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Granzyme B expression correlates with poor prognosis in ovarian serous cystadenocarcinoma

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Objective: The purpose of this study was to explore the expression of Granzyme B (GZMB) and its impact on the survival and prognosis of patients with ovarian serous cystadenocarcinoma (OSC) and to evaluate whether it could be used as a potential prognostic marker for OSC. *Methods*: The study included 43 cases of high-grade serous ovarian cancer (HGSOC) and 415 cases of OSC. Immunohistochemical analysis was performed to assess GZMB expression in cancer tissues, and the biological functions of GZMB were explored through biometric analysis. *Results*: In HGSOC tissues, later Federation International of Gynecology and Obstetrics (FIGO) staging was associated with lower GZMB expression ($p < 0.05$). Moreover, GZMB expression was significantly related to clinical stage, distant metastasis, histological grade, and survival status ($p < 0.05$), whereas it was not related to age ($p > 0.05$). GZMB was also identified as a regulator of the immune response in the tumour microenvironment. *Conclusions*: GZMB is closely related to the occurrence and development of OSC, participating in immune regulation; hence, it could be a potential prognostic biomarker of OSC.

Keywords

Biomarker; Granzyme B; Immunity; Ovarian serous cystadenocarcinoma; Prognosis

1. Introduction

Although the incidence of ovarian cancer ranks third among gynaecological malignancies, it is the deadliest malignant gynaecologic cancer [1]. Ovarian cancer (OC) can be classified into five main subtypes: high-grade serous carcinoma, low-grade serous carcinoma, mucinous carcinoma, endometrioid carcinoma, and clear cell carcinoma. Ovarian serous cystadenocarcinoma (OSC), which accounts for approximately 90% of all OC cases [2] is an aggressive cancer that is associated with poor prognosis. Even with active treatment, the 5-year survival rate of patients with OSC is approximately <40% [3]. OC incidence in China has been increasing every year and has become a chronic disease that seriously threatens the physical and mental health of women [4]. Owing to the insidious onset and rapid development of OC, most patients are at an advanced stage at the time of diagnosis; therefore, prognosis of OC is relatively poor [5, 6], with a very low survival rate. The age-standardized 5-year net survival rate in high-income countries is 36–46% [7]. Presently, the indicators commonly used to judge the prognosis of patients with OC are the Federation International of Gynecology and Obstetrics (FIGO) staging, tumour grade, tumour histological typing, patient age, surgical residual tumour, and presence or absence of peritoneal effusion [8]. However, there is an urgent need for the development of novel biomarkers that can effectively predict the therapeutic response and prognosis of patients with OC [9, 10].

Recently, miRNA [11] and lncRNA [12] , as well as the tumour suppressor gene [13] were identified as OC prognostic biomarkers. Granzyme B (GZMB) is a serine protease released by T cytotoxic lymphocytes and natural killer cells, which plays a vital role in apoptosis by activating intracellular caspases [14]. Studies have reported that active human GZMB can enter target cells through perforin or receptors and target various structural and functional proteins containing Asp-x or Gln-x sites, thereby triggering apoptosis. Thus, the potential use of GZMB for gene therapy to target and kill abnormal cells would be of great significance in cancer treatment [15]. Noteworthy, GZMB expression in human epithelial tumour cells, such as lung, breast [16], and gastric [17] cancers, was reported. GZMB has a wide range of important biological functions, including anti-viral, anti-tumour, and in transplantation immunity [18–20]. In recent years, several cancer-targeting therapeutic drugs using human GZMB as an apoptosis inducer were evaluated and these paved a new path in cancer treatment [21]. GZMB has also been widely used for the treatment of viral and parasitic infections [22]. In transplant rejection, Clément *et al.* [23] detected GZMB expression in infiltrating lymphocytes in transplanted kidneys and used it as an early diagnostic marker of lymphocyte activation. Based on this, a more comprehensive dynamic study of GZMB role in pathology is needed.

Due to the undesirable poor prognosis of patients with OC [24], current treatment options include conventional radiotherapy, chemotherapy, hormone therapy, and surgery. In recently years, gene targeted therapy, especially RNA interference technology, has become a research hotspot, with some positive outcomes [25]. Thus, further studies to identify novel prognostic and predictive biomarkers are needed to help identify high-risk patients, predict OC prognosis, and potentially provide therapeutic guidance [26].

This study aimed to assess GZMB as a potential OSC prognostic biomarker.

2. Materials and Methods

2.1 Patients and data

A total of 43 patients (aged 38–87 years) with ovarian tumours who were treated in the Department of Gynaecology of the First Affiliated Hospital of Zhengzhou University, from January 2017 to January 2019 and had complete clinical data were included in the study. They all had high-grade serous ovarian cancer (HGSOC), according to the post-operative pathology, and had not receive chemotherapy or radiotherapy before surgery. According to the FIGO staging standard, among the 43 cases, 20 were at stage II, 10 at stage III, and 9 cases were at stage IV. Based on tissue differentiation, 12 cases were of moderately and highly differentiated and 31 were poorly differentiated cancers. A total of 22 cases had lymph node dissection, including 15 cases with lymph node metastasis and 7 cases without lymph node metastasis. In the shortest time possible, after the tissue specimen was isolated, it was cut into 4 mm × 4 mm pieces, fixed with 10% formaldehyde solution, and then subjected to immunohistochemistry experiments. The study was approved by the ethical review board of the local institution, and all patients participating in the study provided written informed consent.

2.1.1 Immunohistochemical analysis of tissue specimens

Xylene and ethanol were used to deparaffinise and dehydrate the paraffin-embedded tissue sections. To restore the antigen, the tissue sections were placed in citrate buffer at 95 °C for 5 min, washed with phosphate buffered saline (PBS) three times, and bovine serum albumin blocking solution was added dropwise, and incubated at room temperature for 10 min. Next, the samples were incubated with the primary antibody overnight at 4 °C and then washed with PBS three times. The secondary antibody was incubated at 37 °C for 1 h and then washed with PBS solution three times. Afterward, the water around the tissue sample was removed and DAB (3,3'-diaminobenzidine) substrate was added to it dropwise and rinsed with tap water immediately after colour development. Lastly, the slides were counterstained with haematoxylin and fixed with dibutyl phthalate xylene.

2.1.2 Assessment of immunohistochemistry results

Two experienced pathologists performed double-blind reading without being informed of the tumour grade. The reading teacher randomly selected 10 fields of view in each case for observation. The result of immunohistochemistry was judged according to the staining intensity and the percentage of stained positive cells to achieve a comprehensive score. The staining intensity score was defined with 0–3 points (0: no staining; 1: light yellow; 2: brownish yellow; 3: brown), and the percentage of the number of positive cells was defined with 0–4 points (0: ratio of positive cells <5% percent; 1: between 5% and 25%; 2: between 25% and 50%; 3: between 50% and 75%; 4: >75%). The result was considered positive if the product of the two scores was greater than 2 and negative if less than or equal to 2 [27].

2.2 The Cancer Genome Atlas (TCGA) database information

TCGA (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) is a project jointly initiated by the National Cancer Institute and the National Human Genome Research Institute Home in 2005 to provide access to original clinical data for scientific research [28], comprising 11,000 clinical tumour samples with genetic, histological, and epigenetic data, as well as sequencing data covering half of the cancer clinical gene mutations [29]. Herein, the clinical features (tumour information, OS information, and RNA-sequencing data), as well as GZMB levels of 415 patients with OSC were obtained from the TCGA database. The patients were divided into two groups according to the GZMB expression (high and low levels).

2.3 Gene Ontology (GO) annotation and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analyses

GO is an updated bioinformatics system that provides high-quality functional gene annotation for all species [30, 31]. KEGG (http://www.genome.jp/) is an online database of genes and genomes that can supply information related to genomes, metabolism, biological pathways, diseases, and chemical substances [32]. The database for annotation, visualization and integrated discovery (DAVID) is an online platform for inquiry access that merges biologically abundant data from a large-scale dataset [33]. In this study, DAVID was used as a functional annotation instrument to conduct GO annotation and KEGG pathway enrichment analyses.

2.4 Gene Set Enrichment Analysis (GSEA)

To evaluate the function of the candidate genes, GSEA was performed using the GSEA4.0.3 software (http://www.broadinstitute.org/gsea/). GSEA analysis adopts gene sets collected by the molecular signatures database (MSigDB, http://software.broadinstitute.org/gsea/msigdb/), which comprises a set of annotated genes, with seven main sets [34]. To determine the mean expression in the low GZMB group, data from patients with high-grade serous ovarian cancer (HSGOC) were analysed using the GSEA3.0 software. RNA-sequencing data containing 20,530 genes were extracted from TCGA and GSEA was performed, with a false discovery rate (FDR) <0.05 set as meaningful. Significance was calculated using ranking statistics; the number of permutations was set to 1000, and the phenotypic marker was GZMB-low versus GZMB-high.

2.5 Protein-Protein Interaction (PPI) network analysis

To uncover the functional associations between proteins found to be related to OSC across the genome, the PPI network was evaluated using the online STRING database (https://string-db.org) [35]. The identified differentially expressed genes (DEGs) were used as input in STRING, and the confidence score was set at ≥0.4, with the maximum number of interactors at 50 as threshold. In the PPI network, each

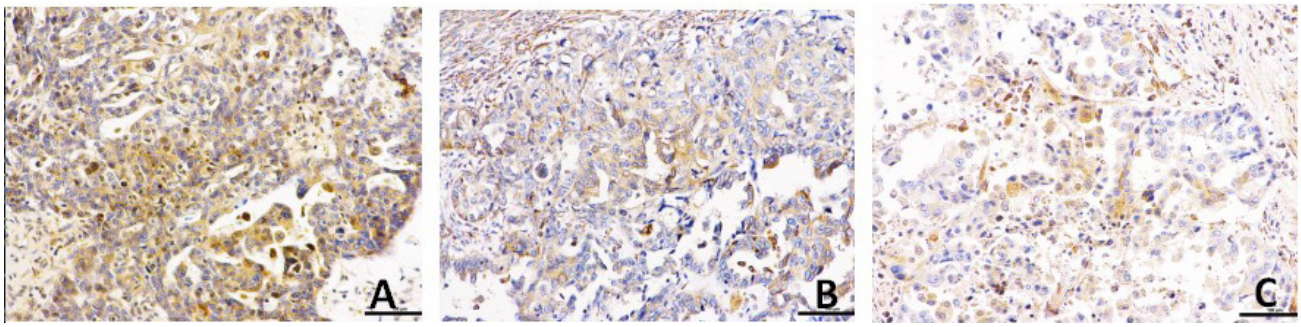

Fig. 1. Expression of GZMB protein in different FIGO stages of high-grade serous ovarian cancer. (A) FIGO II. (B) FIGO III. (C) FIGO IV. SP×400.

node indicates a protein and each edge indicates an interaction involving pairwise proteins. Nodes with comparatively large amounts of edges were determined to be hub proteins.

2.6 Immune cell infiltration

The relationship between GZMB expression and that of various immunity-related genes was determined using the tumour immune estimation resource (TIMER) database (https://cistrome.shinyapps.io/timer/), which is a comprehensive resource used to systematically analyse the immune infiltration of different cancer types and estimate the abundance of six types of immune cells (B cells, $CD8^+$ T cells, $CD4^+$ T cells, dendritic cells, macrophages, and neutrophils).

2.7 Statistical analysis

IBM SPSS 23.0 software for Windows (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Patients with OSC were divided into two groups according to the median value of GZMB expression in tumour cells. The two groups were compared using *t*-test and Chi-square test or Fisher's exact test. Kaplan–Meier curves were used to determine whether GZMB expression in tumour cells had prognostic significance for OS in patients with OSC. To assess whether the standalone prognostic element in OSC was GZMB expression, univariate and multivariate Cox proportional hazard regression analysis models were used to draw Kaplan–Meier curves, and log-rank test was used to compare the differences in OS and progression-free survival between the two groups. Differences were considered statistically significant when the *p*-value was <0.05. All charts and statistical analyses were conducted using Prism software (GraphPad Software, San Diego, CA, USA) and bioinformatics analyses were performed using R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Immunohistochemistry and clinical correlation analysis of 43 cases of HGSOC

Immunohistochemical analysis of tissue samples collected from 43 patients diagnosed with HGSOC showed that GZMB was mainly distributed in the cytoplasm, with a granular pattern, and its expression was mainly manifested by the intensity of the staining (Fig. 1).

Detailed clinical correlation analysis further revealed that the expression of GZMB was significantly lower in FIGO stage IV patients than in FIGO II and III patients ($p = 0.003$). The positive expression rates in FIGO II, III, and IV tissues were of 75.0%, 71.4%, and 11.1%, respectively. Thus, the later the surgical pathological stage, the lower the positive expression rate of GZMB protein. Moreover, the positive expression rate of GZMB in HGSOC was significantly related to FIGO staging, histological grade, and presence or absence of lymph node metastasis ($p < 0.05$), whereas it was not related to age ($p > 0.05$) (Table 1).

Table 1. Positive expression of GZMB protein in HGSOC.

Characteristics	N	GZMB protein		
		Positive rate (%)	X^2	*p*
Age (years)			0.398	0.528
≤52	22	15 (68.2)		
>52	21	8 (38.1)		
FIGO stage			11.642	0.003
II	20	15 (75.0)		
III	14	10 (71.4)		
IV	9	1 (11.1)		
Histologic grade			5.063	0.025
G1/2	12	5 (41.7)		
G3	31	7 (22.3)		
Lymph node metastasis			5.495	0.019
Yes	15	5 (33.3)		
No	7	5 (71.4)		

3.2 Clinical characteristics of patients with OSC in the TCGA database

The clinical and histological characteristics of 415 patients (median age: 58 years; range: 30–87 years) with OSC from TCGA database are shown in Table 2. Among the OSC patients, 23 cases (5.5%) had stage II tumours, 330 cases (79.5%) had stage III, and 62 cases (15.0%) had stage IV tumours, according to the FIGO staging guidelines. According to the histological grading guidelines, 53 cases (12.8%) were G1/G2, 355 cases (85.5%) were G3/G4, and 7 cases (1.7%) were GX/GB. Furthermore, 62 patients (15.0%) had distant metastases and 353 (85.0%) had no distant metastases. At the

end of the study, 231 deaths had been reported (55.7%, of which high and low GZMB expression accounted for 43% and 57%, respectively), and 184 surviving cases (44.3%, of which high and low GZMB expression accounted for 59% and 41%, respectively). The median expression of GZMB in cancer tissues was 1.816 ng/mL, ranging within 0–6.6193 ng/mL.

Table 2. Clinical characteristics of patients with OSC in the TCGA database.

Clinical features	Total
Number of patients	415
Age (years)	
Median	58
Range	30–87
Clincial stage (no.)	
II	23
III	330
IV	62
Histologic grade (no.)	
G1/G2	53
G3/G4	355
GX /GB	7
Distant metastasis (no.)	
Yes	62
No	353
GZMB erpression (ng/mL)	
Median	1.816
Range	0–6.6193
Survival status (no.)	
Died	231
Alive	184

3.3 GZMB can be a diagnostic indicator of OSC

Receiver operating characteristic (ROC) curve analysis was performed using the expression of GZMB in 415 tumour samples and 10 normal samples from the TCGA database. As shown in Fig. 2, the area under the curve (AUC) was 0.755 ($p = 0.006$), which shows that GZMB has obvious diagnostic ability to distinguish tumour tissues from normal controls.

3.4 Correlation analysis between GZMB expression and OSC clinical characteristics

By surveying the association between clinicopathological variables and GZMB expression, relationships between the clinical stage, distant metastasis, lymphatic invasion, histologic grade, and survival status were identified (Table 3). GZMB expression was highly correlated with clinical stage ($p = 0.003$), as well as distant metastasis ($p < 0.0001$), histologic grade ($p < 0.0001$), and survival status ($p < 0.0001$). In addition, to further verify the possibility of GZMB as an independent risk factor for the prognosis of OSC, the expression of GZMB and the clinicopathological characteristics of OSC (clinical data downloaded from TCGA database) were combined to construct a single-factor and multi-factor Cox survival regression analysis model (Table 4). Overall, low

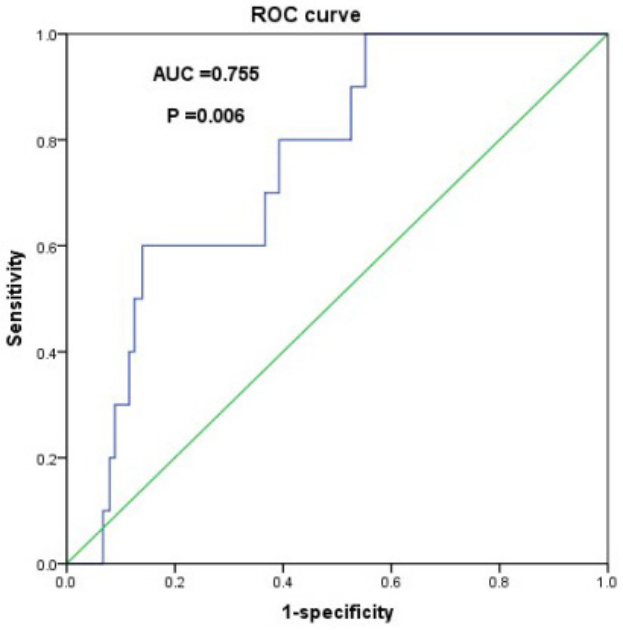

Fig. 2. ROC curve analysis of GZMB. ROC curve shows that GZMB has obvious diagnostic ability to distinguish tumor tissues from normal controls.

Table 3. Association between GZMB expression and clinicopathological characteristics in patients with OSC.

Characteristics	Total	GZMB expression		*p* Value
	N	High	Low	
Age				0.96
≤58	209	105	104	
>58	206	103	103	
Clinical stage				0.003**[b]
II	23	13	10	
III/IV	392	195	197	
Distant metastasis				<0.0001****
No	353	183	170	
Yes	62	25	37	
Histologic grade				<0.0001****
G1/2	53	27	26	
G3/4	355	177	178	
GX/GB	7	4	3	
Survival status				<0.0001****
Alive	184	109	75	
Died	231	99	132	*

[b] indicates the Fisher test, and the other test was the Chi-square test.
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

GZMB expression and age at diagnosis were found to be potential independent risk factors for OSC. In agreement with this finding, for patients with OSC, the age of diagnosis was previously reported as a clinically relevant independent risk factor. In summary, the results of this multi-factor Cox risk ratio regression model once again show that low expression of GZMB may be an independent risk factor for the prognosis of OSC. GZMB has the potential to become an important

Table 4. COX regression model of prognostic factors in patients with OSC.

Characteristics	Univariate			Multivariate		
	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Age	1.017	1.005–1.029	0.006	1.016	1.004–1.029	0.008
Clinical stage (III/IV vs II)	2.212	1.041–4.699	0.039	2.042	0.960–4.344	0.064
Distant metastasis (Yes vs No)	1.264	0.895–1.785	0.183	-	-	-
Histologic grade (G3/4 vs G1/2)	1.295	0.905–1.852	0.158	-	-	-
GZMB (Low vs High)	1.416	1.091–1.839	0.009	1.395	1.074–1.812	0.013

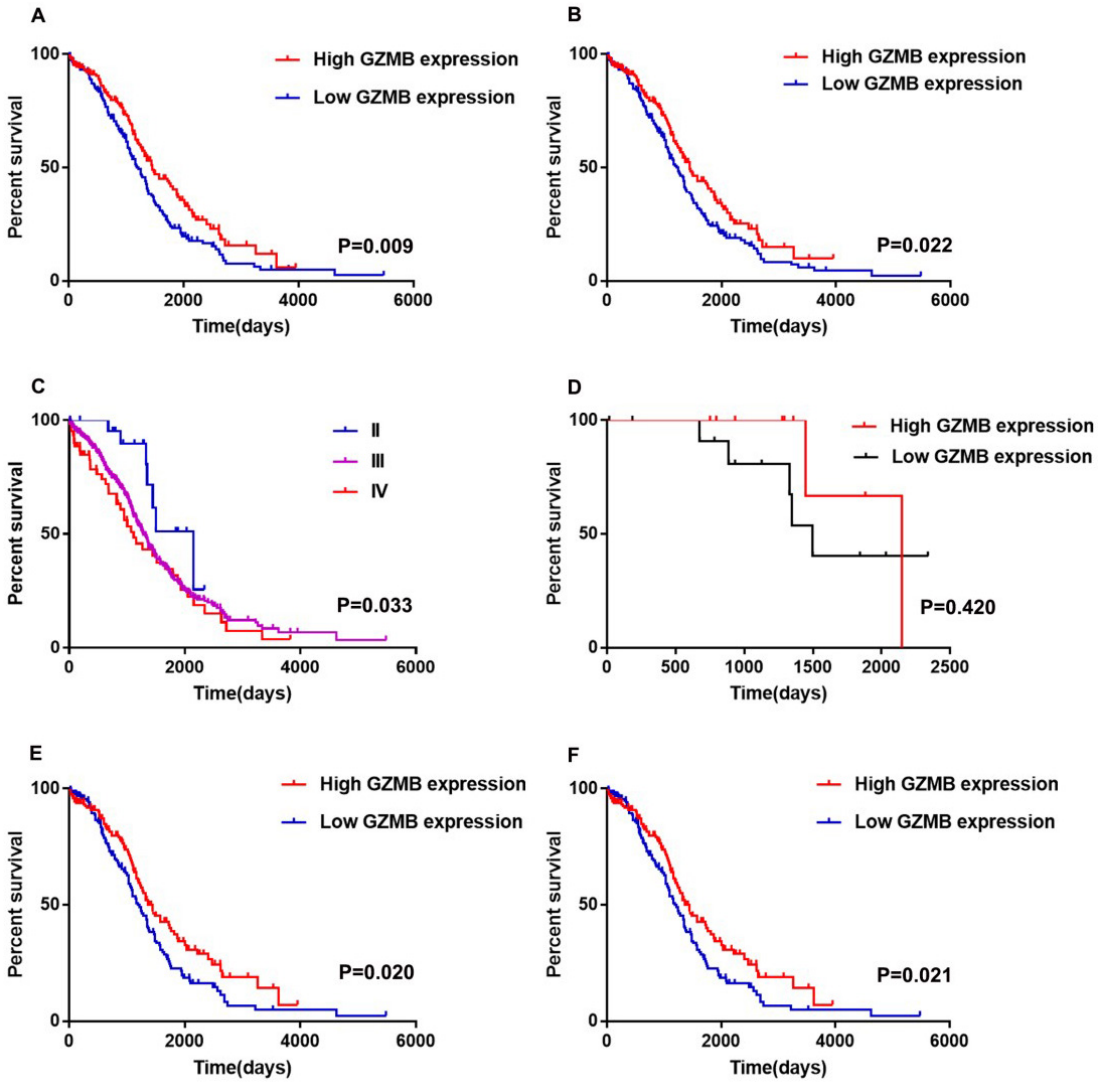

Fig. 3. Kaplan-Meier analysis of GZMB. (A) The Kaplan-Meier (KM) curve illustrates the association between GZMB expression and the OS of patients with OSC. (B) The KM curve illustrates the association between GZMB expression and DFS of patients with OSC. (C) The KM curve illustrates the association between FIGO stages and the OS of patients with OSC. (D) The KM curve illustrates the association between stage II and the OS of patients with OSC. (E) The KM curve illustrates the association between stage III and the OS of patients with OSC. (F) The KM curve illustrates the association between stage IV and the OS of patients with OSC.

molecular marker for the prognosis of OSC and is worth further research and discussion.

3.5 Kaplan–Meier survival curve of GZMB

To further investigate the influence of GZMB on the survival and prognosis of patients with OSC, the relationship between the expression of GZMB with OS and disease-free survival (DFS) of patients with OSC was analysed. In this experiment, the x-tile software was used to convert GZMB expression data from a continuous to a binary variable. The best cut-off value was 1.816. Based on this value, the OSC

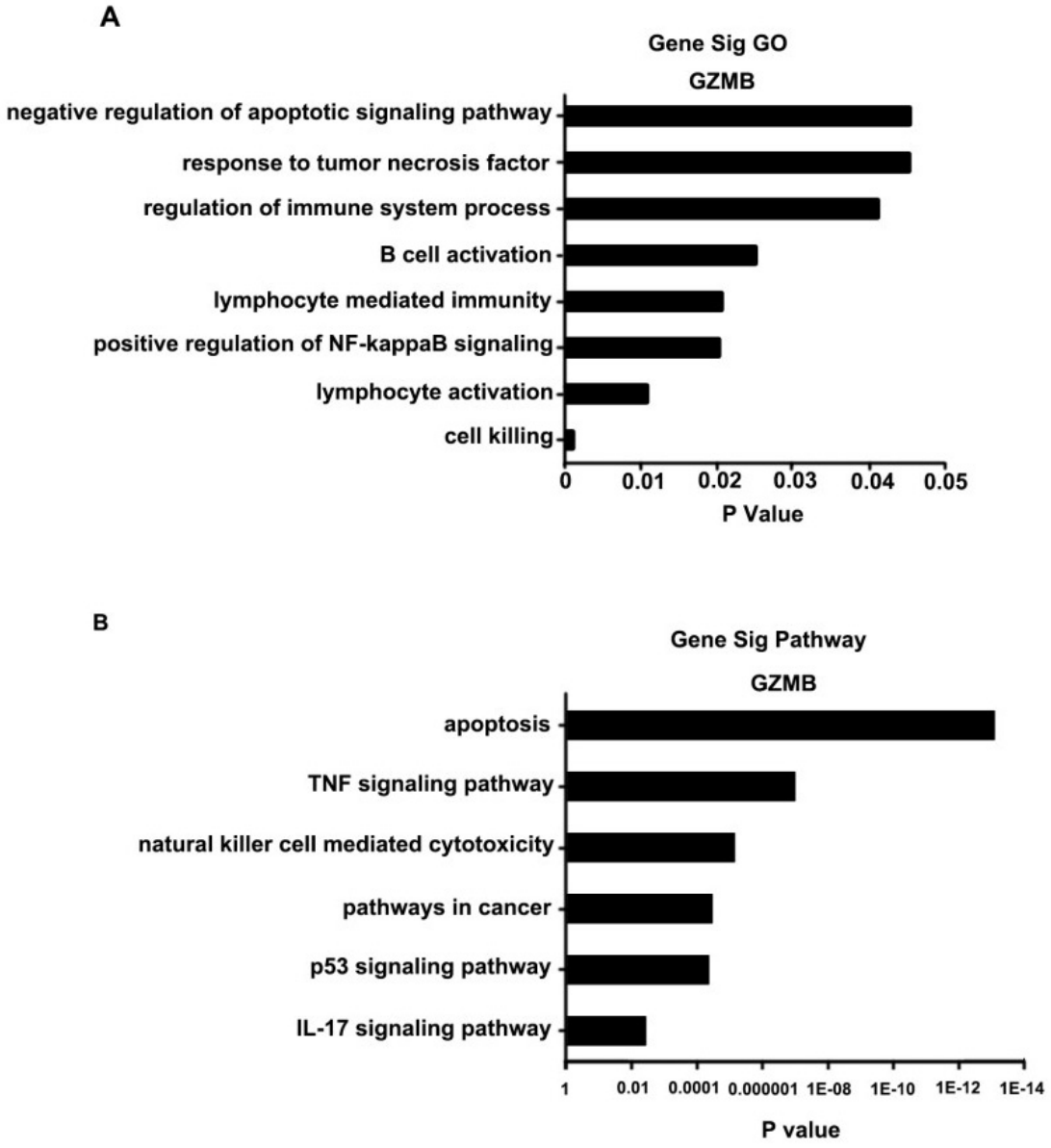

Fig. 4. Functional associations of GZMB. (A) Gene ontology analysis revealed that GZMB is involved in cell killing, lymphocyte activation, positive regulation of NF-κB signaling, and lymphocyte-mediated immunity, B cell activation, regulation of immune system process and response to tumor necrosis factor negative regulation of apoptotic signaling pathway. (B) KEGG analysis showed that GZMB was associated with the IL-17 signaling pathway, p53 signaling pathway, pathways in cancer, natural killer cell mediated cytotoxicity, TNF signaling pathway, and apoptosis.

patients included in the study were divided into the high and low expression groups and the respective Kaplan–Meier survival curves were drawn. Compared with the GZMB high expression group, the GZMB low expression group had shorter OS (Fig. 3A, $p = 0.009$) and shorter DFS (Fig. 3B, $p = 0.002$). Moreover, the later the tumour FIGO stage, the shorter the OS period (Fig. 3C, $p = 0.003$). In addition, the GZMB low expression group had a shorter OS period in FIGO III and IV stage than the GZMB high expression group ($p = 0.020$, $p = 0.021$) (Fig. 3E–F), whereas in FIGO II, the OS period was not statistically different among the two groups ($p > 0.05$) (Fig. 3D). The median OS of the patients in the GZMB high expression group was 31.0 months, and the median OS of the patients in the low expression group was 27.0 months, which suggests that GZMB low expression has an adverse effect on the prognosis of patients with OSC. Thus, GZMB has the potential to represent a valuable molecular marker for the prognosis of patients with OSC.

3.6 Association of GZMB with biological processes of the immune system

GO annotations and KEGG pathway enrichment analysis were used to assess the potential biological impact of the differentially expressed GZMB in OSC to indicate whether GZMB is involved in the biological processes. From the rel-

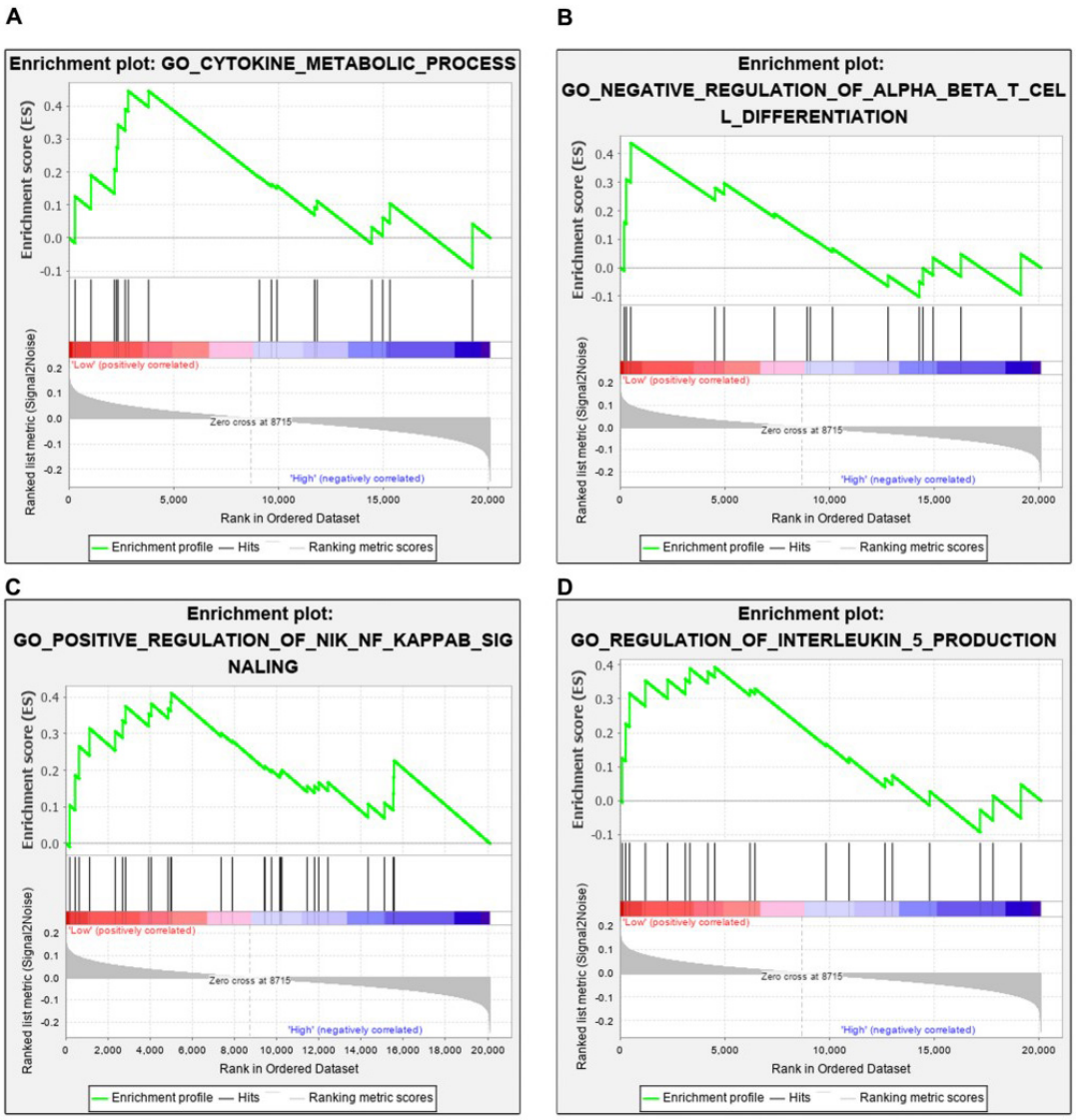

Fig. 5. GSEA analysis of GZMB. (A) Enrichment-plot:cytokine metabolic process. (B) Enrichment-plot: Negative regulation of alpha-beta T cell differenition. (C) Enrichment-plot: Positive regulation of Nik /NF-κB signal. (D) Enrichment-plot: Regulation of IL-5 production. Gene set enrichment analysis (GSEA) showed that GZMB was associated with cytokine metabolic process, negative regulation of alpha-beta T cell differentiation, positive regulation of NIK/NF-κB signaling, and regulation of interleukin 5 production.

evant functional pathways presented in Fig. 4A–B, we found that GZMB was significantly associated with factors such as cell killing, lymphocyte activation, positive regulation of NF-κB signalling, lymphocyte-mediated immunity, B cell activation, regulation of immune system process, response to tumour necrosis factor, and the negative regulation of the apoptotic signalling pathway. GO and KEGG enrichment analyses were carried out to investigate the key pathways. The results showed that main biological pathways are involved including IL-17, p53 and Tumor Necrosis Factor (TNF) signalling pathways as well as pathways in cancer, natural killer cell mediated cytotoxicity, and apoptosis, overall suggesting that GZMB potentially regulates the immune system.

3.7 GZMB analysis by Gene Set Enrichment Analysis

As the results implied that GZMB can play a significant role in the prognosis of OSC, the biological functions of GZMB were explored next using the GSEA approach. Overall, GZMB was found to be related to cytokine metabolic processes, negative regulation of alpha-beta T cell differentiation, positive regulation of Nik/NF-κB signalling, and the regulation of IL-5 production. The aforementioned functions had a positive effect on the low expression of GZMB (Fig. 5). This finding confirmed that GZMB expression may be used as a prognostic marker in OSC.

3.8 PPI network construction

To further define the interactions between GZMB and other hub proteins, a PPI network of GZMB we constructed

based on the information in the STRING protein query from public databases. The PPI network consisted of 12 interacting nodes with 30 edges. The findings showed that GNLY, NOTCH2, CREB1, CAPS3, CASP7, CASP10, BID, CASP8, DFFA, PRF1, and GZMB were closely linked (Fig. 6).

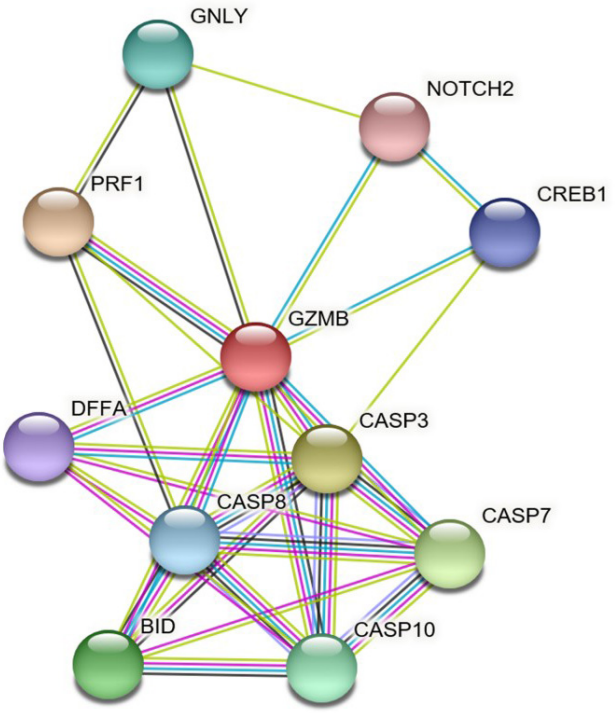

Fig. 6. A protein-protein interaction (PPI) network shows the interaction between the GZMB. Each node represents one gene; the edge indicates the interaction relationship.

3.9 Analysis of the correlation between GZMB and immune infiltration

Next, the expression profiles of GZMB and various immune infiltrations were explored. The data obtained showed the expression of immune markers, such as CD8, macrophages, neutrophils, and dendritic cells (Fig. 7). The results show that GZMB is positively correlated with immune infiltration of OSC.

4. Discussion

High-throughput technologies are increasingly used as very important tools in cancer investigations as means to identify therapeutic targets, for cancer stage classification, early cancer diagnosis, and prognosis prediction [36, 37], which can be quickly and effectively be adapted to clinical applications. Previous studies have identified prognostic biomarkers for patients with OC, whereby patients can be classified and then receive individualized treatment according to the prognostic factors. However, these biomarkers have not yet considerably reduced the social burden caused by OC.

In this study, immunohistochemical methods were used to detect the expression of GZMB in cancer tissues, which revealed that lower GZMB expression was associated with later tumour stages. Analysis of publicly available data from TCGA database further demonstrated that lowed GZMB expression was associated with a poor prognosis in patients with OSC. Whether GZMB is involved in immunoregulation in OSC is currently unclear. To evaluate the function of GZMB in promoting disease progression and to highlight the role of GZMB in the tumour microenvironment, correlations between candidate targets and immune cells using GO analysis, KEGG pathway enrichment and GSEA were performed. GO analysis revealed that GZMB is involved in cell killing, lymphocyte activation, positive regulation of NF-κB signalling, lymphocyte-mediated immunity, B cell activation, regulation of immune system process, response to tumour necrosis factor, and negative regulation of the apoptotic signalling pathway. Hence, GZMB is potentially associated with immunity in OSC.

This study reports GZMB as a potential prognostic marker for OSC and as an immunoregulatory factor in the OSC tumour microenvironment. However, the mechanisms underlying these two findings warrant further validation to completely elucidate the potential of GZMB-targeted therapy in OSC.

There are certain limitations to this study. First, the study lacked data from *in vivo* animal models to validate the current bioinformatics results. Second, the upstream regulators of GZMB and the regulatory roles of GZMB downstream of some signalling pathways in OSC remain to be explained. Owing to the potential benefits of this prognostic biomarker, further investigations and experimental validations are warranted to fully elucidate the roles of GZMB in OSC.

5. Conclusions

GZMB is closely related to the occurrence and development of OSC, and it may be involved in the related immune regulation process. Hence, GZMB may represent a promising prognostic marker to tackle this cancer.

Author contributions

AJL contributed to the conception and design; YMX contributed to provision of study materials and patients, collection and analysis of data. All authors contributed to manuscript writing and final approval of manuscript. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (approval number: 2021-KY-0553-002).

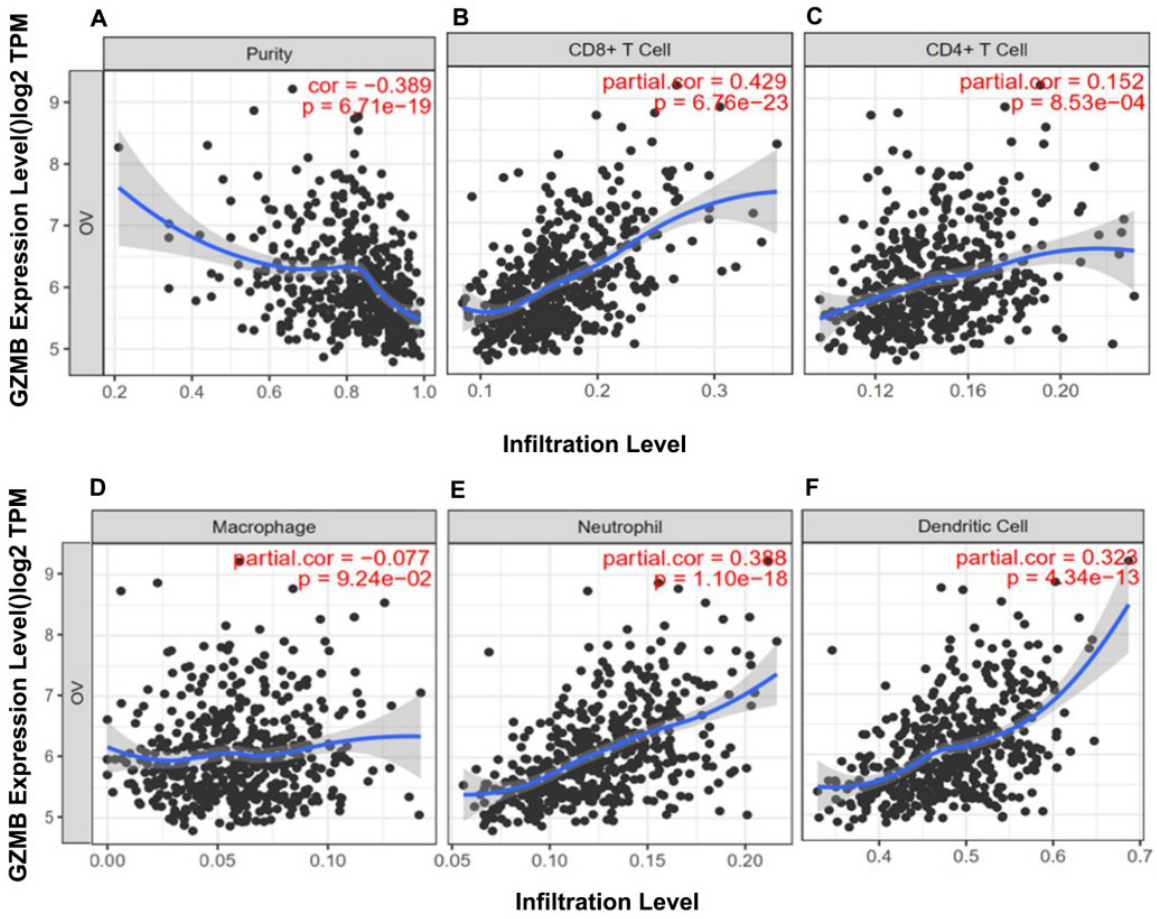

Fig. 7. Relationships between GZMB and immune cell infiltration. (A) Correlation between *GZMB* expression and tumor purity ; (B) Correlation between *GZMB* expression and $CD8^+$ T cell; (C) Correlation between *GZMB* expression and $CD4^+$ T cell; (D) Correlation between *GZMB* expression and macrophage; (E) Correlation between *GZMB* expression and neutrophils; (F) Correlation between *GZMB* expression and dendritic cells.

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Conflict of interest

The authors declare no conflict of interest.

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