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Original Research

Profiles of immune infiltration in ovarian cancer and their clinical significance: a gene expression-based study

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Objective: The effect of immune cell infiltration on the prognosis of ovarian cancer (OC) has not yet been intensively investigated. *Methods*: In the present study, by using a deconvolution algorithm (known as CIBERSORT) and clinical annotated expression profiles (GEO and TCGA), we comprehensively analyzed the tumor-infiltrating immune cells (TIICs) present in OC and their effect on the prognosis and progression of OC. *Results*: A fraction of 22 immune cell subpopulations was evaluated to determine the associations between each cell type and survival. Of the cell subpopulations investigated, follicular helper T cells, monocytes, and macrophages M0 and M1 significantly varied between normal ovarian surface epithelium and OC tumors. The proportion of CD8+ T cells and M0 macrophages was moderately correlated (Pearson correlation = 0.48) in the TCGA. Resting dendritic cells were higher in grade 1 cells after GEO datasets were pooled. Resting dendritic cells showed higher proportion in stage II than in stage III/IV tumors. In TCGA, a higher proportion of neutrophils cell implied poor prognosis compared with the lower. In GEO datasets, the high proportions of CD8 T cells, activated dendritic cells, and plasma cells indicated better survival time comparing with the lower. *Conclusion*: Collectively, our data suggest that subtle differences appear to exist in the cellular composition of the immune cell infiltrate in OC, and these differences are likely to be the important determinants of both prognosis and response to treatment.

Keywords

Immune cell; Infiltration; Ovarian cancer; GEO; TCGA

1. Introduction

Globally, more than 200,000 women are diagnosed with ovarian cancer (OC) every year, and most of them have poor prognosis. This is especially true for the subgroup of women with advanced OC in whom the 5-year survival rate is below 20% [1, 2]. Although this estimate is partly or largely affected by the variation in the registration of cancer cases across different countries, OC is still responsible for approximately 150,00 deaths per year [1, 2]. Unfortunately, the clinical outcome of OC for individual cases remains unsatisfactory. At present, the most effective approach to cure OC is lefting tumor < 1 cm after surgery [1]. Most women with OC will experience tumor recurrence after the first-line therapy, and in almost all of them, resistance to chemotherapy will eventually develop, leading to death [1].

Previous studies have intensively investigated genomic and biological mutations in cancer cells and the effect of these mutations on patients' prognosis and treatment response [3]. The tumor-related microenvironment has also been intensively studied [4, 5]. It was found that 22 types of immune cells, known as tumor-infiltrating immune cells (TIICs), were recruited to the tumor area, with other immune components to form a complex biological process that promotes or inhibits tumor growth [6, 7]. In 2007, Hamanishi *et al.* reported that patients with OC who showed a high expression of PD-L1 and low intraepithelial CD8+ T lymphocyte count had a significantly poor prognosis [8]. Another study indicated that the expression of CD20, FoxP3, and TIA-1 in immune cells was a positive prognostic factor for patients with OC. T regulatory (T_{reg}) cell infiltration was also found to be associated with advanced stage OC [9]. However, there are very few studies on the infiltration of immune cells in OC. Therefore, by using TCGA and GEO databases, we systematically analyzed the expression of 22 types of immune cells in OC and the association of these cells with tumor cell grade, clinical stage, and patients' survival time.

2. Materials and methods

2.1 Data acquisition

The data for this study were downloaded from TCGA and GEO databases from April 10 to 15, 2019. Patients with missing or insufficient data on tumor cell grade, clinical stages (FIGO stage), survival time, and disease-free survival time were excluded from subsequent analysis. Preprocessing and aggregation of raw data were normalized using the "limma" package of R software. Details of the study design and the samples included at each stage of analysis are illustrated as a flowchart in Fig. 1.

2.2 Evaluation of TIICs

The normalized gene expression data were used to infer the relative proportions of 22 types of TIICs by using the CIBERSORT algorithm as previously reported [10–13]. Briefly, gene expression datasets were prepared using standard annotation files and data uploaded to the CIBERSORT web portal (http://cibersort.stanford.edu/), with the algorithm run using the default signature matrix at 1000 permu-

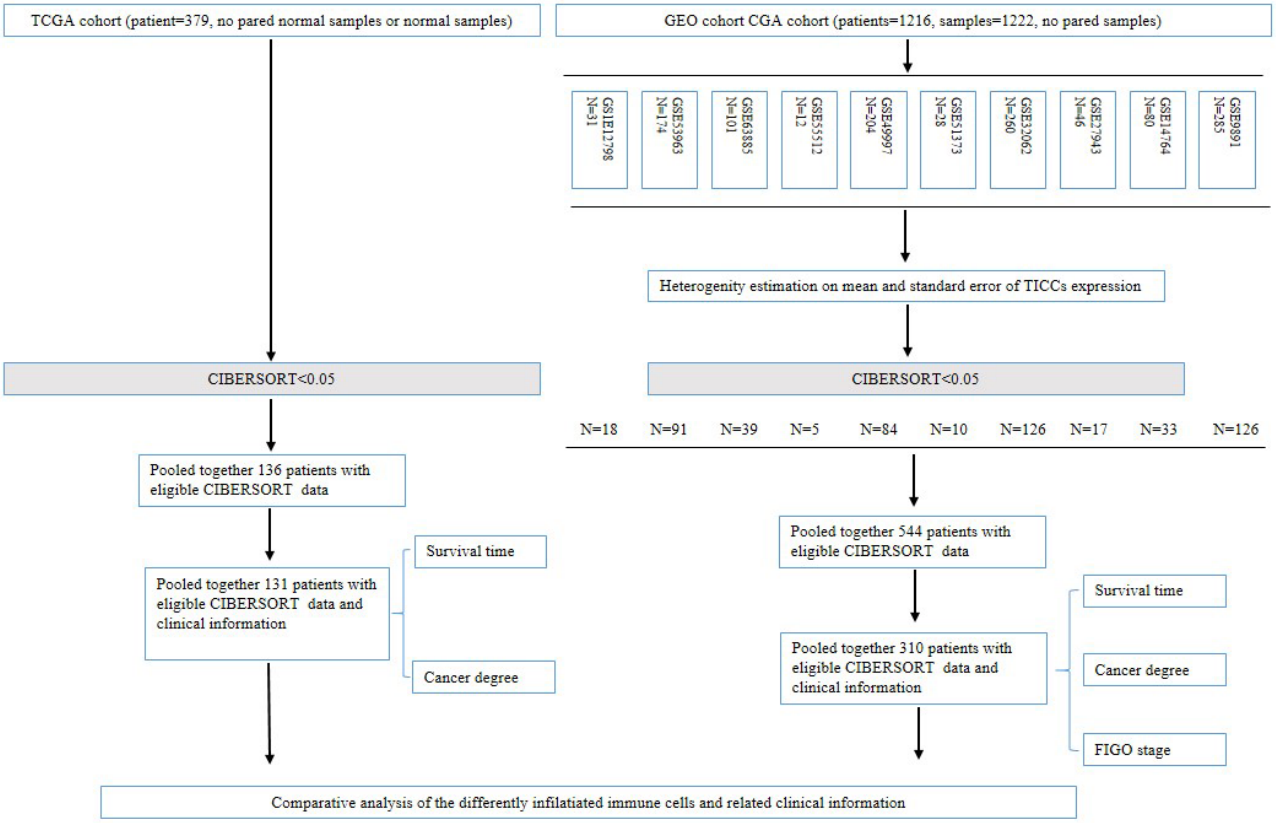

Fig. 1. Study flowchart detailing the flow of samples at each stage of analysis in TCGA.

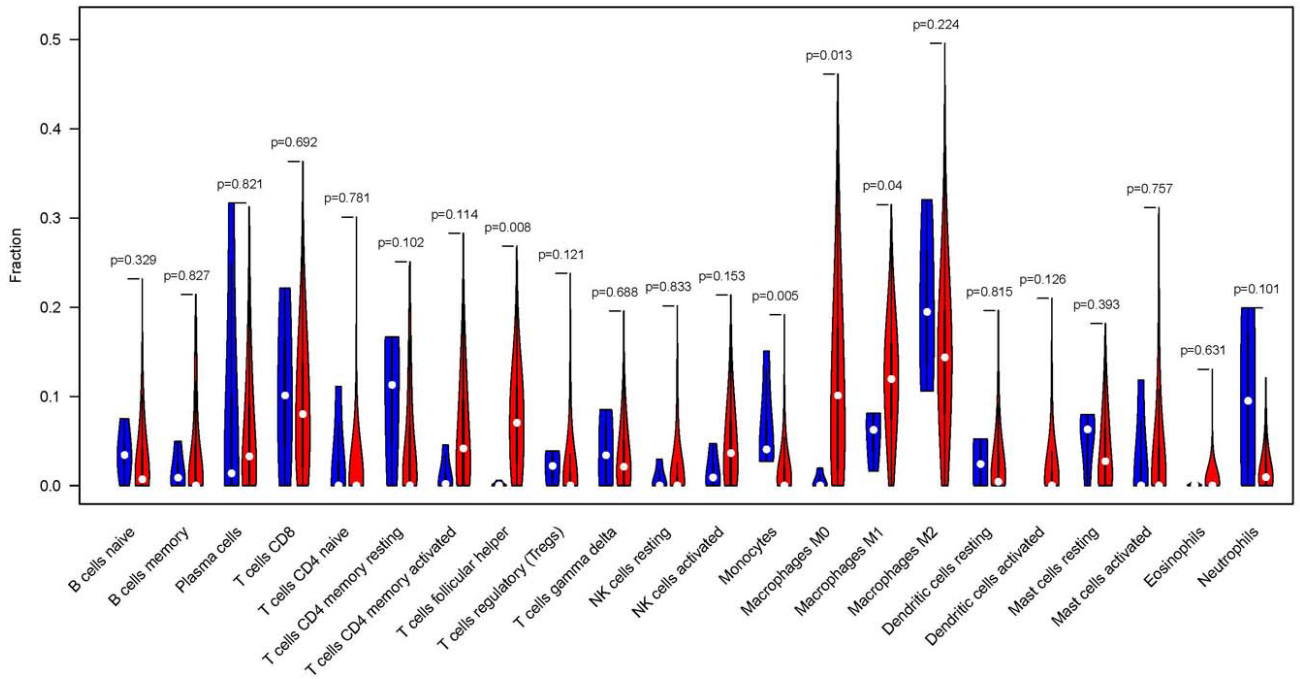

Fig. 2. Proportions of 22 tumor-infiltrating immune cells (TIICs) between cancer sample and ovarian surface epithelium (GSE54388).

tations [11]. CIBERSORT calculates a *P*-value for the deconvolution of each sample by using Monte Carlo sampling, thus providing a measure of confidence in the results.

2.3 Statistical analyses

Cases with a CIBERSORT *P*-value of < 0.05 were included in the main survival analysis. The associations that

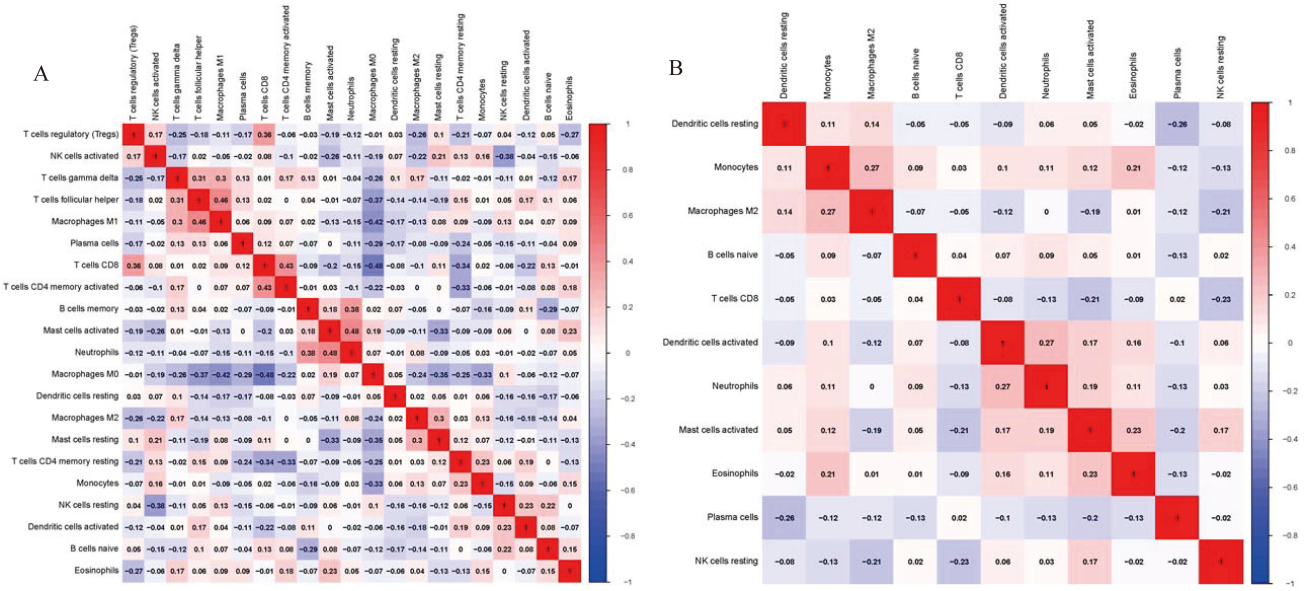

Fig. 3. Correction of cytolytic activity in TCGA (A) and GEO (B).

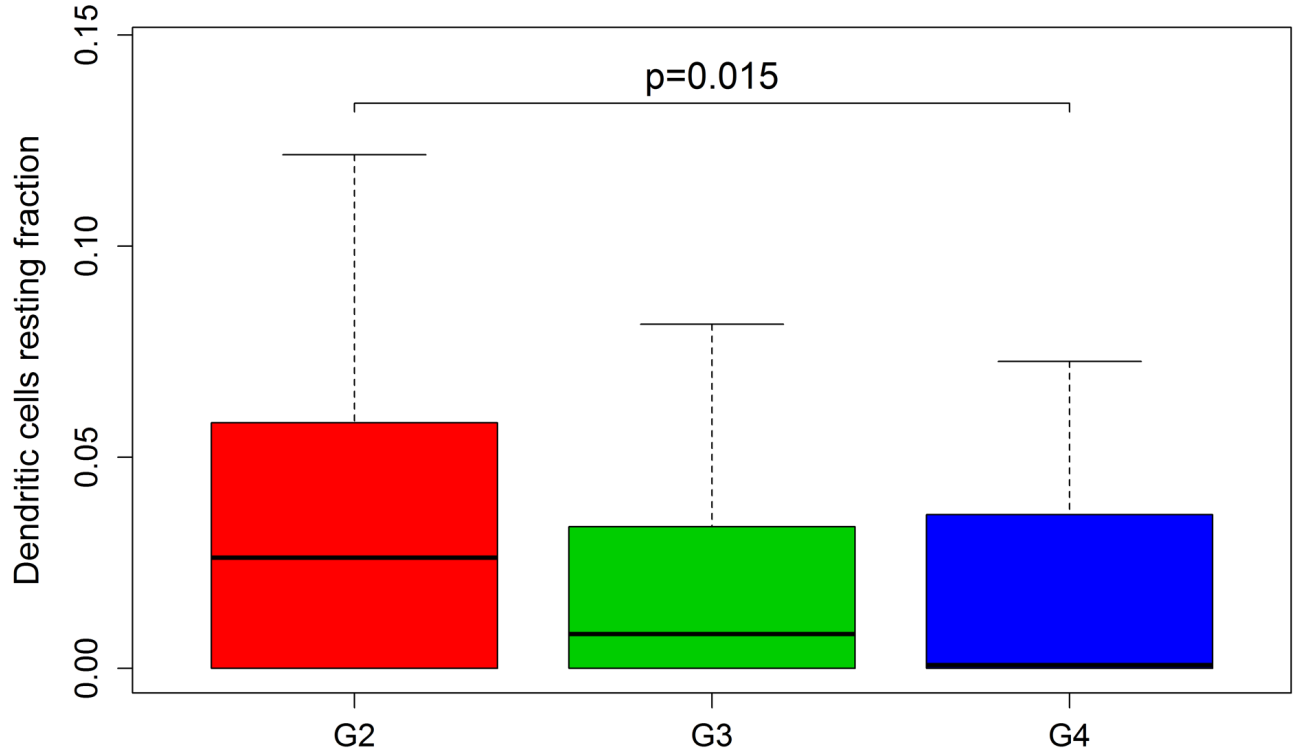

Fig. 4. Proportion of resting dendritic cells was significantly higher in grade 1 than in grade 2 and 3 in GEO datasets.

differentiated tumor cell grades, clinical stage, patients' survival times and inferred proportions of immune cell types were tested using Cox regression analysis. Immune cell subsets that were significantly associated with outcome in unadjusted analyses were included in the multivariate models. Multivariate analyses were adjusted for survival time, differentiated tumor cell grades, and clinical stage. The association between infiltrated immune cells and the corresponding disease-free survival time was analyzed by Kaplan-Meier survival curves and evaluated using the log-rank test.

All analyses were conducted using R version 3.5. All statistical tests performed were two-sided, and P values < 0.05 were considered to be statistically significant.

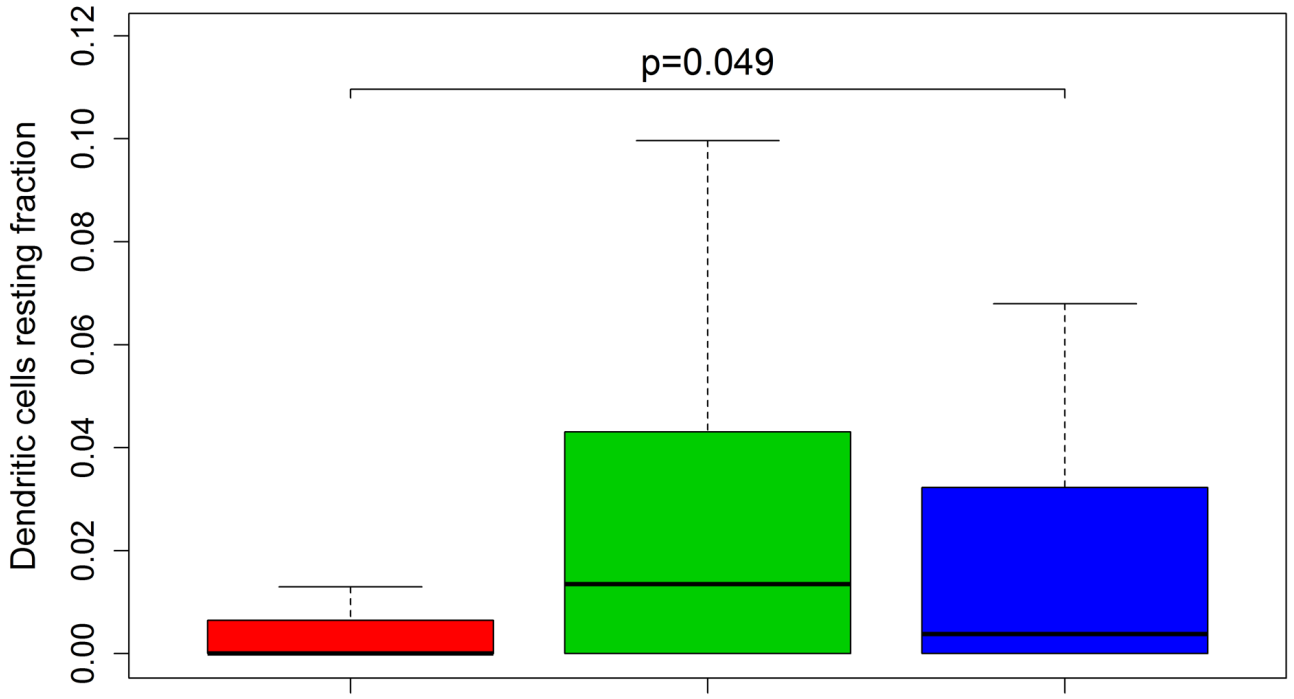

Fig. 5. Proportion of resting dendritic cells was higher in stage II tumor than in stage III/IV tumors in GEO datasets after TIICs were excluded because of heterogeneity.

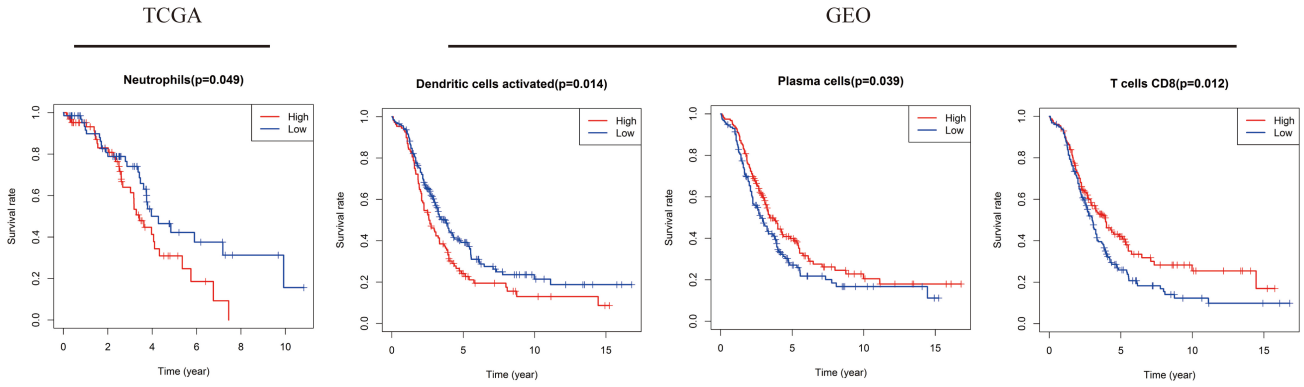

Fig. 6. The subpopulations of TIICs were associated with patients' survival time.

3. Results

3.1 Performance of CIBERSORT for characterizing the composition of TIICs in OC

CIBERSORT coupled with 22 kinds of lymphocytes enables highly sensitive and specific differentiation of human leukocyte subsets, and this tool has already been used in many previous studies [12–15]. The analysis of TCGA OC genomic data by CIBERSORT revealed that one of the LMs (CD4 naïve T cells) was not expressed in OC (Fig. S1A). The analysis of GEO datasets revealed that 22 LMs were present in the OC sample (Fig. S1B). A heterogeneity analysis of these cells according to their proportion revealed the following cell types: activated dendritic cells, CD8+ T cells, neutrophils, resting dendritic cells, eosinophils, monocytes, resting NK cells, activated Mast cells, and plasma cells.

The cell population of M2 macrophages and naïve B cells could be pooled together because of no significant heterogeneity between GEO multiarray (Table 1).

3.2 Profile of immune infiltration in OC

By using CIBERSORT algorithm, we investigated the difference in immune cell infiltration between cancer sample and normal ovarian surface epithelium among 22 subpopulations of immune cells (GSE54388). It was found that the proportion of follicular helper T cells, monocytes, and M0 and M1 macrophages significantly varied between normal ovarian surface epithelium and OC sample (Fig. 2). This finding, however, could not be confirmed in TCGA because of shortage of normal tissue datasets.

Table 1. The heterogenity in GEO after pooling multiarray.

sample	n	effect size (es)	95% Confidence interval (CI)	*P* value
Mast cells resting	549	0.034	0.027, 0.041	0
NK cells activated	549	0.04	0.031, 0.049	0
Macrophages M0	549	0.107	0.088, 0.125	0
Macrophages M1	549	0.111	0.1, 0.121	0
T cells regulatory (Tregs)	549	0.013	0.009, 0.018	0.001
T cells gamma delta	549	0.031	0.025, 0.036	0.001
T cells follicular helper	549	0.071	0.062, 0.081	0.001
T cell CD4 memory resting	549	0.041	0.033, 0.048	0.025
T cell cd4 memory actived	549	0.052	0.044, 0.059	0.025
T cell CD4 naïve	549	0.01	0.007, 0.014	0.034
B cell memory	549	0.031	0.024, 0.037	0.04
Dendritic cells activated	549	0.015	0.011, 0.019	0.066
T cell CD8	549	0.087	0.078, 0.096	0.076
Neutrophils	549	0.018	0.015, 0.02	0.17
Dendritic cells resting	549	0.023	0.019, 0.026	0.172
Eosinophils	549	0.004	0.003, 0.005	0.261
Monocytes	549	0.014	0.012, 0.016	0.305
NK cells resting	549	0.013	0.01, 0.015	0.531
Mast cells activated	549	0.028	0.023, 0.032	0.669
Plasma cell	549	0.054	0.048, 0.059	0.793
Macrophages M2	549	0.142	0.135, 0.15	0.875
B cell naïve	549	0.023	0.014, 0.033	0.901

3.3 CIBERSORT P-values reflect the overall proportion of immune cells

Cytolytic activity was moderately correlated with the proportion of CD8+ T cells and M0 macrophages (Pearson correlation = 0.48) in the TCGA dataset with a CIBERSORT P value of < 0.05 (Fig. 3A). However, it's no clear correction after excluding the TIICs because of great heterogeneity in GEO datasets (Fig. 3B).

3.4 Identification of different cancer cell diffeneratiated grades of TIICs in OC

We found that resting dendritic cells were higher in grade 1 cells after GEO datasets were pooled (Fig. 4A). However, we did not found any significant difference of the propertions of TIICs among OC differentiated grade in TCGA (Table 2).

3.5 Identification of prognostic subsets of TIICs in clinical stages of OC

Because shortage of clinical stage samples of OC in TCGA, we determined the association between TIICs and the clinical stages of OC in GEO datasets. We found that resting dendritic cells had a higher proportion in stage II than in stage III/IV tumors (Fig. 5).

3.6 Association between prognostic subsets of TIICs and survival time of patients with OC

Next, we investigated whether variation in TIIC subsets affects patients' prognosis. In TCGA, the higher proportion of neutrophils cell implied poor prognosis comparing with the lower (Fig. 6A). In GEO datasets, the high proportion of CD8 T cells, activated dendritic cells and plasma cells indicated better survival time comparing with the lower (Fig. 6B).

4. Discussion

Tumor-related microenvironment involves a large variety of cells and an abundant accumulation of cytokines, chemokines, and growth factors [16, 17]. It's difficult to systematically analyze the network of indegrities function in cancer. Recently, CIBERSORT, a computational tool, has been used to analyze the proportion of TIICs in tumors [6], the previous studies has proved its violation [10, 18–20]. This tool could overcome the limitation of traditional immunohistochemistry (IH)-based methods that define cell types mainly based on one or two cell markers. However, a few studies did not test the heterogeneities of genomic data after acquiring multiarray data from GEO database [13, 20]. Therefore, we excluded TIICs with a high heterogeneity in order to maintain the consistency of results.

To exclude multiarrays with potential heterogeneity, we mainly analyzed one array of GEO dataset and compared it with TIICs in normal tissue and OC sample. We found that the proportion of follicular helper T cells, monocytes, and M0 and M1 macrophages was significantly higher in the OC sample than in normal tissues. Follicular helper T cells are one of the CD4+ T cells; they provide specialized help to B cells and are essential for the generation of memory B cells and long-lived antibody-secreting plasma cells. Monocytes are direct precursors of hematopoietic stem cell-derived macrophages [21]. After their recruitment into the tumor tissue, they can differentiate into tumor-associated macrophages, a very heterogeneous cell population in terms of phenotype and pro-tumor function, thus supporting tumor initiation, local progression, and distant metastasis [22].

Table 2. The comparsion of tumor-infiltrating immune cells (TIICs) propertion on tumor cell diffeneratiated grade in TCGA.

TIICs	*P* value
B cells naive	0.503756
B cells memory	0.427281
Plasma cells	0.657612
T cells CD8	0.88371
T cells CD4 memory resting	0.600152
T cells CD4 memory activated	0.789448
T cells follicular helper	0.300062
T cells regulatory (Tregs)	0.314243
T cells gamma delta	0.806908
NK cells resting	0.887291
NK cells activated	0.397064
Monocytes	0.960479
Macrophages M0	0.245618
Macrophages M1	0.190304
Macrophages M2	0.21711
Dendritic cells resting	0.399246
Dendritic cells activated	0.264313
Mast cells resting	0.181067
Mast cells activated	0.731538
Eosinophils	0.256253
Neutrophils	0.182936

One of the most important roles of macrophages in tumor growth seems to be the promotion of angiogenesis [21]. Tumor-associated macrophages play a role in multiple stages of tumor development-from promoting tumor cell invasion and metastasis [23]. The association between monocytes/macrophages and tumor metastasis has been established by extensive clinical correlations [23]. Collectively, our results further proved that these cells play an important role in carcinogenesis and development of OC.

Resting dendritic cells could induce immune tolerance. Dendritic cells play a critical role in priming antitumor T-cell immunity and thereby represent a major therapeutic target for cancer immunotherapy [24]. Resting dendritic cells were found the higher proportion in diffenerated grade 1 than grade 2 and 3 in GEO dataset, but this conclusion could not been confirmed in TCGA dataset. Thus, further investigation is needed to validate this result.

In patients with solid cancers, a large number of neutrophils proliferate in both the tumor microenvironment and the body system, and they are generally associated with poor prognosis [25]. This result was confirmed in OC after we analyze TCGA data. However, the same result was not observed for GEO dataset, and we found that the lower proportion of activated dendritic cells, higher proportion of plasma cells, and higher proportion of CD8+ T cells were associated with better prognosis comparing with the lower each of them. Elevated levels of cytotoxic CD8+ T cells in the tumor microenvironment showed a good prognosis in various cancer types [26]. Dendritic cells play a key role in inducing and maintaining antitumor immunity, but in the tumor environment, their antigen-presenting function may be lost or become inefficient [27]. Dendritic cells might also be polarized into immunosuppressive/tolerogenic regulatory dendritic cells, which limit the activity of effector T cells and support tumor growth and progression [27]. These results could explain why lower proportion of activated dendritic cells and higher proportion of CD8 T cells imply better prognosis in OC. Tumor-infiltrated plasma cells show two side effects in solid tumors. The elevated level of plasma cells indicate favorable prognosis in OC [28, 29] and various other cancers [6]. Our results were consistent with these conclusions. On the other hand, plasma cells also play inhibitory roles in cancer via the immunosuppressive cytokines IL10 and IL35 [30].

Despite the significant results obtained in the present study, we did not find similar results for GEO and TCGA. This might due to data heterogeneity. The function of TIICs in tumor was based not only on their proportion but also on their location. For example, Hamanishi *et al.* found that a high number of intraepithelial CD8+ T lymphocytes indicated a better prognosis than stromal CD8+ T lymphocytes [8]. Further studies are needed to completely understand the role of TIICs in tumor.

Author contributions

XZ and BY conceived and designed this experiment. XZ, YZ and ZJL completed the data acquisition, analysis and interpretation. XZ and BY drafted the article, BY revised the article and final approval of the version to be published.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

This paper has not been published elsewhere in whole or in part. All authors have read and approved the content, and agree to submit for consideration for publication in the journal. There are no any ethical/legal conflicts involved in the article. This manuscript has been edited and proofread by International Science Editing Limited.

Availability of data and materials

The datasets supporting the conclusions of this article are available in the in Gene Expression Omnibus (GEO) database with the accession numbers listed as follows: GSE14764, GSE9891, GSE27943, GSE32062, GSE51373, GSE49997, GSE55512, GSE63885, GSE53963, and GS1E12798. All the previous studies were approved by their respective institutional review boards.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://ejgo.imrpress.com/EN/10.31083/j.ejgo.2021.02.5347.

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