. M2 subgroup chemokines, including CCL2, CCL17 and CCL22, promote the recruitment of Tregs. Therefore, M2 macrophages have characteristics associated with the promotion of tumour development[12]. In the present study, we found that M1 macrophages abundantly infiltrated gastric cancer tissues, increasing gradually with pathological grade but decreasing with increasing clinical stage. The results imply that during tumour progression, the effect on prognosis not only depends on the location of macrophages (in the tumour core or in the stroma) but also may reflect the transition from the M1 phenotype to the M2 phenotype in the local distribution, which may change the tumour microenvironment and promote tumour progression[10]. Further attention is needed in future research.

We found that high levels of CD8 T cells infiltrated gastric cancer tissues, which was associated with an improved OS. CD8 T cells are also significantly associated with pathological grade. The composition fractions gradually decreased at the advanced clinical stage (T4). Previous studies have shown that the use of quantitative IHC identified CD8 T cell infiltration as an important prognostic factor for predicting markers of gastric cancer patients[13]. Endogenous CD8+ T tumour-infiltrating cells (CD8+ TILs) have been identified in a small number of patients with advanced gastric cancer[14]. These naturally occurring CD8+ TILs can specifically recognize cells derived from autologous tumours, providing a basis for developing immunotherapy for patients with advanced gastrointestinal malignancies.

The distribution of immune cells also varies by tumour type. In colorectal cancer, non-small-cell lung cancer, melanoma and head and neck tumours, all subsets of T cells are distributed in the tumour core and infiltrating margins. In colorectal cancer, the density of CD8+ T cells decreases with local invasion of the tumour (i.e., the density of immune infiltrating cells is lower at the T4 stage than at the T1 stage). In contrast, a primary tumour with high CD8+ T cell infiltration will have a reduced immune cell infiltration fraction after recurrence, especially in the core area[15]. High density of CD8+ T cells indicates prolonged overall survival in colorectal cancer patients with liver and/or lung metastases[16] but may also predict shortened overall survival in clear-cell renal carcinomas with liver and/or lung metastasis[17, 18]. These facts indicate that even in different individuals with the same cancer type, the immune microenvironment

is affected by changes in the density and location of immune cells between tumours. Different immune cell populations may have different roles in different tumours, thus affecting clinical outcomes.

Therapeutic interventions in the immune microenvironment are likely to lead to changes in tumours. Knowledge of the immune microenvironment or the corresponding molecular structure in tumour patients will enable clinicians to propose the most appropriate treatment. For example, in patients with melanoma treated with anti-PD-1, reactive tumours are characterized by pre-existing CD8+ T cells entering the tumour core from the tumour margins[19], indicating that immunological data such as immune cell type, density and location found in tumour samples can be good predictive markers[20].

B cells, which are part of the immune core network and are associated with prolonged survival, showed a dual effect on recurrence and tumour progression[21]. Different B cell subtypes can play different roles in the tumour microenvironment. B cells can regulate various effects of pro-inflammatory secretion, such as inhibition of CTL activity, perturbation of the Th1/Th2 CD4+ T cell lineage, and differential recruitment and activation of immune cells, ultimately promoting tumour progression[22]. B cells regulate the T cell response through antigen presentation and co-stimulation and cytokine production. B cells can mediate the proliferation of CD4+ T cells and influence the contraction of the CD4+ T cell immune response. B cells affect the development, expansion and survival of Tregs and enhance the T cell response effect, thereby helping to control tumour invasion and metastasis[21, 23]. However, no significant correlation between B cells and clinical features in gastric cancer was found in our study. Further research is needed.

However, there were some limitations in the present study. First, TCGA is a public database compiled by Western countries. The data included were mostly for white individuals, with relatively little data for Asian and black individuals; there are some differences associated with race and ethnicity. Second, the clinical characteristics included in this database are not comprehensive, which may lead to potential errors or bias.

Conclusions

In summary, we analysed the infiltration patterns of 22 infiltrating immune cell subgroups in gastric cancer samples by using CIBERSORT software based on the TCGA database. Furthermore, we identified that some tumour-infiltrating immune cells were associated with clinicopathological features. We determined that CD8 T cells can be prognostic markers for gastric cancer, providing a new perspective for personalized treatment options for cancer patients.

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:Not Applicable.

Authors' contributions

YanYan Li conceived and designed the study. Xiaoli Wu obtained the datasets and conducted data analysis. HongBo Ma made the figures and wrote the manuscript , YanYan Li revised the manuscript. All authors reviewed and approved the final manuscript.

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Table 1

Table 1 characteristics of gastric cancer samples

characteristics	Number of patients	percentage（%）
age		
<60	133	29.60%
≥60	307	68.40%
Unknown	9	2%
gender		
male	290	64.60%
female	159	35.40%
grade		
G1	12	2.70%
G2	159	35.40%
G3	263	58.60%
unknown	15	3.30%
Stage		
Stage I	59	13.10%
Stage II	131	29.20%
Stage III	184	41.00%
Stage IV	44	9.80%
unknown	31	6.90%

Figures

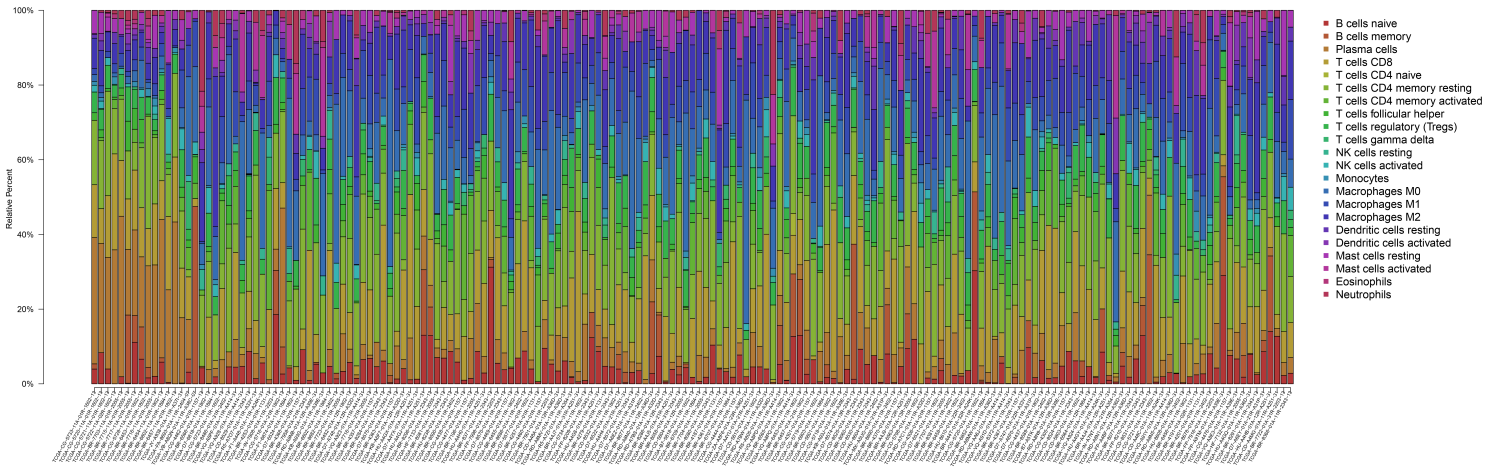

Figure 1

Bar plot of the proportions of 22 immune cells in all samples

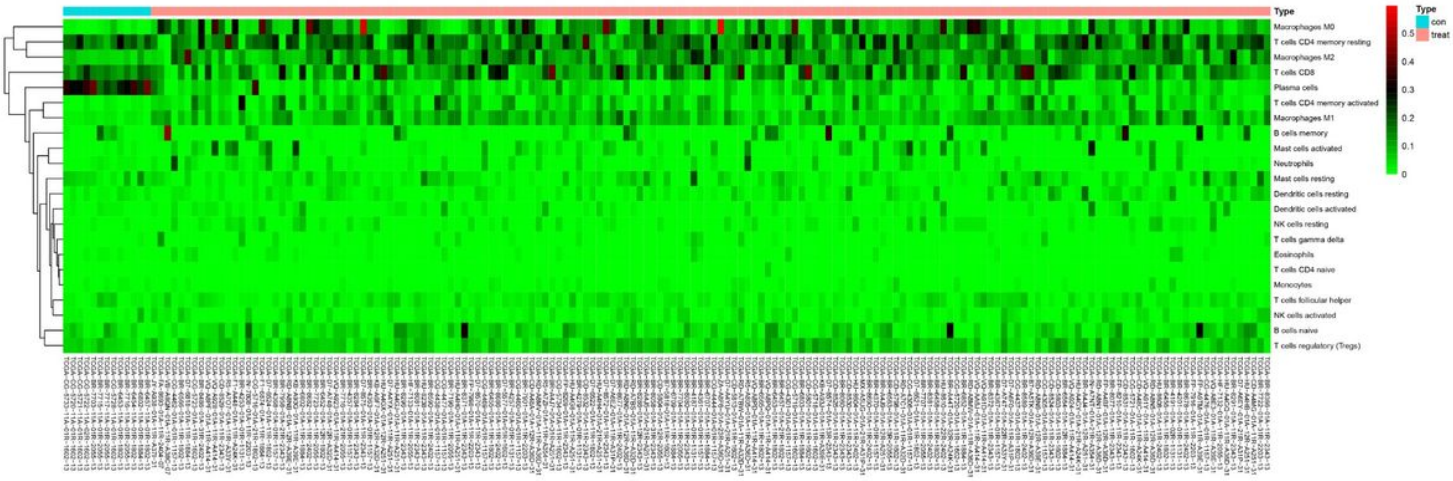

Figure 2

Relative immune cell proportions evaluated in gastric cancer samples

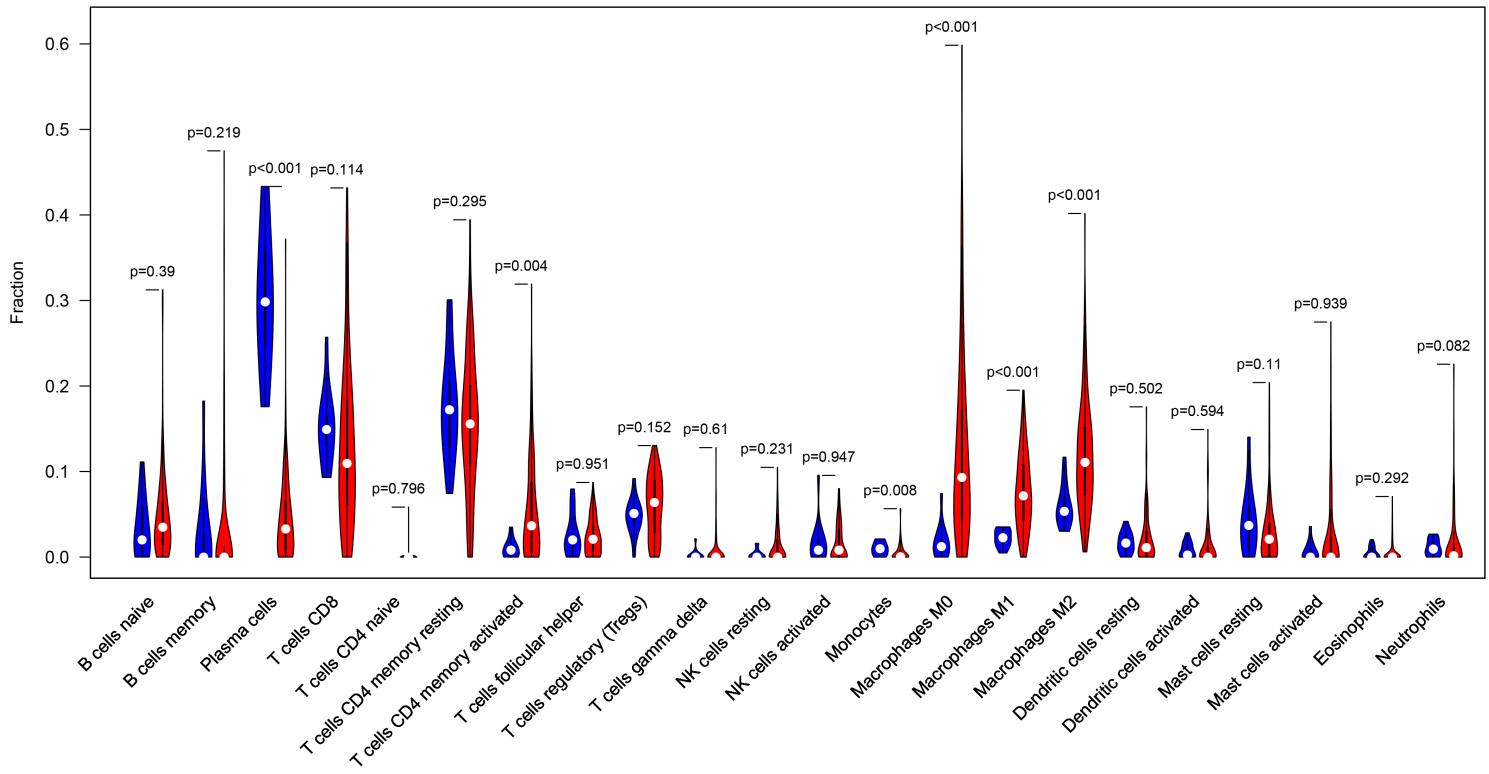

Figure 3

Violin plot of immune cells in gastric carcinoma and normal tissues

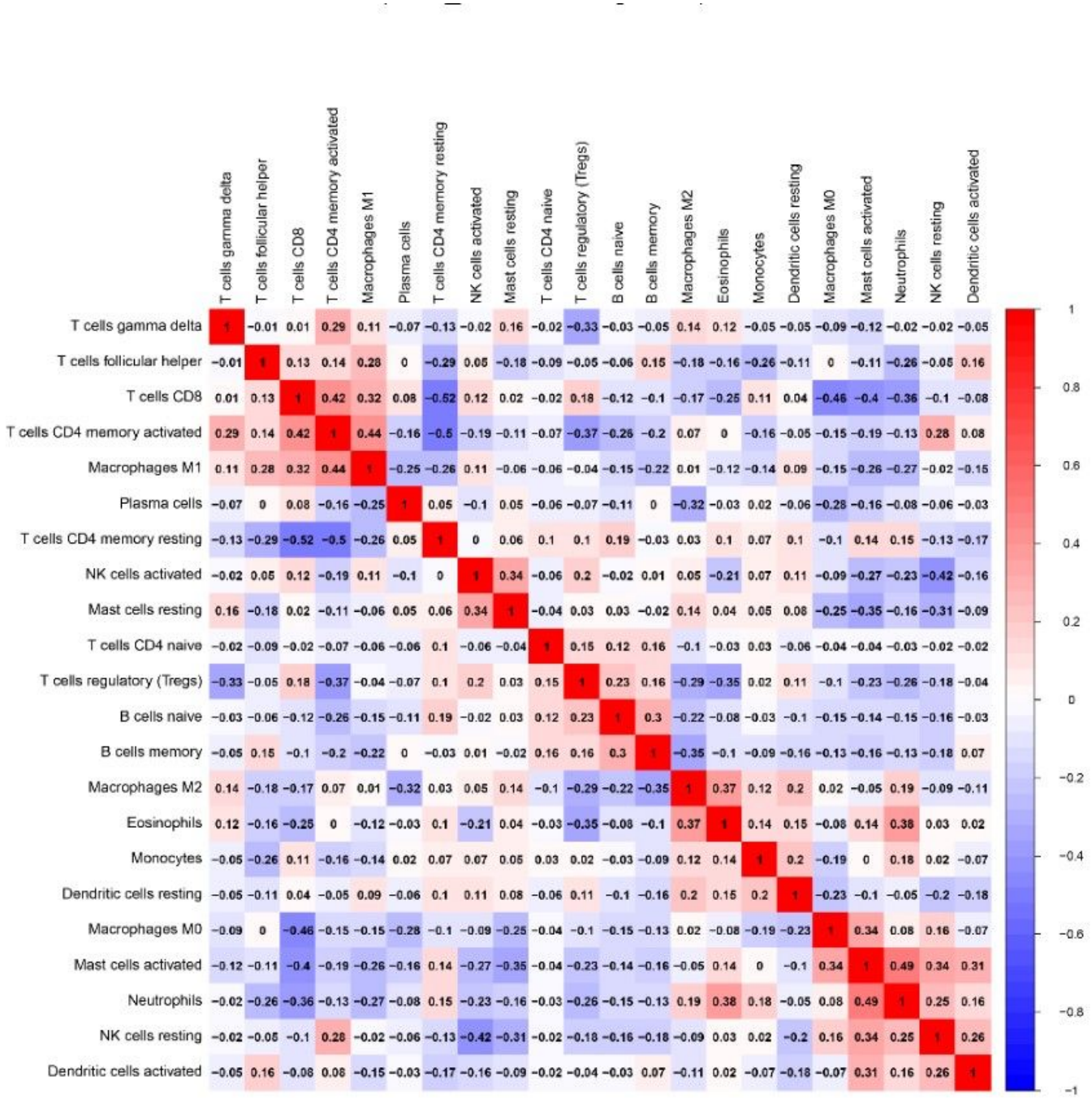

Figure 4

Correlation of the proportion of each immune cell in gastric carcinoma tissue samples

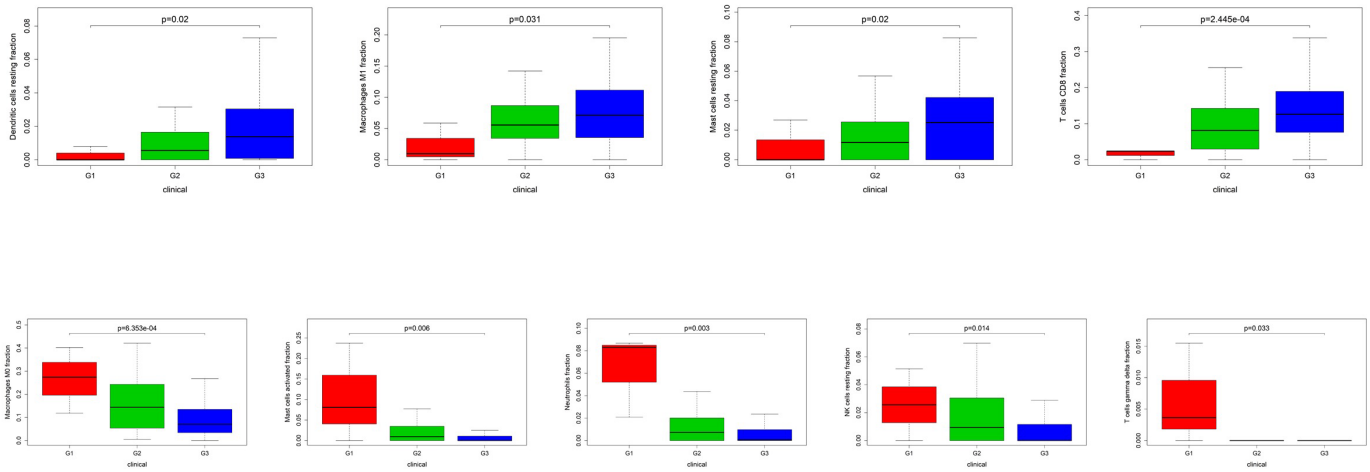

Figure 5

Correlation between immune cells and pathological grade in gastric cancer patients

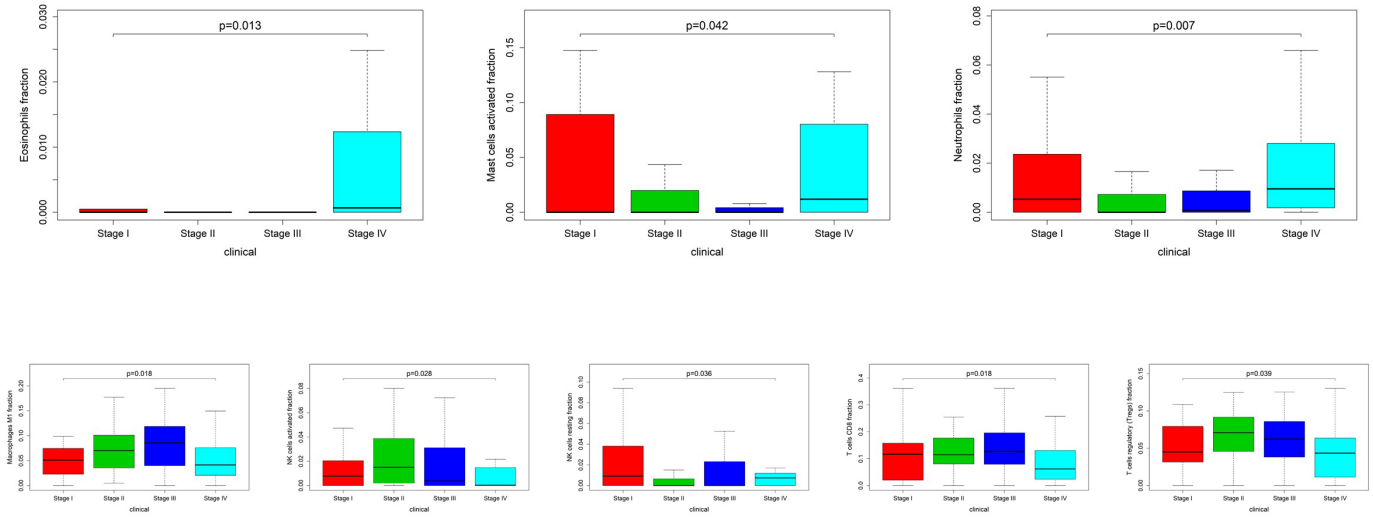

Figure 6

Correlation between immune cells and clinical stage in gastric cancer patients

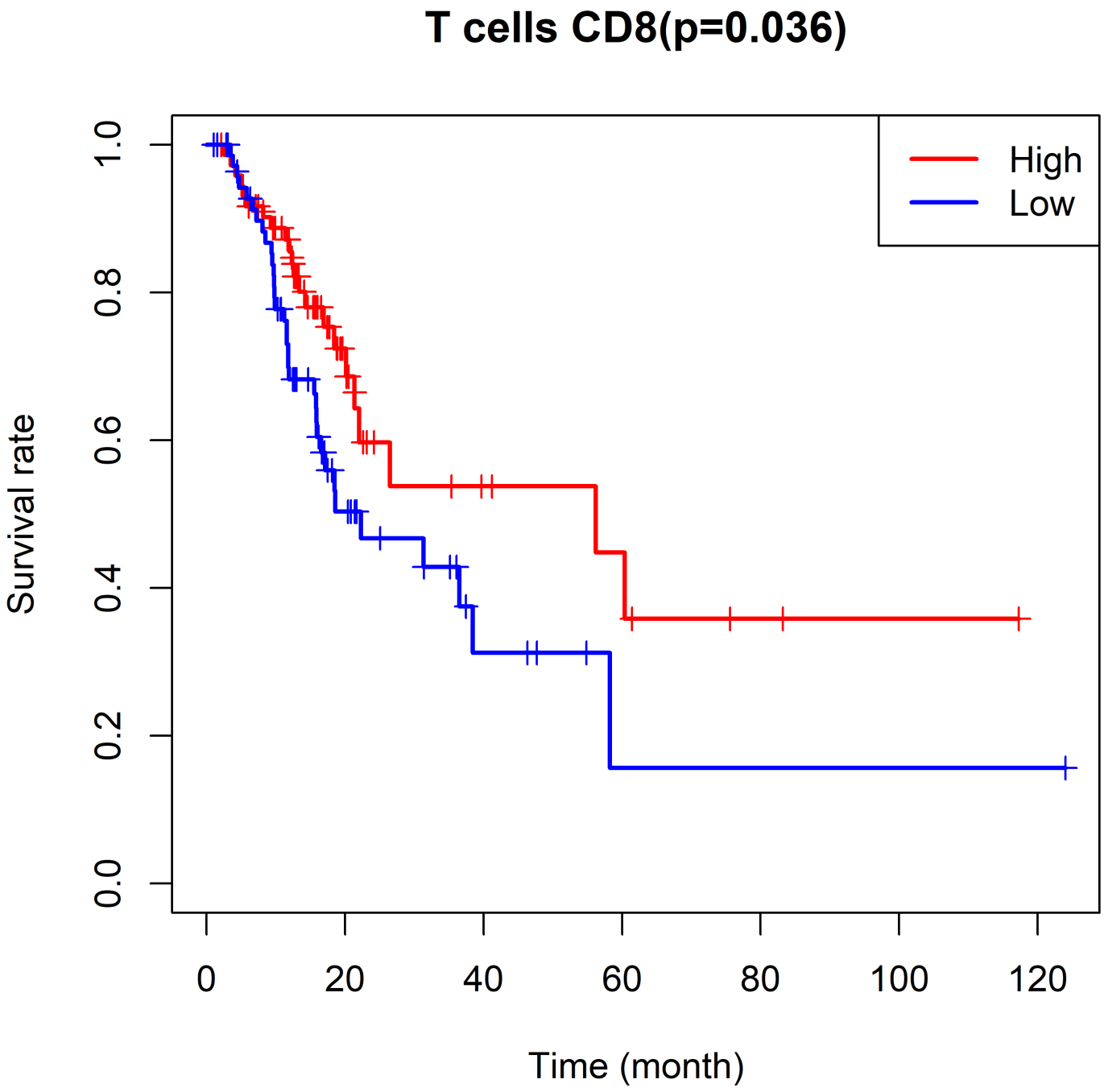

Figure 7

K-M survival plots of CD8 T cells