Identification Of The Molecular Targets And Immunophenotype Of Gastric Cancer By Bioinformatics Analysis

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Abstract

Background: Gastric cancer (GC) is the most lethal tumor of gastrointestinal tract worldwide. Despite advances in various therapies, the prognosis of GC remains poor. Moreover, only a small fraction of GC patients benefit from immunotherapy. Therefore, it is urgent to deeply understand the molecular characteristics and immunophenotype of GC.

Methods: We analyzed the gene expression profile of GSE118916 from GEO database, including the mRNA expression profiles of 15 pairs of GC tumor and adjacent non-tumor tissues. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the online website DAVID. And then the survival prediction values of the top 10 up-regulated genes were analyzed using Kaplan–Meier plotter database. Finally, the immune cells infiltration was analyzed using CIBERSORT online tool.

Results: A total of 1156 DEGs were identified, including 633 up-regulated genes and 523 down-regulated genes. The up-regulated genes were mainly enriched in cell adhesion, proliferation, migration and inflammation response. In addition, the up-regulated genes were significantly enriched in acid metabolism, complement and coagulation cascades, cell adhesion and p53 signaling pathway, which were all significant in tumor progression, relapse and metastasis. In addition, the up-regulated genes *CTSL* and *PIEZO1* were associated with poor prognosis in GC patients. Moreover, a unique immune-suppressive microenvironment was identified in GC tissues.

Conclusions: *CTSL* and *PIEZO1* might be potential biomarkers and therapeutic targets in GC patients.

Introduction

Gastric cancer is the most lethal tumor of gastrointestinal tract and the fourth most common tumor worldwide[1]. Despite the rapid development of surgery, chemotherapy and targeted therapy, the 5-year survival of patients with GC remains only about 10%[2]. Therefore, further understanding the molecular characteristics of GC might be urgent to develop novel therapeutic strategies to improve the prognosis of GC patients.

Currently, several core genes and pathways involved in the development of GC have been identified, including Wnt pathway[3], Hedgehog pathway[4], EGFR pathway[5], transforming growth factor-beta signaling[6] and so on. Recently, an integrated genomic analysis of microarray data was used to identify of core genes and outcome in GC[7]. The results showed that focal adhesion, ECM-receptor interaction and PI3K/Akt signaling pathway were mainly associated with GC development, and that the genes (BGN, MMP2, COL1A1 and FN1) were correlated with poor prognosis of GC patients. However, the molecular mechanisms of GC have not been fully understood.

In last decade, immunotherapy has revolutionized a promising landscape by blocking immune checkpoints (PD-1, PD-L1 and CTLA-4) in several solid tumors[8]. However, the antibodies targeting PD-1

or PD-L1 have had impressive and durable effects only in a small subset of gastric cancer patients[9]. It is well known that immunotherapy's efficacy seems to be associated to the immune microenvironment of the tumor and its immunogenicity. Thus, it might be extremely urgent to explore the immune microenvironment of gastric cancer.

In this study, a latest published mRNA microarray dataset (GSE118916) [10]downloaded from GEO database was analyzed to identify the molecular targets and immunophenotype of gastric cancer. Herein, the differentially expressed genes (DEGs) and biological process and KEGG pathways of these DEGs were analyzed. Moreover, the prognostic value of the hub genes was determined in gastric cancer patients. We found that the genes *CTSL* and *PIEZO1* might be the key genes and molecular targets in gastric cancer progression. In addition, a unique immuno-suppressive microenvironment was illustrated to further guide the immunotherapy for GC.

Methods

1. Microarray Datasets

A public and freely available dataset (GSE118916) was downloaded from GEO database (http://www.ncbi.nlm.nih.gov/geo/). The dataset included the mRNA expression profiles of 15 pairs of GC tumor and adjacent non-tumor tissues.

2. Data Processing Of DEGs

The original probe-level data were firstly converted into gene level data. Then R language was used to identify the DEGs between GC and CEPI samples. Furthermore, Bonferroni method in multtest package was utilized to adjust raw p value into false discovery rate (FDR), and DEGs were selected with the cutoff criteria of |log2 fold change (FC)| >1 and FDR < 0.05. In addition, the expression values of the identified DEGs, top 10 upregulated DEGs, and top 10 downregulated DEGs were compared between GC and CEPI groups by using the t test. The criterion for this analysis was $p < 0.05$.

3. GO function and KEGG pathway analysis of DEGs

GO function and KEGG pathway enrichment of DEGs were analyzed using DAVID database (http://david.abcc.ncifcrf.gov/). The core biological processes, molecular functions, cellular components and pathways were visualized among those DEGs. $P < 0.05$ was set as the cut-off criterion.

4. Survival Analysis

The survival prediction values of the top 10 up-regulated genes were analyzed using Kaplan–Meier plotter database (http://kmplot.com/analysis/). The database contains 1065 gastric cancer patients with a mean follow-up of 33 months. The hazard ratio (HR) with 95% confidence intervals (95%CI) and log rank P value were calculated and displayed on the website.

5. Analysis Of Immune Cells Infiltration

The immune cells infiltration was analyzed using CIBERSORT online tool (http://cibersort.stanford.edu). This tool provides 22 kinds of gene characteristics, which represents 22 kinds of white cell subtypes, including B cells, T cells, NK cells and so on. The statistic significance of proportion of immune cells was analyzed by students't test. $P < 0.05$ was set as significant difference.

Results

1. Identification of DEGs

To explore the molecular mechanisms of GC, the DEGs between GC and normal stomach tissues were firstly analyzed in a public dataset (GSE118916), which containing 15 pairs of GC tumor and adjacent non-tumor tissues. A total of 1156 DEGs were identified from GSE118916, including 633 up-regulated genes and 523 down-regulated genes (Fig. 1A). Besides, the top 10 up- and down-regulated genes were shown respectively (Fig. 1B).

2. Functional And Pathway Enrichment Analysis

To further understand the function of DEGs, the GO function and KEGG pathway enrichment analysis was performed using DAVID. The results showed that the up-regulated genes in GC were associated with a series of biological processes, including cell adhesion, proliferation, migration and inflammation response (Fig. 2A). Moreover, the up-regulated DEGs were significantly enriched in 25 pathways mainly about acid metabolism, complement and coagulation cascades, cell adhesion, p53 signaling pathway and so on (Fig. 2B).

3. The Prognostic Value Of DEGs Analysis

To assess the prognostic value of DEGs in GC, we analyzed the top 10 up-regulated genes using Kaplan–Meier plotter database, which containing 1065 gastric cancer patients. We found that in these genes, *CTSL* and *PIEZO1* over-expression were associated with poor overall survival in GC patients (Fig. 3), while other eight genes were not (data not shown). This implied that *CTSL* and *PIEZO1* might be the key genes and molecular targets in gastric cancer progression.

4. The Infiltration Of Immune Cells In GC

Recently, immune checkpoint blockers (ICBs) have been proven exciting anti-tumor efficacy in many cancers. However, the antibodies targeting PD-1 or PD-L1 have had impressive and durable effects only in a small subset of gastric cancer patients. Thus, the analysis of immune cell infiltration would be better understanding the immunophenotype of GC. As anti-tumor immune cells, B cells, $CD8^+$ T cells and NK cells infiltrated less in GC than that in adjacent normal tissues, while M1 macrophages and dentritic cells infiltrated more in GC (Fig. 4A). In addition, pro-tumor immune cell, including γδT cells, M2 macrophages

and neutrophils, infiltrated more in GC than that in normal tissues, excluding Tregs (Fig. 4B). These results implied that there existed a unique immuno-suppressive microenvironment in GC.

Discussion

Despite advances in various therapies, the prognosis of GC remains poor in the past decades[11]. Moreover, the efficacy of novel immunotherapy has proven so limited in GC patients[12]. Therefore, understanding of the molecular characteristics and immunophenotype of GC might be extremely urgent to develop novel therapeutic strategies.

In this study, a total of 1156 DEGs were identified from a GC microarray dataset (GSE118916), including 633 up-regulated genes and 523 down-regulated genes. The up-regulated genes were mainly enriched in cell adhesion, proliferation, migration and inflammation response. In addition, the up-regulated genes were significantly enriched in acid metabolism, complement and coagulation cascades, cell adhesion and p53 signaling pathway, which were all significant in tumor progression, relapse and metastasis. The survival analysis showed that the up-regulated genes *CTSL* and *PIEZO1* were associated with poor prognosis in GC patients. Moreover, an immune-suppressive microenvironment was found in GC tissues, which might be useful for immunotherapy development.

CTSL is one of the members of Cysteine cathepsins (CTSs) famaily, which are involved in the degradation and remodeling of the extracellular matrix[13]. Overexpession of CTSL has been reported in various tumors, including ovarian cancer[14], lung cancer[15], breast cancer[16] and gastric cancer[17]. The elevated levels of CTSL might be associated to proliferation, invasion, metastasis and chemoresistance of cancer cells[18]. In addition, knockdown of CTSL could sensitize cancer cells to chemotherapy and target therapy[19, 20]. Our study found that CTSL was up-regulated in gastric cancer compared to normal tissues, which is consistent with the findings in previous studies[21]. Moreover, CTSL overexpression was found to negatively correlate with prognosis of gastric cancer in this study, which might be explained by that CTSL could induce the migration, invasion and epithelial-mesenchymal transition of cancer cells[18, 22].

PIEZO1 is highly expressed in epithelial cells of skin, bladder, kidney, and lung, which are important for maintaining cell homeostasis of epithelial monolayers[23]. Besides, PIEZO1 have been implied to be related to initiation and progression in many cancers[24–26]. Numerous studies have reported that PIEZO1 might regulate cancer cell mobility by various mechanisms. Previous study found that PIEZO1 was suggested to promote cell migration by interacting Trefoil factor family 1 (TFF1). In addition, a recent study reported that PIEZO1 could promote proliferation, migration and chemo-sensitivity of gastric cancer cells by regulating the activity of Rho GTPase family members[27]. Moreover, the study found that increase of PIEZO1 was associated with poor disease specific survival in gastric[27], which also support our conclusion that PIEZO1 might be a novel clinical therapeutic target for GC.

Immunotherapy has recently been a novel and promising approach in cancer treatment. However, a large fraction of GC patients do not benefit from ICBs[12]. To date, there are many predictive biomarker of ICBs,

including PD-L1 expression, TMB (tumor mutation burden), TILs infiltration, and so on[28]. Herein, we found an immune-suppressive microenvironment in GC tissues, which are consistent with the results in previous studies. Moreover, the less infiltration of $CD8^+$ T cells indicated a “cold tumor” phenotype, which predicts poor response to immunotherapy. The results implied that a pretreatment of enhancing the immunogenicity might be essential for improving the efficacy of immunotherapy for GC.

Conclusions

This study was intended to identify DEGs with comprehensive bioinformatics analysis to find the potential target and immune phenotype of GC. A total of 1156 DEGs were identified, and *CTSL* and *PIEZO1* might be the key genes and molecular targets in GC progression. Moreover, a “cold tumor” phenotype was found in GC tissue. Our results might be useful to provide new cutes for diagnosis and treatment of GC patients.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing Interests

No potential conflicts of interest were disclosed.

Fundings

No funding was received.

Authors’ Contributions

Conception and design: Fang Li, Tao Yang.

Development of methodology: Tao Yang, KeGang Zhang.

Acquisition of data (provided managed patients, provided facilities, etc.): Rui Xu, Junhao You.

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): KeGang Zhang, Rui Xu.

Writing, review, and/or revision of the manuscript: KeGang Zhang, Rui Xu.

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Junhao You, Tao Yang.

Study supervision: Fang Li, Tao Yang.

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Figures

Figure 1

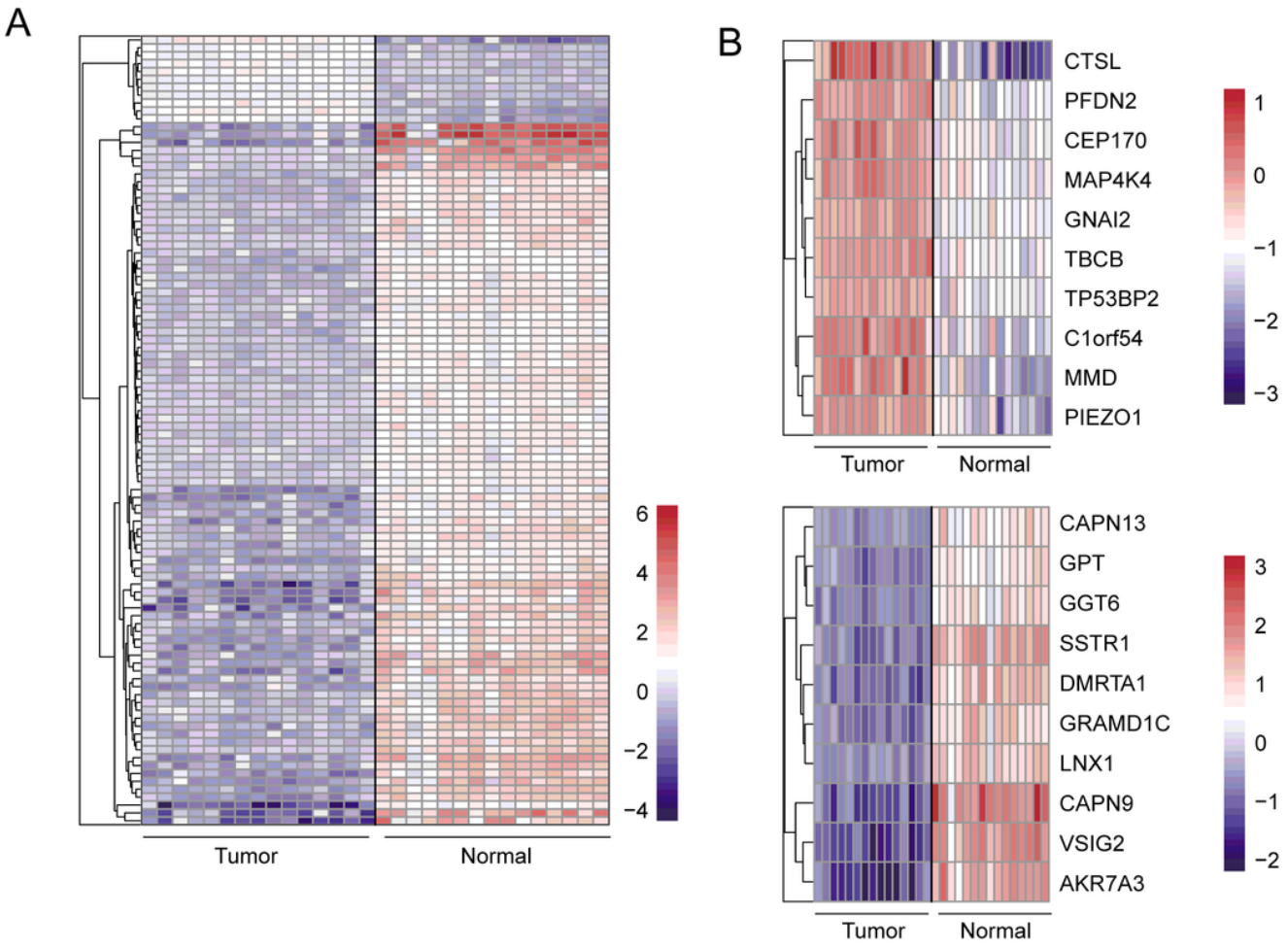

Figure 1

Identification of differentially expressed genes in mRNA expression profiling datasets GSE118916. A. a total of 1156 DEGs were identified, including 633 up-regulated genes and 523 down-regulated genes. B. the top 10 up- and down-regulated genes were shown respectively.

Figure2

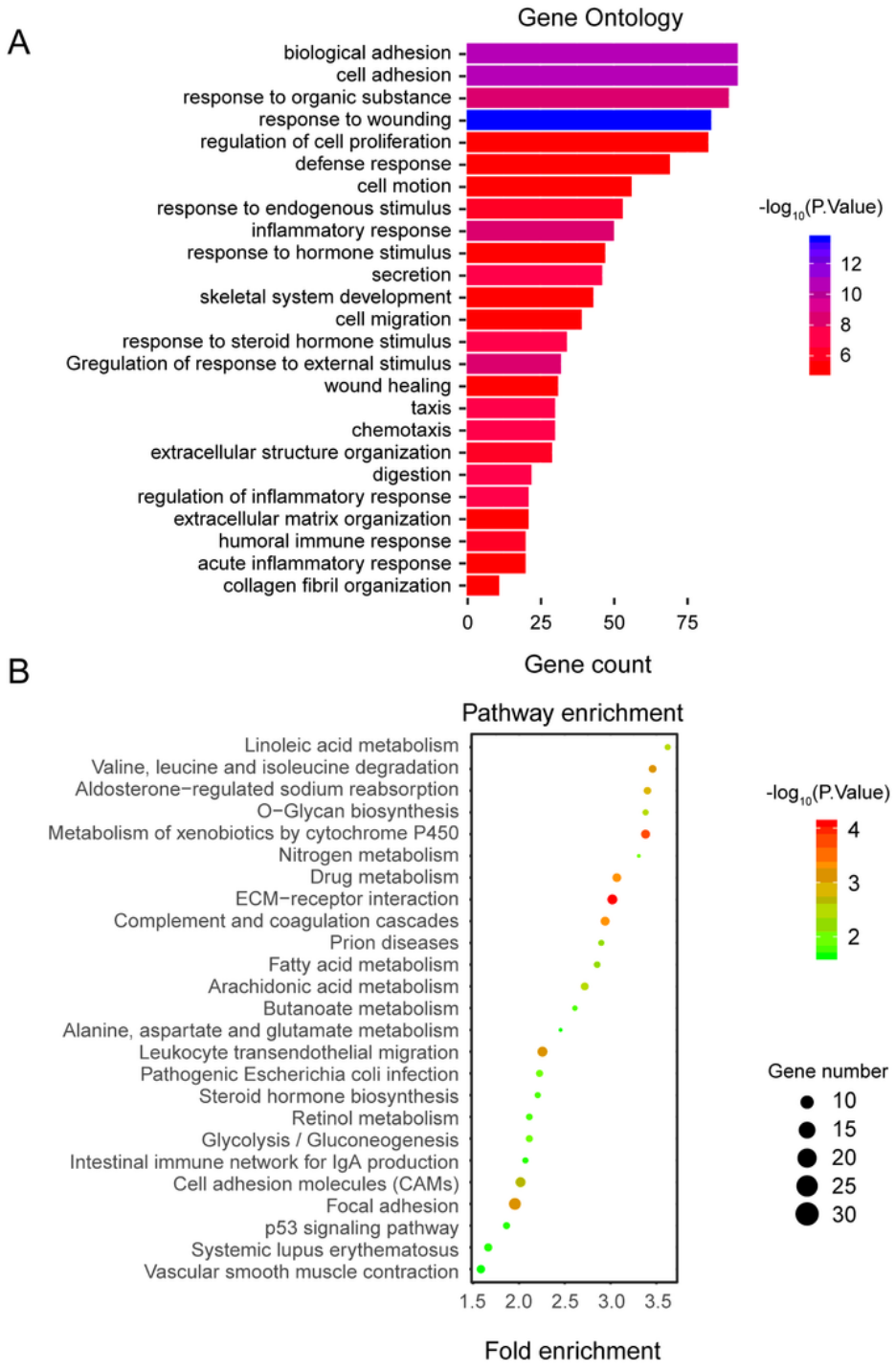

Figure 2

The function analysis of DEGs. GO function (A) and KEGG pathway enrichment (B) of up-regulated genes were analyzed using DAVID database.

Figure3

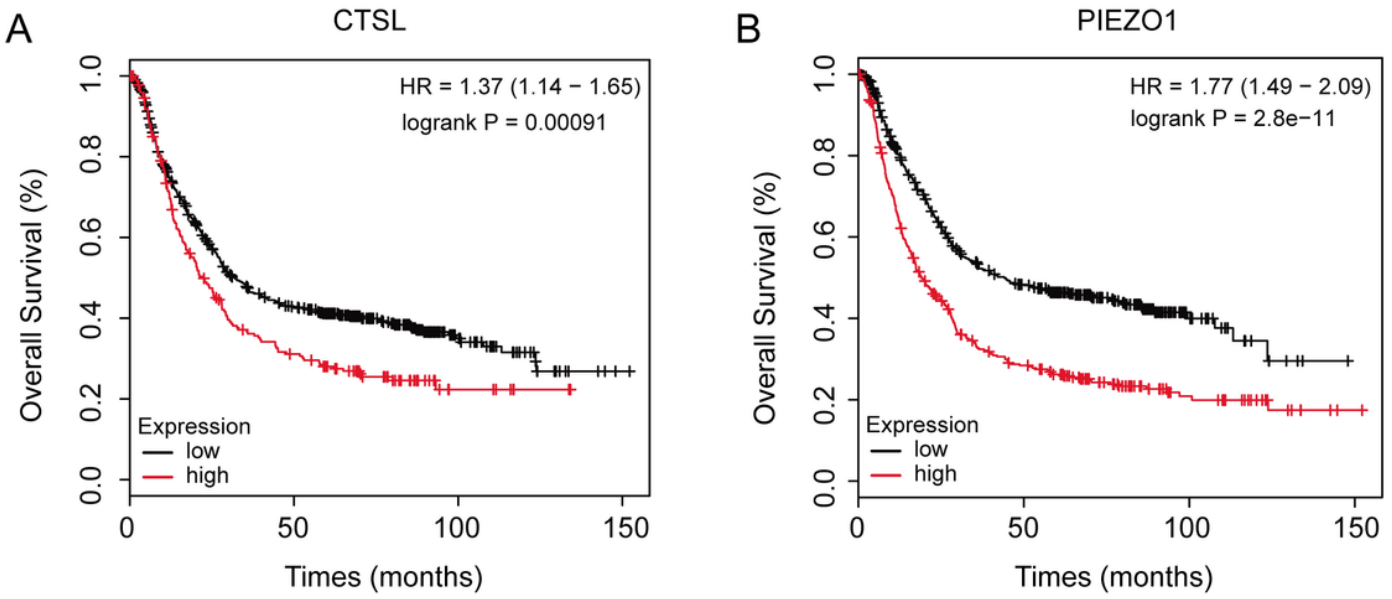

Figure 3

Prognostic value of two genes in gastric cancer patients. Prognostic value of CTSL (A) and PIEZO1 (B) were obtained in Kaplan–Meier plotter database. HR, hazard ratio.

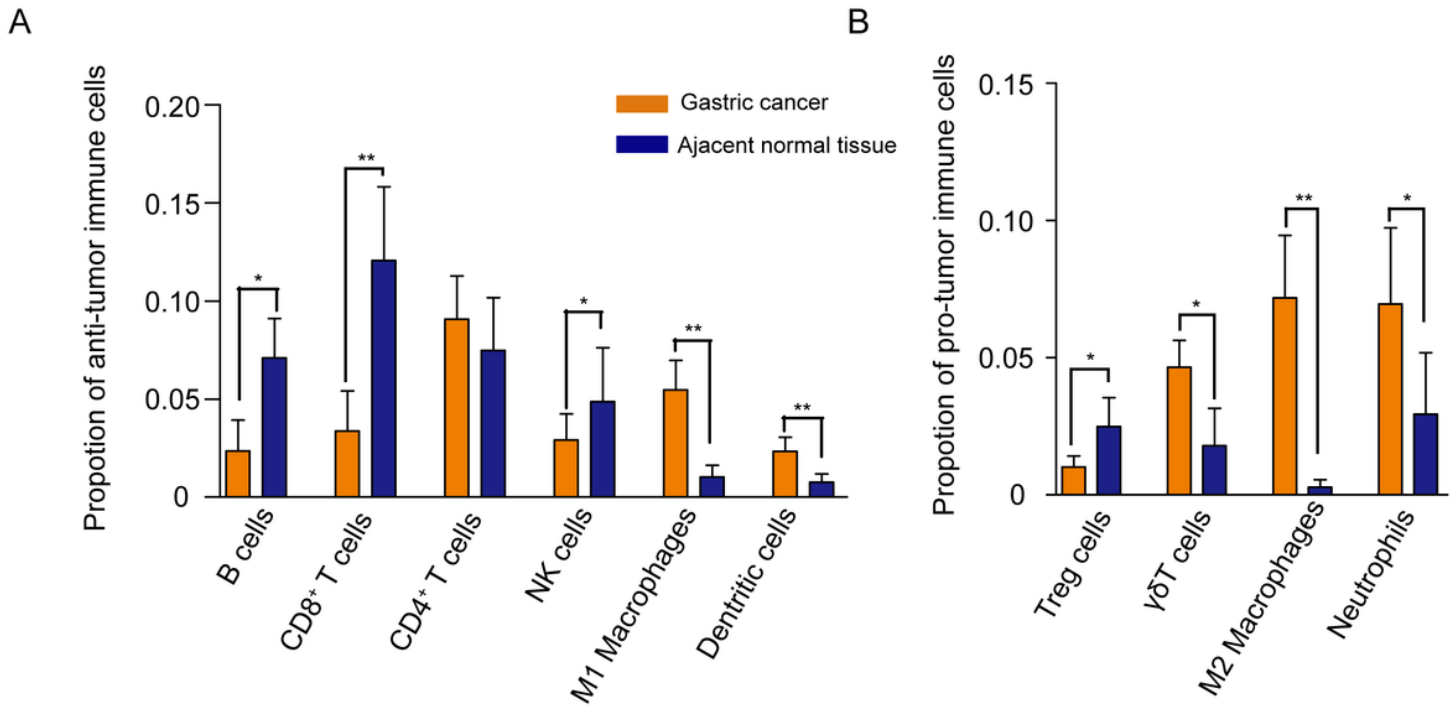

Figure 4

The infiltration of immune cells in gastric cancer and normal tissues. The immune cells infiltration was analyzed using CIBERSORT online tool. The infiltration of anti-tumor immune cells (A) and pro-tumor

immune cells were shown respectively. *P<0.05, **P<0.01.