Classification of Ovarian Cancer Associated with BRCA1 Mutations, Immune Checkpoints and Tumor Microenvironment Based on Immunogenomic Profiling

Yousheng Wei [Equal first author, 1], **Tingyu Ou** [Equal first author, 1], **Yan Lu** [1], **Guangteng Wu** [1], **Ying Long** [1], **Xinbin Pan** [2], **Desheng Yao** [Corresp. 1]

[1] Department of Gynecologic Oncology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi, China

[2] Department of Radiation Oncology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi, China

Corresponding Author: Desheng Yao
Email address: yaodesheng@gxmu.edu.cn

Background: Ovarian cancer constitutes the leading cause of fatalities among gynecological malignancies, and new effective treatment strategies are required. Recently, immunotherapy has attracted mounting research attention worldwide; however, its therapeutic effect in ovarian cancer has not been satisfactory. Thus, it is necessary to conduct profound investigations on the immune landscape of patients, to improve treatment efficacy.

Methods: The expression profiles, somatic mutation data, as well as clinical information were mined from The Cancer Genome Atlas. We classified ovarian cancer based on 29 immune-associated gene sets, which represented different immune cell types, functions, and pathways. Single-sample gene set enrichment (ssGSEA) was used to quantify the activity or enrichment extents of the gene sets in ovarian cancer, and the unsupervised machine learning method was used to implement the classification. Validation of this classification was then engaged using the Gene Expression Omnibus datasets.

Results: According to the ssGSEA score, we divided ovarian cancer into three subtypes, the subtype 1(immunity low), subtype 2(immunity median), and subtype 3(immunity high) . It was revealed that most tumor-infiltrating immune cells and immune checkpoint molecules were upgraded in the subtype 3 compared with that in the other subtypes. Notably, the tumor mutation burden (TMB) was not significantly different among the three subtypes; however, all patients with BRCA1 mutations were detected in the subtype 3. Furthermore, most immune signature pathways, such as T and B cell receptor signaling, the PD-1 checkpoint pathway in cancer, PD-L1 expression, the NF-κB signaling axis, Th17 cell differentiation and the interleukin-17 signaling pathway, and the TNF signaling axis were hyperactivated in the subtype 3.

Conclusion: The findings of the ovarian cancer subtypes based on immune biosignatures could guide the development of novel therapeutic strategies for ovarian cancer.

Classification of Ovarian Cancer Associated with BRCA1 Mutations, Immune Checkpoints and Tumor Microenvironment Based on Immunogenomic Profiling

Yousheng Wei[1], Tingyu Ou[1]*, Yan Lu[1], Guangteng Wu[1], Ying Long[1], Xinbin Pan[2], Desheng Yao[1]

[1]Department of Gynecologic Oncology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi 530021, P.R. China

[2]Department of Radiation Oncology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi 530021, P.R. China

Correspondence to: De-Sheng Yao, Department of Gynecologic Oncology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi 530021, P.R. China, email: yaodesheng@gxmu.edu.cn

Abbreviations: TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; ssGSEA, Single-sample gene set enrichment; TMB, tumor mutation burden; ICIs, immune checkpoint inhibitors; ORR, objective response rate; FPKM, Fragments Per Kilobase of transcript per Million fragments mapped; ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data; CIBERSORT, Cell-type Identification by Estimating Relative Subsets of RNA Transcripts; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

Key words: Ovarian cancer, tumor microenvironment, immune checkpoint, immunogenomic profiling, classification

Abstract

Background: Ovarian cancer constitutes the leading cause of fatalities among gynecological malignancies, and new effective treatment strategies are required. Recently, immunotherapy has attracted mounting research attention worldwide; however, its therapeutic effect in ovarian cancer has not been satisfactory. Thus, it is necessary to conduct profound investigations on the immune landscape of patients, to improve treatment efficacy.

Methods: The expression profiles, somatic mutation data, as well as clinical information were mined from The Cancer Genome Atlas. We classified ovarian cancer based on 29 immune-associated gene sets, which represented different immune cell types, functions, and pathways. Single-sample gene set enrichment (ssGSEA) was used to quantify the activity or enrichment extents of the gene sets in ovarian cancer, and the unsupervised machine learning method was used to implement the classification. Validation of this classification was then engaged using the Gene Expression Omnibus datasets.

Results: According to the ssGSEA score, we divided ovarian cancer into three subtypes, the subtype 1(immunity low), subtype 2(immunity median), and subtype 3(immunity high). It was revealed that most tumor-infiltrating immune cells and immune checkpoint molecules were upgraded in the subtype 3 compared with that in the other subtypes. Notably, the tumor mutation burden (TMB) was not significantly different among the three subtypes; however, all patients with BRCA1 mutations were detected in the subtype 3. Furthermore, most immune signature pathways, such as T and B cell receptor signaling, the PD-1 checkpoint pathway in cancer, PD-L1 expression, the NF-κB signaling axis, Th17 cell differentiation and the interleukin-17 signaling pathway, and the TNF signaling axis were hyperactivated in the subtype 3.

Conclusion: The findings of the ovarian cancer subtypes based on immune biosignatures could guide the development of novel therapeutic strategies for ovarian cancer.

Introduction

Ovarian cancer constitutes the leading cause of fatalities among gynecological malignancies. It is estimated that approximately 21,750 new cases of ovarian cancer will be diagnosed and 13,940 ovarian cancer fatalities will occur in 2020 in the U.S. *(Siegel, Miller & Jemal, 2020)*. Due to a

lack of symptoms, most ovarian cancer cases are diagnosed at an advanced stage and with a 5-year relative survival of only around 40% *(Bray et al., 2018; Lheureux et al., 2019; Torre et al., 2018)*. Research has deepened our understanding of ovarian cancer; nonetheless, the 5-year survival rate has only improved modestly over the past few decades *(Ghisoni et al., 2019; Holmes, 2015)*. The standard treatment strategy for ovarian cancer includes surgery and platinum-based chemotherapy. Most patients can achieve complete remission from initial treatment; however, the majority ultimately recur *(Odunsi, 2017)*. Therefore, novel therapeutic approaches are urgently needed to improve the quality of life, as well as the survival of these patients.

Cancer immunotherapy has been recently considered as a promising treatment across multiple solid tumors *(Bellmunt et al., 2017; Reck et al., 2016)*. Compared with traditional therapies, cancer immunotherapy eliminates cancer by primarily targeting the immune system or the tumor microenvironment, but not on tumor cells. Cancer cells affect the process of antigen presentation, disrupt the regulatory cascades of T cells, mobilize immune-suppressing cells, and produce active cytokines with immune repressive effects, thereby weakening the immune system, modifying immune regulation, and benefiting tumor cells *(Antonia, Larkin & Ascierto, 2014; Odunsi, 2017)*. The immune checkpoint inhibitor (ICI)-based antibodies, directed at cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death 1 ligand 1 (PD-L1) receptors, as well as programmed cell death 1 (PD-1), have enhanced the survival for patients with different forms of cancer, such as malignant melanoma, bladder cancer, and lung cancer, by initiating the immune cell function and normalizing the tumor microenvironment *(Bellmunt et al., 2017; Borghaei et al., 2015; Robert et al., 2015)*. However, the response rate to ICIs for ovarian cancer patients remains unsatisfying, in which the objective response rate (ORR) was <15% *(Hamanishi et al., 2015; Matulonis et al., 2019)*. In the phase II KEYNOTE-100 study of 376 patients with advanced recurrent ovarian cancer it was found that pembrolizumab monotherapy was linked to an ORR of 8.0% (95% CI, 5.4-11.2), and higher PD-L1 expression level was linked to higher response *(Matulonis et al., 2019)*. It seems that single agent ICIs have exhibited only modest findings in this type of malignancy. In fact, genomic features, such as PD-L1 expression, tumor mutation burden

(TMB), neoantigen load, as well as the defects in DNA damage repair, have so far proven to be associated with tumor immunotherapeutic responsiveness in ovarian cancer *(Ghisoni et al., 2019; Odunsi, 2017; Tian et al., 2020)*.

Here, we classified ovarian cancer based on 29 immune signatures, which represented different immune cell kinds, functions, as well as pathways. Single-sample gene set enrichment (ssGSEA) was used to quantify the activity or enrichment degrees of the gene sets in cancer, then ovarian cancer was classified into three subtypes: subtype 1(immunity low), subtype 2(immunity median), and subtype 3(immunity high). After that we compared the tumor microenvironment, immune cells, immune checkpoint molecules, TMB, BRCA1/2 mutation, prognosis, gene ontology and pathways. Our findings may assist with selecting patients with ovarian cancer who would benefit from immunotherapy.

MATERIALS AND METHODS

Data

Gene expression profiles were mined from The Cancer Genome Atlas (TCGA) repository (https://tcga-data.nci.nih.gov/tcga/) consisting of normalized gene expression patterns for 379 ovarian cancer samples mapped using fragments per kilobase of transcript per million fragments. Clinical data constituting age, survival, stage, and tumor grade were also mined from TCGA. The somatic mutation data were also obtained from single nucleotide polymorphism (SNP) data in TCGA repository using MuTect. The expression data of the validation dataset was retrieved from the Gene Expression Omnibus (GEO) repository (GSE51088), which contains 172 ovarian cancer samples. All computational and statistical analyses were accomplished in the R software (version 3.6.1, http://www.R-project.org).

ssGSEA and Clustering

We obtained 29 immune-correlated gene sets, which typified different immune cell types, functions, as well as pathways, comprising of 707 genes in total (Additional file 1) *(He et al.,*

119 *2018; Yue, Ma & Zhou, 2019)*. Single-sample gene set enrichment analysis (ssGSEA), as
120 accomplished using the GSVA R package (version 1.34.0), was employed in calculating the
121 enrichment scores of the 29 immune biosignatures for each sample in the tumor microenvironment
122 *(Barbie et al., 2009; Hänzelmann, Castelo & Guinney, 2013)*. ssGSEA calculates gene signature
123 overexpression scores by contrasting the level of genes in the signature compared with that in all
124 the other genes in the transcriptome. An unsupervised machine learning method was used to
125 performed hierarchical clustering of ovarian cancer into three clusters. Then, according to the
126 immune scores, the clusters were distributed into three distinct subtypes: subtype 1, subtype 2, and
127 subtype 3.
128
129 ***ESTIMATE and CIBERSORT***
130 Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data
131 (ESTIMATE) *(Yoshihara et al., 2013)* is an approach that employs gene expression biosignatures
132 to deduce the proportion of stromal, as well as immune cells in tumor samples, which form the
133 major non-tumor constituents of tumor samples. By performing ssGSEA, it calculates stromal, as
134 well as immune scores to estimate the level of invading stromal, as well as immune cells, which
135 forms the rationale for the ESTIMATE score to deduce tumor purity in the tumor tissue. By using
136 "ESTIMATE" in R package, each ovarian cancer sample from the immune and stromal scores,
137 and tumor purity was calculated based on the gene expression data. Cell-type Identification by
138 Estimating Relative Subsets of RNA Transcripts (CIBERSORT) *(Newman et al., 2015)* is a
139 biological tool, that uses the deconvolution strategy to compute the fractions of the 22 human
140 immune cell types. Here, we set 1000 permutations and data with $P<0.05$, as the maxim for the
141 successful deconvolution of a sample. The Kruskal-Wallis test was employed in comparing the
142 proportions of immune cell types among ovarian cancer subtypes.
143
144 ***Calculation of TMB scores***
145 TMB is the overall enumeration of mutations per million bases in tumor tissue. Typically, it is the

mutation density of tumor genes, i.e., the enumeration of mutations in the tumor genome, entailing the total enumeration of genetic coding errors, base substitutions, and gene insertions or deletions. Herein, we computed the mutation frequency with the enumeration of variants/the length of exons (38 million) for every sample with Perl (v5.30.1, https://www.perl.org/).

Survival analyses

We retrieved the follow-up information of patients from the clinical data and calculated the significance of survival time via the log-rank test, and differences using a threshold of $P<0.05$. For the relationship between related immune gene sets score and prognosis, we used the median, as the cut-off value to cluster the samples into high score or low score levels. We plotted the Kaplan-Meier curves to indicate the survival period differences.

Gene-set enrichment evaluations

Gene-set enrichment analysis of TCGA datasets was conducted in the GSEA (R implementation). *(Subramanian et al., 2005)*. Kyoto Encyclopedia of Genes and Genomes (KEGG), as well as Gene Ontology (GO) analyses were employed in assessing the functional role of the differentially expressed genes between subtype 1 and subtype 3. Differential gene set enrichment was inspected in the limma R package. $P<0.05$ was used as the cut-off value.

Results

Immunogenomic profiling identifies three ovarian cancer subtypes

Using ssGSEA, we obtained enrichment scores for the 29 immune-associated gene sets, for each sample in the tumor microenvironment. Then, according to the immune scores, we hierarchically clustered ovarian cancer into three classes. Interestingly, three classes were separated clearly then, we confirmed the three classes as subtype 1, subtype 2, and subtype 3 which represented immunity low, immunity median, and immunity high, respectively (Fig.1A). Applying the ESTIMATE algorithm, we determined the immune, stromal, and ESTIMATE scores, and tumor purity. We

established that the immune and stromal scores were the highest in the subtype 3 and the lowest in subtype 1, while tumor purity was the highest in the subtype 1 and the lowest in subtype 3, and the difference was significant (P<0.001) (Fig.1B). These findings indicated that immune and stromal cells have the highest content in the subtype 3, while tumor cells have the highest content in the subtype 1.

Furthermore, we found that most of the human leukocyte antigen (HLA) gene expression levels were the lowest in the subtype 1 and the highest in subtype 3(P<0.001) (Fig.S1A). In addition, the expression levels of many immune cell subgroup biomarker genes, such as FOXP3 [regulatory T cell (Treg)], CD45RO (memory T cell), CD8A (cytotoxic T cell), CD20 (B cell), CD1A [immature dendritic cell (iDC)], CXCR5 (Tfh cell), IL3RA [plasmacytoid dendritic cell (pDC)] were remarkably higher in the subtype 3 and markedly lower in the subtype 1(Fig.S1B).

Three subtypes show differential expression of immune checkpoint genes

We analyzed the expression levels of the checkpoint receptors, which decreased T cell bioactivity, including PDCD1 (PD1), CTLA4, LAG-3, and TIM-3 in the three ovarian cancer subtypes. Then, the PDCD1 ligand CD274 (PD-L1), PDCD1LG2 (PD-L2), the CTLA4 ligand CD86, and CD80 were also analyzed. We found that the expression levels of these 8 immune checkpoint genes were all remarkably lower in the subtype 1 and markedly elevated in the subtype 3(P<0.001) (Fig.2). This result indicated that the immunophenotype of our hierarchical cluster could be clearly distinguished, and the ovarian cancer subtype 3 might respond more effectively to checkpoint inhibitor therapy.

Analysis of the TMB and BRCA mutations among the 3 subtypes of ovarian cancer

TMB has been considered as a predictor of tumor behavior and immunological response in a diverse range of cancers *(Goodman et al., 2017)*. In general, tumors with a high TMB have elevated levels of neoantigens, which play an important role in immunotherapy activities *(Goodman et al., 2017; Schumacher & Schreiber, 2015)*. We mined the somatic mutation profiles of 436 ovarian

cancer patients from the SNP data in TCGA using MuTect, and then the TMB was calculated using the enumeration of the mutation incidences per million bases. Then, the TMB between the 3 subtypes of ovarian cancer were analyzed, and we found that the three subtypes were not remarkably related to TMB (P=0.732) (Fig.3A).

An escalating number of reports have documented that targeted therapies can stimulate the immune response of the host. The discussion of the relationship between BRCA mutations and immunity is being investigated at present. Here, we analyzed the connection linking BRCA1 and BRCA2 mutations in the 3 subtypes. The BRCA1 and BRCA2 mutation data were mined from the SNP data in TCGA via MuTect, and there were 23 patients with the BRCA1 mutation and 20 patients with the BRCA2 mutation, out of 436 ovarian cancer patients. Using the intersection between the mutation data and the immunity clusters data samples, we found 13 patients with the BRCA1 mutation and 13 patients with the BRCA2 mutation, out of 274 ovarian cancer patients. Surprisingly, we found that all the BRCA1 mutation patients were in the subtype 3, and the difference was remarkable (χ^2 test, P=0.0016) (Fig.3B). The BRCA2 mutation ratio was greater in the subtype 2 and subtype 3 compared with that in the subtype 1, but the difference was not statistically remarkable (χ^2 test, P=0.577) (Fig.3C).

Different immune cells among the 3 subtypes of ovarian cancer

CIBERSORT can deduce 22 types of human immune cells, such as B cells, myeloid subset cells, T cells, macrophages, NK cells, as well as, DCs, according to the gene expression data using the gene-based deconvolution algorithm method *(Newman et al., 2015)*. Here, we set 1000 permutations and data with $P < 0.05$, as the maxim for the successful deconvolution of a sample. Consequently, CD8 T cells, CD4 memory activated T cells, Tregs, macrophages M1, resting dendritic cells were all at the highest level in the subtype 3 and at the lowest levels in the subtype 1($P< 0.01$). However, activated dendritic cells had an opposite trend (Fig. 4).

Prognostic analysis of the ovarian cancer subtypes and immune-associated gene sets

Survival analyses indicated that the three ovarian cancer subtypes had a considerable difference in prognosis. The subtype 2 had the worst survival prognosis among the three subtypes; however, there was no remarkable difference in survival between the subtype 1 and the subtype 3. Furthermore, we analyzed the prognostic value of the different immune gene sets expression score in predicting patient survival. We found that high expression level of check-point, major histocompatibility complex (MHC) class I, APC co-inhibition, T cell co-inhibition, Th1 and Th2 cells, Tfh, inflammation-promoting, and Tregs was associated with a better prognosis compared with that with the low expression levels, and the difference was remarkable (P<0.05) (Fig.5).

Identification of the ovarian cancer subtype-specific pathways and GO

GSEA identified 628 GO and 56 KEGG terms in the subtype 1 and subtype 3. The GO analysis indicated that the immunoglobulin complex, circulating immunoglobulin complex, the MHC class II protein complex, immunoglobulin receptor binding and the MHC protein complex were the top 5 significantly enriched biological processes in the subtype 3. In addition, glucuronidation, metabolic process and methyl-CpG binding were the most enriched terms in the subtype 1. (Fig.6A, B). The GSEA result showed that the immune-correlated cascades were most active in the subtype 3, consisting of Th17 cell differentiation, the NF-κ B signaling axis, the B cell receptor signaling cascade, the T cell receptor signaling cascade, PD-L1 expression and the PD-1 checkpoint axis in cancer, the IL-17 signaling cascade and the tumor necrosis factor (TNF) signaling axis. This result verified that immune activity was increased in the subtype 3. However, the subtype 1 was enriched in pathways, such as maturity onset diabetes of the young, ascorbate and aldarate metabolism, pentose and glucuronate interconversions, fat digestion and absorption, porphyrin and chlorophyll metabolism (Fig.6C, D). This suggests that these cascades could be inversely linked to ovarian cancer immunity.

Validation of external datasets

The same method was used to hierarchically cluster ovarian cancer in the GSE51088 dataset,

254 which includes 172 ovarian cancer samples. Interestingly, it showed a similar clustering result,
255 with three clusters separated (Fig.7A). We established that the immune and stromal scores were
256 remarkably higher in the subtype 3 and markedly lower in the subtype 1, while tumor purity had
257 an opposite result (Fig.7B). Consistent with TCGA datasets, most HLA genes and CD8A, CD1A,
258 CD45R, IL3RA expression levels were significantly lower in the subtype 1 and significantly
259 higher in the subtype 3(Fig.S2A, Fig.S2B). Furthermore, the expression of the immune checkpoint
260 genes, entailing PDCD1, CD274, TIM-3, CTLA4, CD80, CD86, LAG-3 were all remarkably
261 lower in the subtype 1 and significantly higher in the subtype 3(Fig.7C), these were also similar to
262 previous research. These results suggested that there were different subtypes of immune status in
263 ovarian cancer, and they might have different effects on the treatment of immune checkpoints.
264
265 **Discussion**
266 Recently, an escalating number of reports have identified ovarian cancer subtypes based on
267 genomic profiling to achieve individualized treatment and improve patient survival *(Schwede et*
268 *al., 2020; Yang et al., 2018; Zheng et al., 2020)*. However, few studies have classified ovarian
269 cancer based on immune signatures. In this study, we sought to identify immune-correlated ovarian
270 cancer subtypes in TCGA-ovarian cancer cohort based on 29 immune-linked gene sets, which
271 typified different immune cell types, functions, as well as pathways. Using ssGSEA, we could
272 classify ovarian cancer into three subtypes, with an immune score range from low to high.
273 Furthermore, it was reproducible and predictable in the external dataset, GSE51088.
274 We found that the immune microenvironment of the subtype 3 was strengthened, and the
275 immune cell invasion, as well as anti-tumor immune activities was stronger, such as high levels of
276 cytotoxic T cells and B cell invasion. Furthermore, the levels of expression of most of the HLA
277 genes were highest in the subtype 3. A core step in the threshold of the immune response is the
278 recognition and expression of tumor antigens on effector cells, such as CD8+ T cells. HLA serves
279 a central role in providing effector CD8+ T cells with natural intracellular proteins or neoantigens
280 produced by the cancer cells *(Koşaloğlu-Yalçın et al., 2018)*. In most human tumors, down

modulation of the expression of HLA class I participate in the escape from the host immune system, as well as immunotherapy resistance *(Chowell et al., 2018; Lhotakova et al., 2019)*. In addition, many immune cell subgroup marker genes, such as FOXP3 (Treg), CD45RO (memory T cell), CD8A (cytotoxic T cell), CD20 (B cell), CD1A (iDC), CXCR5 (Tfh cell), IL3RA (pDC) were strengthened in the subtype 3. Furthermore, we found that CD8 T cells, macrophages M1, CD4 memory activated T cells, Tregs, resting dendritic cells were markedly higher in the subtype 3 and remarkably lower in the subtype 1. These results further confirmed that there were different subtypes of immune status in ovarian cancer, and the immune activity of the subtype 3 was strengthened. The survival analyses showed that the most dismal prognosis was found in the subtype 2; however, there was no significant survival difference between the subtype 1 and the subtype 3. This suggested that the immune-enhanced subtypes may not have the best outcome in ovarian carcinoma, which was consistent with the findings from Zheng *et al (Zheng et al., 2020)*.

To date, numerous studies have demonstrated that immune checkpoint serves a pivotal role in the immune escape of cancer. It is well-known that, PD-1, CTLA4, LAG-3, VISTA, TIM-3, and BTLA are the most common immune checkpoint receptors. It was previously reported that blocking PD1/PD-L1 was more effective when it was utilized in combination with other agents, particularly other checkpoint suppressors *(Boutros et al., 2016; Doo, Norian & Arend, 2019; Huang et al., 2017)*. Clinical studies have shown that the effect of treatment in patients with advanced melanoma could be improved when combined with the anti-PD-1/PD-L1 antibody and the CTLA-4 inhibitor *(Boutros et al., 2016)*. A previous study showed that PD-1 blocking alone was insufficient in controlling murine ovarian tumor growth; nevertheless, dual blocking of the PD-1-LAG-3 or PD-1-CTLA-4 cascades could delay murine ovarian tumor growth and that blocking of 3 PD-1-CTLA-4-LAG-3 cascades was superior if the PD-1 pathway was entirely blocked *(Huang et al., 2017)*. Here, we identified that the expression level of the checkpoint genes, entailing PDCD1, CD274, TIM-3, CD80, PDCD1LG2, CTLA4, LAG-3, and CD86 was remarkably higher in the subtype 3. The data revealed that the subtype 3 may be linked to the

intrinsic immune escape of ovarian cancer, which may unearth novel insights for the treatment of ovarian cancer with immune checkpoint blockers.

Many studies have discovered that a higher level of TMB was associated with higher neoantigen loads, which have been verified to be the target of ICIs *(Brown et al., 2014; Gubin et al., 2014; Samstein et al., 2019)*. TMB generates new antigens resulting in the enrichment of the immune cells in tumors, which could predict survival across diverse kinds of human cancer, e.g., non-small cell lung cancer, melanoma, and bladder cancer, and is applicable in patients under the treatment of either anti-CTLA-4 or anti-PD-1 therapies *(Samstein et al., 2019)*. Contrary to conventional views, we failed to detect an association between TMB and tumor infiltrating immune cells and no significant difference was found in TMB among the three immune ovarian cancer subtypes. Similarly, Dai *et al (Dai et al., 2018)* found there was no association between TMB and the tumor immune response, represented by cytolytic activity or immune cell infiltration. Therefore, TMB may not serve well as the biomarker for immunotherapies in ovarian cancer. Interestingly, we found that all the patients with BRCA1 mutations were in the subtype 3 and the patients with BRCA2 mutations were primarily in the subtype 2 and subtype 3. It was previously found that ovarian cancer, with BRCA1 or BRCA2 mutations, had increased immune infiltrates compared with those without mutations *(McAlpine et al., 2012)*. Strickland *et al (Strickland et al., 2016)* demonstrated that BRCA1/2-mutated high grade serous ovarian cancer depicted remarkably elevated CD3+ and CD8+ tumor-invading lymphocytes, and elevated levels of expression of PD-1, as well as PD-L1 in the tumor-linked immune cells contrasted with that in homologous recombination proficient tumors. Another study also showed that intraepithelial CD8+ T cells was linked to the presence of a mutation or loss of expression of BRCA1 *(Clarke et al., 2009)*. These findings suggest that BRCA-mutated ovarian cancer may be more sensitive to immune checkpoint blockade therapy.

Using enrichment analysis, we identified 628 GO and 56 KEGG terms in the subtype 1 and subtype 3. The GO analysis indicated that the immunoglobulin complex, the MHC class II protein complex, immunoglobulin receptor binding and the MHC protein complex were primarily

enriched in biological processes in the subtype 3. T cell immunity needs recognition of antigens in the context of MHC class I and class II proteins by CD8+ and CD4+ T cells, respectively *(Koşaloğlu-Yalçın et al., 2018)*. A previous study chronicled that the MHC proteins offer differential sensitivity to CTLA-4 and PD-1 blocking in melanoma *(Rodig et al., 2018)*. The immune-linked cascades were most active in the subtype 3, entailing Th17 cell differentiation, the NF-κB signaling cascade, the B cell receptor signaling axis, the T cell receptor signaling axis, PD-L1 expression and the PD-1 checkpoint cascade in cancer, the IL-17 signaling axis and the TNF signaling cascade. It has been proven that the NF-κB signaling axis is the major cascade involved in ovarian cancer, that enhances chemoresistance, cancer stem cell maintenance, metastasis, as well as immune evasion *(Harrington & Annunziata, 2019)*. Bilska *et al* *(Bilska et al., 2020)* indicated a proinflammatory nature of the ovarian cancer microenvironment, with high levels of IL-17A in the peritoneal fluid and a high percentage of Th17-infiltrating ovarian cancer, and suggested that Th17 cells/IL-17A might serve an advantageous role in ovarian cancer immunity. There have been a number of studies that have proved that the PD-1/PD-L1 cascade, the B cell and T cell receptor signaling axis and the TNF signaling cascade were associated with the immunity of ovarian cancer *(Ghisoni et al., 2019; Gupta et al., 2019; Josephs et al., 2017)*.

Nevertheless, there are some limitations in this study. First of all, the data utilized herein was from public repositories, not generated by ourselves. Secondly, the BRCA1 and BRCA2 mutation ratios in ovarian cancer were relatively low in the SNP data from TCGA. Hence, further research recruiting a larger sample size is required to validate the relevance of BRCA mutations with ovarian cancer immunity. Finally, immunogenomic analysis requires more experimental evidence to verify the role of BRCA1/BRCA2 mutations, checkpoint genes, and the enriched cascades involved in the immune microenvironment.

In conclusion, we identified ovarian cancer subtypes base on immune signatures which were distinct in tumor microenvironment, immune cells, immune checkpoint molecules, BRCA mutation and clinical prognosis. These findings may provide guidance for developing novel strategies of immunotherapy in ovarian cancer.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81760466).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Desheng Yao designed the project and proposed the research concept. Yousheng Wei and Tingyu Ou performed the bioinformatic analysis, constructed the graphic images and data charts, and performed the statistical processing. Yousheng Wei wrote the manuscript. Xinbin Pan and Guangteng Wu jointly performed the bioinformatic analysis and designed the figures. Ying Long and Yan Lu searched and downloaded the data, and performed a literature review. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Availability of data and material

The following information was supplied regarding data availability:

Gene expression profiles and clinical data are available at The Cancer Genome Atlas (TCGA) repository(https://tcga-data.nci.nih.gov/tcga/). Search terms: "Primary Site" IS "ovary" AND "Program" IS "TCGA" AND "Project" IS "TCGA-OV" AND "Data Category" IS "Transcriptome Profiling" AND "Data Type" IS "Gene Expression Quantification" AND "Workflow Type" IS "HTSeq-FPKM".

The somatic mutation data is also available at TCGA repository. Search terms: "Primary Site" IS "ovary" AND "Program" IS "TCGA" AND "Project" IS "TCGA-OV" AND "Data Category" IS "Simple Nucleotide Variation" AND "Data Type" IS "Masked Somatic Mutation" AND "Workflow Type" IS "Mu Tect2 Variant aggregation and Masking".

The expression data of the validation dataset is available at NCBI GEO under accession numbers GSE51088.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

References

Antonia SJ, Larkin J, Ascierto PA. 2014. Immuno-oncology combinations: a review of clinical experience and future prospects. *Clinical cancer research : an official journal of the American Association for Cancer Research* **20:**6258-6268 DOI 10.1158/1078-0432.CCR-14-1457.

Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, Fröhling S, Chan EM, Sos ML, Michel K, Mermel C, Silver SJ, Weir BA, Reiling JH, Sheng Q, Gupta PB, Wadlow RC, Le H, Hoersch S, Wittner BS, Ramaswamy S, Livingston DM, Sabatini DM, Meyerson M, Thomas RK, Lander ES, Mesirov JP, Root DE, Gilliland DG, Jacks T, Hahn WC. 2009. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* **462:**108-112 DOI 10.1038/nature08460.

Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ, Climent MA, Petrylak DP, Choueiri TK, Necchi A, Gerritsen W, Gurney H, Quinn DI, Culine S, Sternberg CN, Mai Y, Poehlein CH, Perini RF, Bajorin DF, KEYNOTE-045 Investigators. 2017. Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. *The New England journal of medicine* **376:**1015-1026 DOI 10.1056/NEJMoa1613683.

Bilska M, Pawłowska A, Zakrzewska E, Chudzik A, Suszczyk D, Gogacz M, Wertel I. 2020. Th17 Cells and IL-17 As Novel Immune Targets in Ovarian Cancer Therapy. *Journal of oncology* **2020:**8797683 DOI 10.1155/2020/8797683.

Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhäufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crinò L, Blumenschein GR Jr, Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, Brahmer JR. 2015. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *The New England journal of medicine* **373:**1627-1639 DOI 10.1056/NEJMoa1507643.

Boutros C, Tarhini A, Routier E, Lambotte O, Ladurie FL, Carbonnel F, Izzeddine H, Marabelle A, Champiat S, Berdelou A, Lanoy E, Texier M, Libenciuc C, Eggermont AM, Soria JC, Mateus C, Robert C. 2016. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. *Nature reviews. Clinical oncology* **13:**473-486 DOI 10.1038/nrclinonc.2016.58.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians* **68:**394-424 DOI 10.3322/caac.21492.

Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, Holt RA. 2014. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome research* **24:**743-750 DOI 10.1101/gr.165985.113.

Chowell D, Morris L, Grigg CM, Weber JK, Samstein RM, Makarov V, Kuo F, Kendall SM, Requena D, Riaz N,

Greenbaum B, Carroll J, Garon E, Hyman DM, Zehir A, Solit D, Berger M, Zhou R, Rizvi NA, Chan TA. 2018. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science* **359:**582-587 DOI 10.1126/science.aao4572.

Clarke B, Tinker AV, Lee CH, Subramanian S, van de Rijn M, Turbin D, Kalloger S, Han G, Ceballos K, Cadungog MG, Huntsman DG, Coukos G, Gilks CB. 2009. Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* **22:**393-402 DOI 10.1038/modpathol.2008.191.

Dai Y, Sun C, Feng Y, Jia Q, Zhu B. 2018. Potent immunogenicity in BRCA1-mutated patients with high-grade serous ovarian carcinoma. *Journal of cellular and molecular medicine* DOI 10.1111/jcmm.13678.

Doo DW, Norian LA, Arend RC. 2019. Checkpoint inhibitors in ovarian cancer: A review of preclinical data. *Gynecologic oncology reports* **29:**48-54 DOI 10.1016/j.gore.2019.06.003.

Ghisoni E, Imbimbo M, Zimmermann S, Valabrega G. 2019. Ovarian Cancer Immunotherapy: Turning up the Heat. *International journal of molecular sciences* **20:** DOI 10.3390/ijms20122927.

Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, Stephens PJ, Daniels GA, Kurzrock R. 2017. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Molecular cancer therapeutics* **16:**2598-2608 DOI 10.1158/1535-7163.MCT-17-0386.

Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, Mulder GE, Toebes M, Vesely MD, Lam SS, Korman AJ, Allison JP, Freeman GJ, Sharpe AH, Pearce EL, Schumacher TN, Aebersold R, Rammensee HG, Melief CJ, Mardis ER, Gillanders WE, Artyomov MN, Schreiber RD. 2014. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* **515:**577-581 DOI 10.1038/nature13988.

Gupta P, Chen C, Chaluvally-Raghavan P, Pradeep S. 2019. B Cells as an Immune-Regulatory Signature in Ovarian Cancer. *Cancers* **11:** DOI 10.3390/cancers11070894.

Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, Kanai M, Mori Y, Matsumoto S, Chikuma S, Matsumura N, Abiko K, Baba T, Yamaguchi K, Ueda A, Hosoe Y, Morita S, Yokode M, Shimizu A, Honjo T, Konishi I. 2015. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **33:**4015-4022 DOI 10.1200/JCO.2015.62.3397.

Harrington BS, Annunziata CM. 2019. NF-κB Signaling in Ovarian Cancer. *Cancers* **11:** DOI 10.3390/cancers11081182.

He Y, Jiang Z, Chen C, Wang X. 2018. Classification of triple-negative breast cancers based on Immunogenomic profiling. *Journal of experimental & clinical cancer research : CR* **37:**327 DOI 10.1186/s13046-018-1002-1.

Holmes D. 2015. Ovarian cancer: beyond resistance. *Nature* **527:**S217 DOI 10.1038/527S217a.

Huang RY, Francois A, McGray AR, Miliotto A, Odunsi K. 2017. Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. *Oncoimmunology* **6:**e1249561 DOI 10.1080/2162402X.2016.1249561.

Hänzelmann S, Castelo R, Guinney J. 2013. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC bioinformatics* **14:**7 DOI 10.1186/1471-2105-14-7.

Josephs DH, Bax HJ, Dodev T, Georgouli M, Nakamura M, Pellizzari G, Saul L, Karagiannis P, Cheung A, Herraiz C, Ilieva KM, Correa I, Fittall M, Crescioli S, Gazinska P, Woodman N, Mele S, Chiaruttini G, Gilbert AE, Koers A, Bracher M, Selkirk C, Lentfer H, Barton C, Lever E, Muirhead G, Tsoka S, Canevari S, Figini M, Montes A, Downes N, Dombrowicz D, Corrigan CJ, Beavil AJ, Nestle FO, Jones PS, Gould HJ, Sanz-Moreno V, Blower PJ, Spicer JF,

Karagiannis SN. 2017. Anti-Folate Receptor-α IgE but not IgG Recruits Macrophages to Attack Tumors via TNFα/MCP-1 Signaling. *Cancer research* **77:**1127-1141 DOI 10.1158/0008-5472.CAN-16-1829.

Koşaloğlu-Yalçın Z, Lanka M, Frentzen A, Logandha Ramamoorthy Premlal A, Sidney J, Vaughan K, Greenbaum J, Robbins P, Gartner J, Sette A, Peters B. 2018. Predicting T cell recognition of MHC class I restricted neoepitopes. *Oncoimmunology* **7:**e1492508 DOI 10.1080/2162402X.2018.1492508.

Lheureux S, Gourley C, Vergote I, Oza AM. 2019. Epithelial ovarian cancer. *Lancet* **393:**1240-1253 DOI 10.1016/S0140-6736(18)32552-2.

Lhotakova K, Grzelak A, Polakova I, Vackova J, Smahel M. 2019. Establishment and characterization of a mouse tumor cell line with irreversible downregulation of MHC class I molecules. *Oncology reports* **42:**2826-2835 DOI 10.3892/or.2019.7356.

Matulonis UA, Shapira-Frommer R, Santin AD, Lisyanskaya AS, Pignata S, Vergote I, Raspagliesi F, Sonke GS, Birrer M, Provencher DM, Sehouli J, Colombo N, González-Martín A, Oaknin A, Ottevanger PB, Rudaitis V, Katchar K, Wu H, Keefe S, Ruman J, Ledermann JA. 2019. Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study. *Annals of oncology : official journal of the European Society for Medical Oncology* **30:**1080-1087 DOI 10.1093/annonc/mdz135.

McAlpine JN, Porter H, Köbel M, Nelson BH, Prentice LM, Kalloger SE, Senz J, Milne K, Ding J, Shah SP, Huntsman DG, Gilks CB. 2012. BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* **25:**740-750 DOI 10.1038/modpathol.2011.211.

Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA. 2015. Robust enumeration of cell subsets from tissue expression profiles. *Nature methods* **12:**453-457 DOI 10.1038/nmeth.3337.

Odunsi K. 2017. Immunotherapy in ovarian cancer. *Annals of oncology : official journal of the European Society for Medical Oncology* **28:**viii1-1viii7 DOI 10.1093/annonc/mdx444.

Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O'Brien M, Rao S, Hotta K, Leiby MA, Lubiniecki GM, Shentu Y, Rangwala R, Brahmer JR, KEYNOTE-024 Investigators. 2016. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *The New England journal of medicine* **375:**1823-1833 DOI 10.1056/NEJMoa1606774.

Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V, Ascierto PA. 2015. Nivolumab in previously untreated melanoma without BRAF mutation. *The New England journal of medicine* **372:**320-330 DOI 10.1056/NEJMoa1412082.

Rodig SJ, Gusenleitner D, Jackson DG, Gjini E, Giobbie-Hurder A, Jin C, Chang H, Lovitch SB, Horak C, Weber JS, Weirather JL, Wolchok JD, Postow MA, Pavlick AC, Chesney J, Hodi FS. 2018. MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. *Science translational medicine* **10:** DOI 10.1126/scitranslmed.aar3342.

Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, Kaley TJ, Kendall SM, Motzer RJ, Hakimi AA, Voss MH, Russo P, Rosenberg J, Iyer G, Bochner BH, Bajorin DF, Al-Ahmadie HA, Chaft JE, Rudin CM, Riely GJ, Baxi S, Ho AL, Wong RJ, Pfister DG, Wolchok JD, Barker CA, Gutin PH, Brennan CW, Tabar V, Mellinghoff IK, DeAngelis LM, Ariyan CE, Lee N, Tap WD, Gounder MM, D'Angelo SP, Saltz L, Stadler ZK, Scher HI, Baselga J, Razavi P, Klebanoff CA, Yaeger R, Segal NH, Ku GY,

DeMatteo RP, Ladanyi M, Rizvi NA, Berger MF, Riaz N, Solit DB, Chan TA, Morris L. 2019. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nature genetics* **51:**202-206 DOI 10.1038/s41588-018-0312-8.

Schumacher TN, Schreiber RD. 2015. Neoantigens in cancer immunotherapy. *Science* **348:**69-74 DOI 10.1126/science.aaa4971.

Schwede M, Waldron L, Mok SC, Wei W, Basunia A, Merritt MA, Mitsiades CS, Parmigiani G, Harrington DP, Quackenbush J, Birrer MJ, Culhane AC. 2020. The Impact of Stroma Admixture on Molecular Subtypes and Prognostic Gene Signatures in Serous Ovarian Cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **29:**509-519 DOI 10.1158/1055-9965.EPI-18-1359.

Siegel RL, Miller KD, Jemal A. 2020. Cancer statistics, 2020. *CA: a cancer journal for clinicians* **70:**7-30 DOI 10.3322/caac.21590.

Strickland KC, Howitt BE, Shukla SA, Rodig S, Ritterhouse LL, Liu JF, Garber JE, Chowdhury D, Wu CJ, D'Andrea AD, Matulonis UA, Konstantinopoulos PA. 2016. Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. *Oncotarget* **7:**13587-13598 DOI 10.18632/oncotarget.7277.

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* **102:**15545-15550 DOI 10.1073/pnas.0506580102.

Tian W, Shan B, Zhang Y, Ren Y, Liang S, Zhao J, Zhao Z, Wang G, Zhao X, Peng D, Bi R, Cai S, Bai Y, Wang H. 2020. Association between DNA damage repair gene somatic mutations and immune-related gene expression in ovarian cancer. *Cancer medicine* DOI 10.1002/cam4.2849.

Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL. 2018. Ovarian cancer statistics, 2018. *CA: a cancer journal for clinicians* **68:**284-296 DOI 10.3322/caac.21456.

Yang L, Wang S, Zhang Q, Pan Y, Lv Y, Chen X, Zuo Y, Hao D. 2018. Clinical significance of the immune microenvironment in ovarian cancer patients. *Molecular omics* **14:**341-351 DOI 10.1039/c8mo00128f.

Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, Treviño V, Shen H, Laird PW, Levine DA, Carter SL, Getz G, Stemke-Hale K, Mills GB, Verhaak RG. 2013. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nature communications* **4:**2612 DOI 10.1038/ncomms3612.

Yue C, Ma H, Zhou Y. 2019. Identification of prognostic gene signature associated with microenvironment of lung adenocarcinoma. *PeerJ* **7:**e8128 DOI 10.7717/peerj.8128.

Zheng M, Hu Y, Gou R, Liu O, Nie X, Li X, Liu Q, Hao Y, Liu J, Lin B. 2020. Identification of immune-enhanced molecular subtype associated with BRCA1 mutations, immune checkpoints and clinical outcome in ovarian carcinoma. *Journal of cellular and molecular medicine* **24:**2819-2831 DOI 10.1111/jcmm.14830.

Figure 1

Immunogenomic profiling identifies three ovarian cancer subtypes.

(A) Ovarian cancer was hierarchically clustered into three clusters in The Cancer Genome Atlas dataset. In the heat map of gene expression, red represents high expression and blue represents low expression. Tumor purity, ESTIMATE score, stromal score, and immune score were calculated using ESTIMATE. (B) The distribution of tumor purity, immune score, and stromal score in the three immune subtypes were compared, respectively. ***P<0.001. ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data.

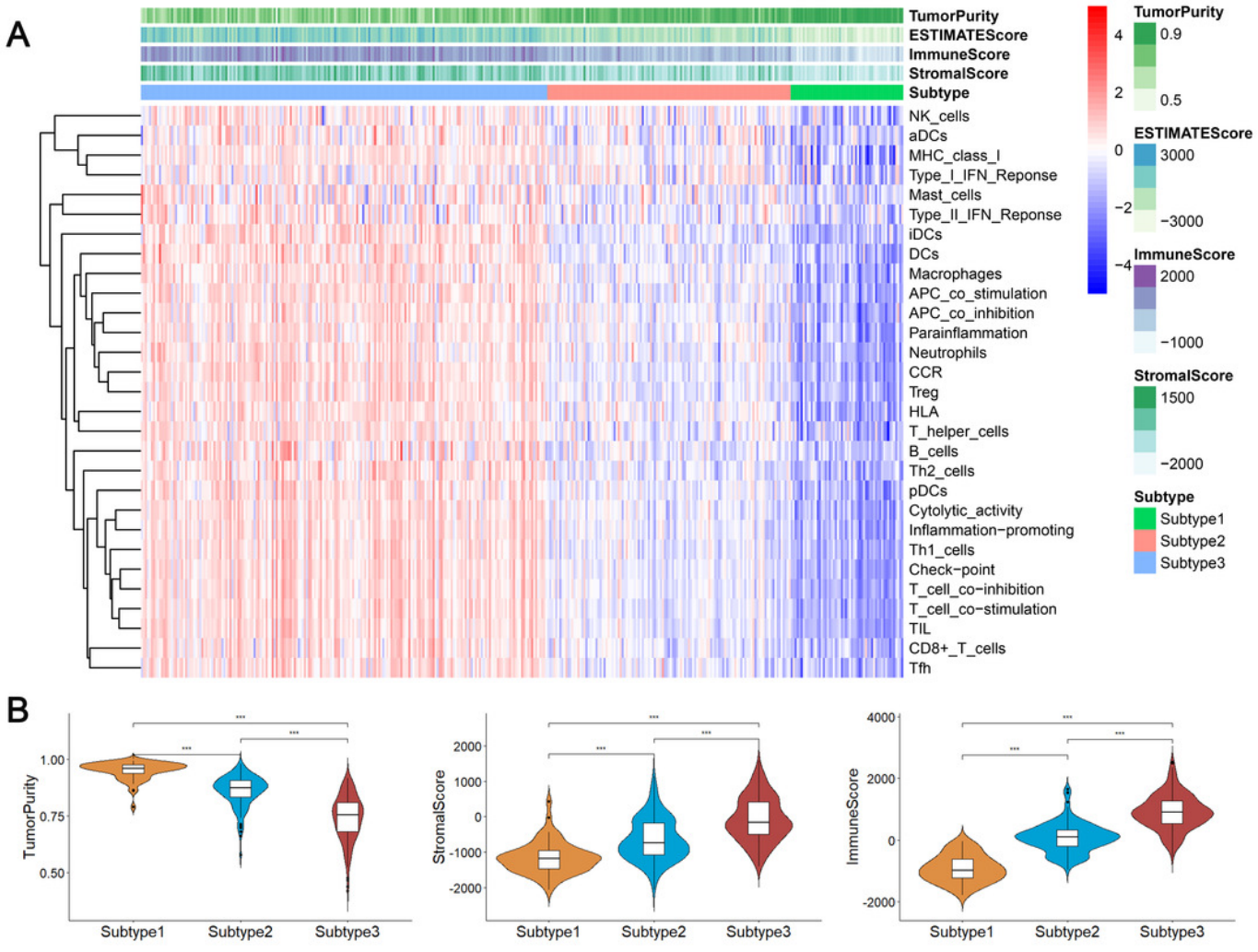


Figure 2

TMB and BRCA mutation among the three subtypes of ovarian cancer.

(A) Three subtypes were not significantly correlated with TMB. (B) All patients with BRCA1 mutations were concentrated in the subtype 3 and the difference was significant (χ^2 test, P=0.0016). (C) The patients with BRCA2 mutations were mainly found in the subtype 2 and subtype 3, but the difference was not significant (χ^2 test, P=0.577). TMB, tumor mutation burden.

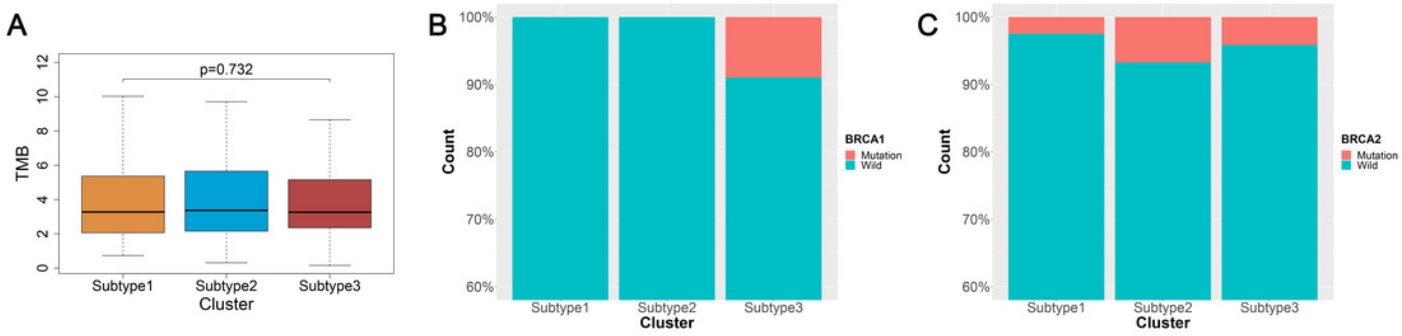


Figure 3

Differential proportions of the immune cells in the three ovarian cancer subtypes.

Resting dendritic cells, macrophages M1, CD4 memory activated T cells, CD8 T cells, regulatory T cells were highest in the subtype 3 and lowest level in the subtype 2, but activated dendritic cells had an opposite trend. **P<0.01, ***P<0.001.

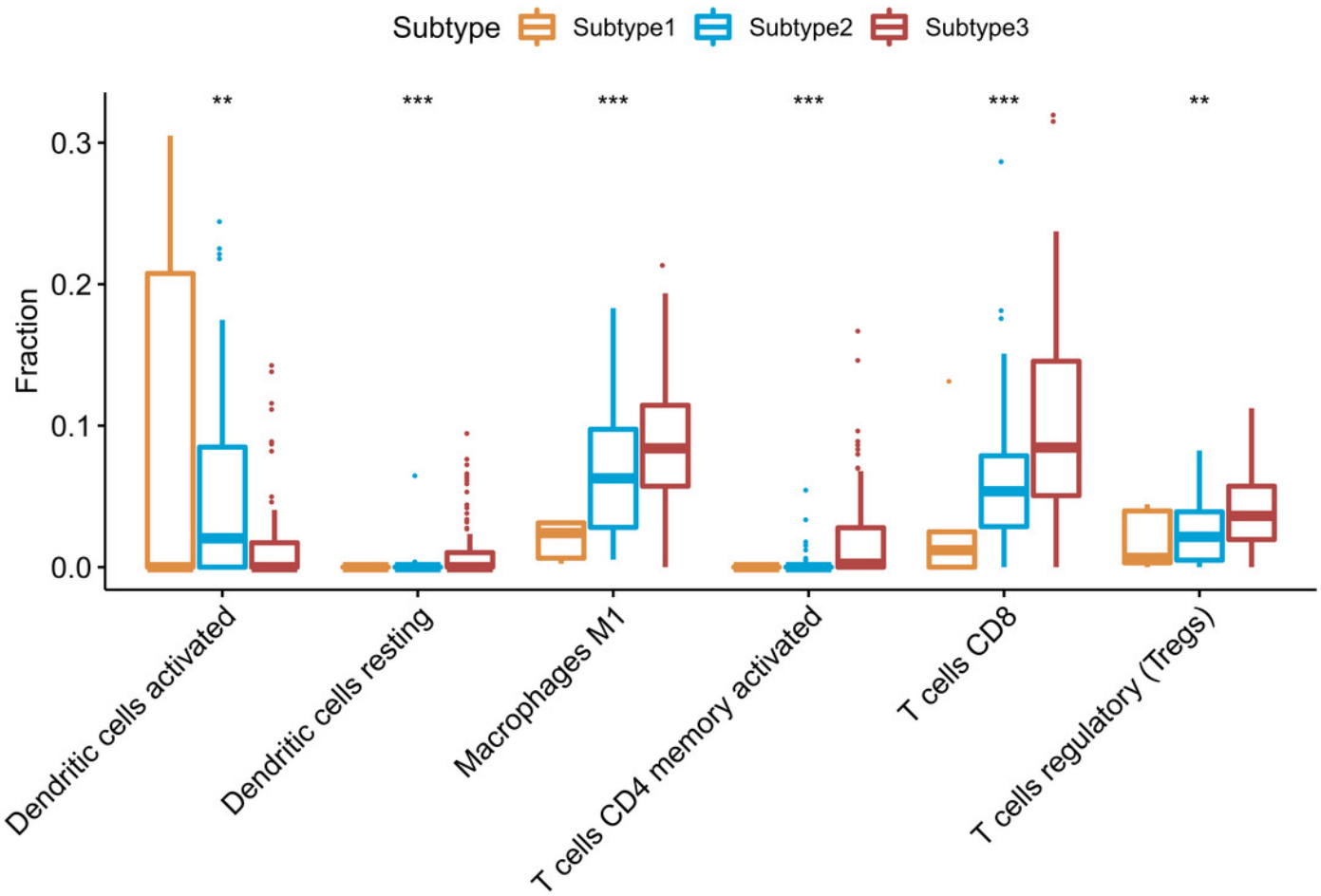


Figure 4

Kaplan-Meier curves showing survival prognosis of the ovarian cancer subtypes and immune-associated gene sets.

The subtype 2 showed the worst survival prognosis among the three subtypes. High level gene expression score of check-point, major histocompatibility complex class I, APC co-inhibition, T cell co-inhibition, Th1 cells, Th2_cells, Tfh, inflammation-promoting, Treg were associated with a better prognosis. Treg, regulatory T cells.

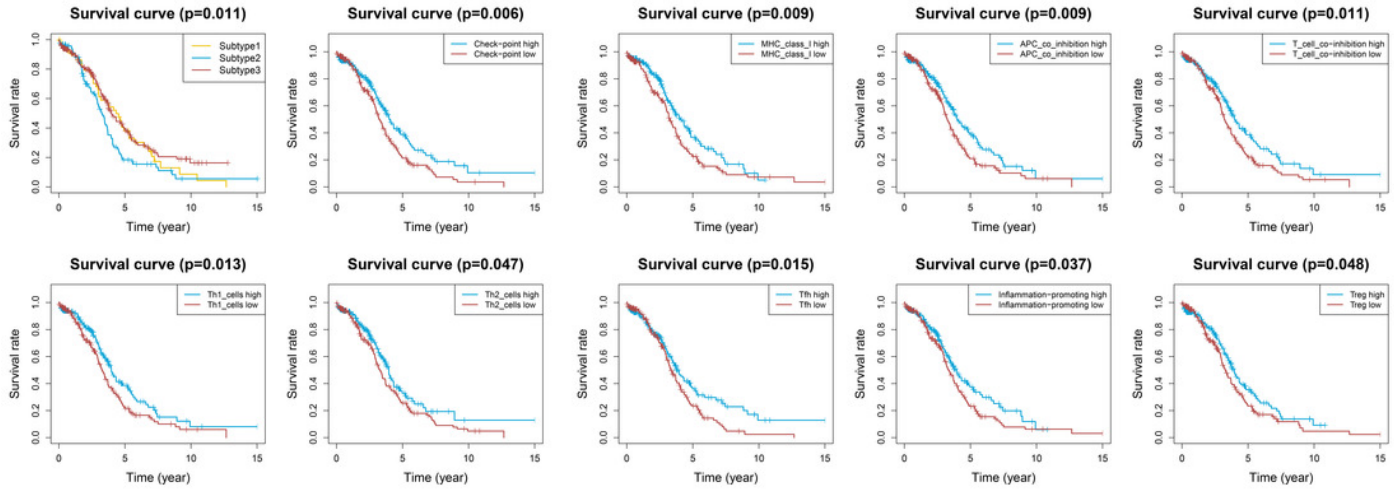


Figure 5

Expression distribution of the eight immune checkpoint genes in the three ovarian cancer subtypes.

The expression level of PDCD1 and its ligands (CD274 and PDCD1LG2), CTLA4 and its ligands (CD86 and CD80), TIM-3, LAG3 were all significantly lower and significantly higher in the subtype 1 and subtype 3, respectively. ***P<0.001.

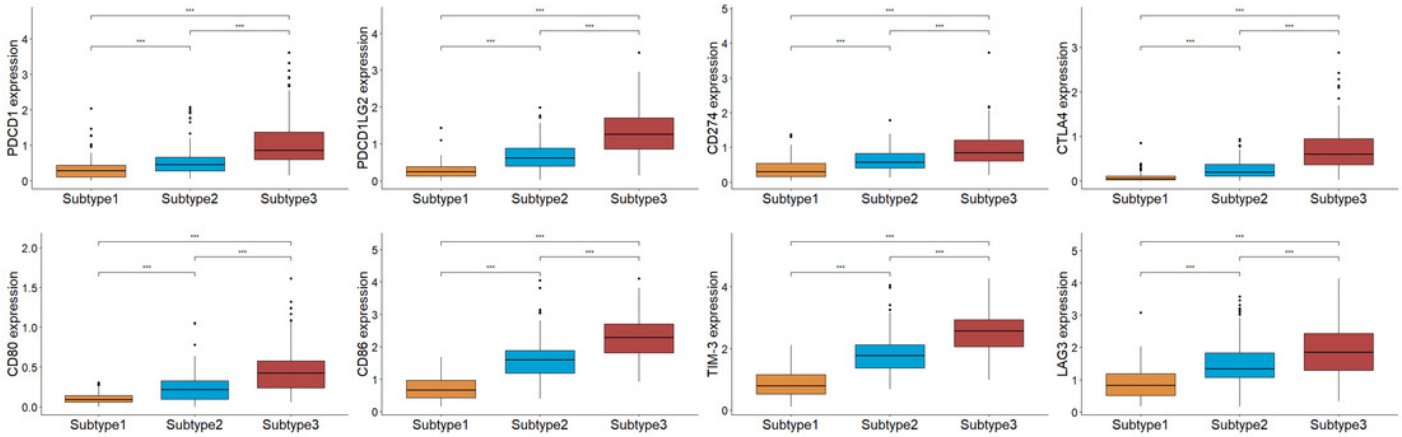


Figure 6

GSEA identified GO and KEGG pathways enriched in the subtype 1 and subtype 3.

(A) GO analysis of the top 5 significantly enriched biological processes in the subtype 3. (B) GO analysis of the top 10 biological processes significantly enriched in the subtype 1 and the subtype 3, respectively. (C) KEGG analysis of the subtype-specific pathways enriched in the subtype 3. (D) KEGG analysis of the top 10 pathways significantly enriched in the subtype 1 and the subtype 3, respectively. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

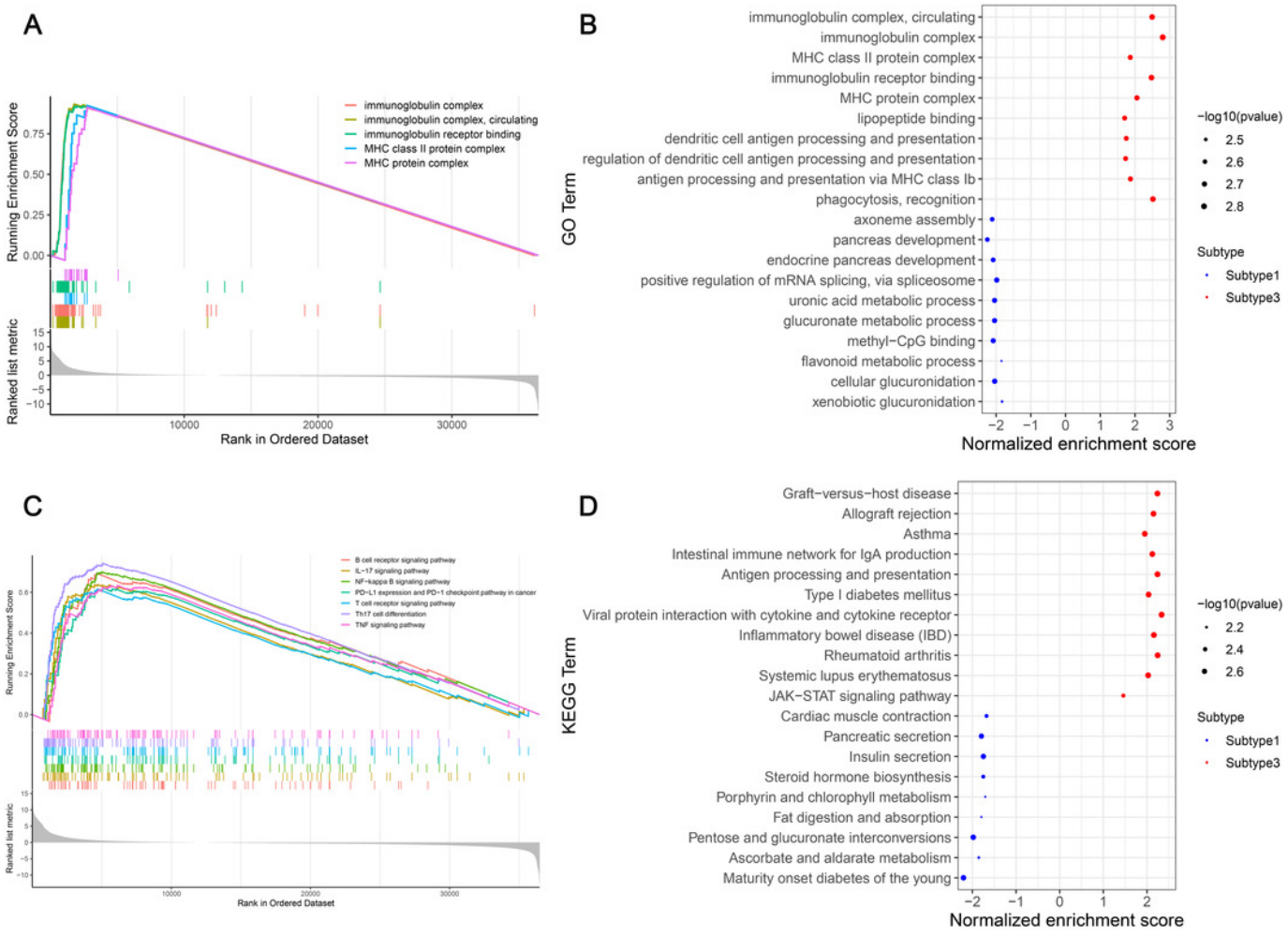
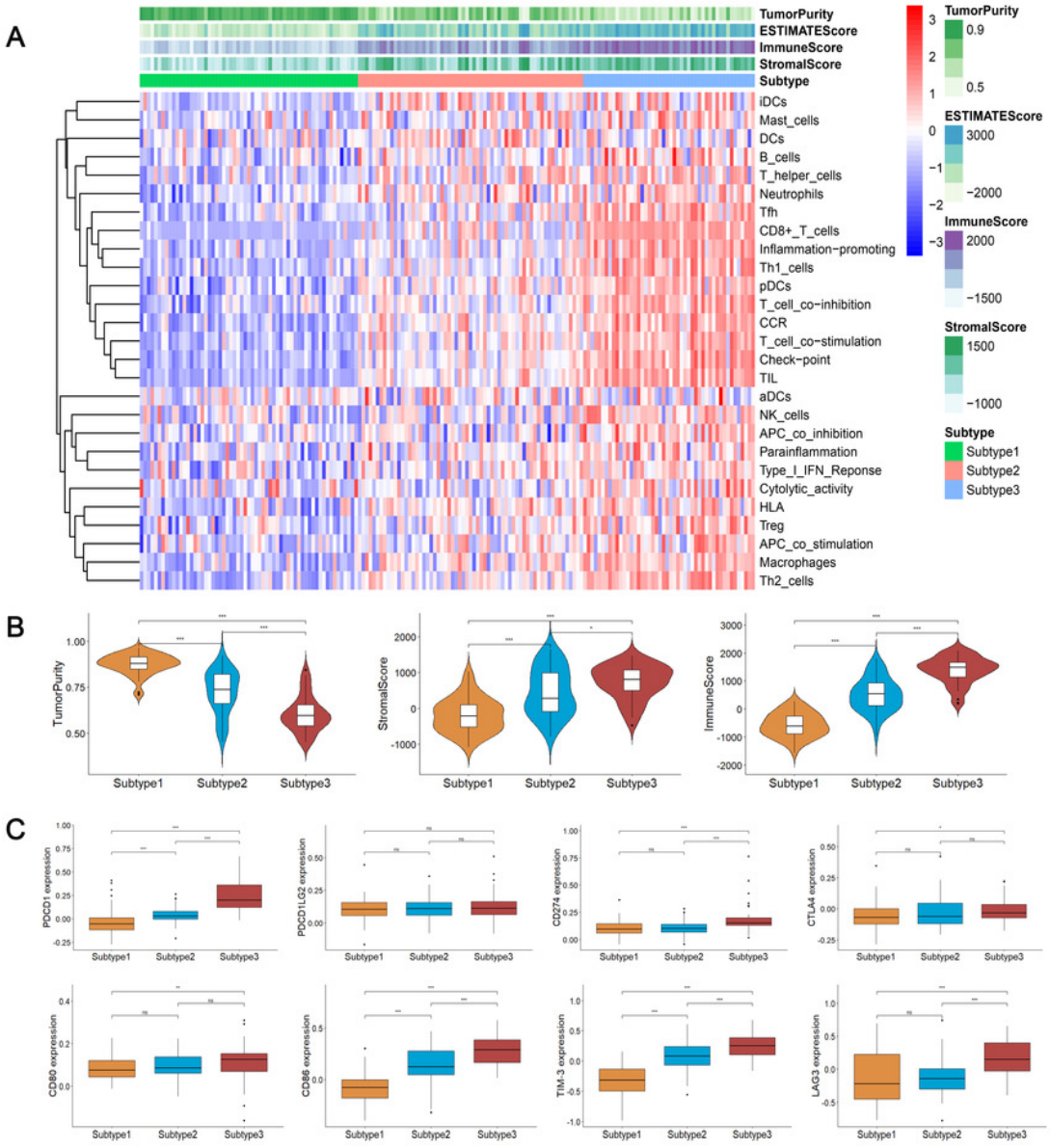


Figure 7

Validation of the external datasets.

(A) Hierarchical clustering of ovarian cancer yields three subtypes in the GEO dataset. Red represents high expression and blue represents low expression. (B) The distribution of tumor purity, immune score, and stromal score were compared in the three immune subtypes in the GEO dataset, respectively. (C) Expression distribution of the 8 immune checkpoint genes in the three ovarian cancer subtypes in the GEO dataset. *P<0.05, **P<0.01, ***P<0.001. GEO, Gene Expression Omnibus.


A
TumorPurity
ESTIMATEScore
ImmuneScore
StromalScore
Subtype
iDCs
Mast_cells
DCs
B_cells
T_helper_cells
Neutrophils
Tfh
CD8+_T_cells
Inflammation-promoting
Th1_cells
pDCs
T_cell_co-inhibition
CCR
T_cell_co-stimulation
Check-point
TIL
aDCs
NK_cells
APC_co_inhibition
Parainflammation
Type_I_IFN_Reponse
Cytolytic_activity
HLA
Treg
APC_co_stimulation
Macrophages
Th2_cells
Subtype1
Subtype2
Subtype3
B
C
PDCD1 expression
PDCD1LG2 expression
CD274 expression
CTLA4 expression
CD80 expression
CD86 expression
TIM-3 expression
LAG3 expression