Immunogenomic analyses of advanced serous ovarian cancer reveal immune score is a strong prognostic factor and an indicator of chemo-sensitivity

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Abstract

Purpose: Ovarian cancer is one of the first human cancers for which in situ immune response was reported to be important for the clinical outcome. To elucidate the mechanistic relationship between immune repertoire and cancer genotype in ovarian cancer, the development of a well-defined immune score for ovarian cancer is required.

Experimental Design: From a collection of 2,203 patient samples of advanced ovarian cancer from public available resources, we evaluated the prognostic values for a compendium of immune marker genes and proposed an immune score. The relationships between immune score, tumor-infiltrating immune cells, cancer genotypes and their impact on patient outcome were characterized.

Results: Loss of chemokine and IFN-γ pathway genes is frequent in ovarian cancer and is significantly associated with low immune score and poor outcome. Chemotherapy can increase the immune score of tumors by inducing the expression of IFN-γ inducible chemokines. High immune score is significantly associated with BRCA1/2 mutation status and the response to chemotherapy. Multivariate analysis revealed that immune score is a strong predictor of patient survival and the response to immunotherapy.

Conclusions: Our results reveal the drivers of the immune repertoire of advanced ovarian cancer and demonstrate the importance of immune score as an independent prognostic signature and a potent indicator of intratumoral immune status.
Translational relevance

This study characterized the immune microenvironments of advanced ovarian cancer from an immunogenomic perspective. We developed immune score to assess the immune status in advanced ovarian tumors, which would be important for up-front stratification of patients in clinical trials of immunotherapy strategies. This study demonstrated that chemokine and IFN-γ pathway genes, whose expression could be stimulated by chemotherapeutic agents, are drivers of immune repertoire in ovarian cancer, suggesting that chemotherapy may benefit patients with a low density of infiltrating immune cells. This study highlights the importance of the assessment of immune microenvironment via immune score in the setting of immunotherapy strategies.
Introduction

Since the first piece of evidence for the infiltration of cytotoxic T cells in epithelial ovarian cancer (EOC) was reported in 1991 (1), many studies have confirmed a spontaneous anti-tumor immune response in EOC patients (2). EOC is one of the first human cancers for which tumor-infiltrating lymphocytes (TILs) was found to be associated with improved patient survival and delayed recurrence of disease (3). Recent studies further confirmed the prognostic value of tumor-infiltrating lymphocytes (TILs) using multiple EOC cohorts (4). In light of the promising effect of immunotherapy in the initial studies of EOC (5,6), especially in patients that are resistant to the conventional therapy, ongoing clinical trials are designed to evaluate the effect of immune-checkpoint inhibitors in EOC patients (7). However, pioneering studies have shown that only a minority of patients is responsive to immunotherapy, and thus the identification of these patients has become critical. A common feature of the patients responsive to immunotherapy is the high activity of pre-existing anti-tumor immunity (8). A high density of TILs, as an indicator of in situ immune activity, is reported to be important in the setting of the immune-checkpoint blockade (8,9). Thus, deeply understanding the determinants of in situ immune activity in EOC would assist the administration of immunotherapy in this disease.

Currently, there is no consensus signature to estimate the immune activity in EOC and to stratify patients accordingly. Molecular subtyping has been proposed and identified an immunoreactive subtype for high-grade serous ovarian cancer (HGSC), but it provides limited prognostic information (10). On the other hand, although numerous prognostic signatures have been developed to stratify EOC patients (11), they are not indicative of the anti-tumor immune activity. In colorectal cancer, a criterion of in situ immune activity, designated “immunoscore” has been demonstrated to be the strongest prognostic factor that may serve as a new component of cancer classification (12,13), indicating that assessment of immune microenvironment via a standard criterion provides a potent prognostic tool. An immune score has many advantages over traditional prognostic signatures in EOC. First, it helps to stratify patients into those suitable for checkpoint blockade and those would benefit more from conventional therapies. Second, a well-defined immune score would facilitate the comparison of different immune modulators and the exploration of the mutual influence between chemotherapy and tumor immune microenvironment (14,15). More importantly, it
facilitates the genomic analyses of genotype-immunophenotype relationships that are now imperative for an improved understanding of the immunogenomic profile of EOC.

Here, we aim to establish an immune score for advanced (late-stage and high-grade) serous ovarian cancer to investigate the relationship between cancer genotype and immune activity. We hypothesize that an immune score that is highly reflective of pre-existing antitumor immunity should be a strong prognostic signature in EOC. Starting from a large-scale investigation for the prognostic value of immune marker genes in 16 cohorts of advanced serous ovarian cancer patients, we developed an immune score that can be used as a potent indicator of the anti-tumor activity of naturally infiltrating immune cells and a strong prognostic marker of EOC patients. Based on the immune score, we investigated the genotype-immunophenotype relationships and identified the genomic determinants of immune infiltration in ovarian cancer. We further demonstrated that immune score predicted the response to chemotherapy, regardless of DNA repair defects such as BRCA1/2 mutations. We also showed that neoadjuvant chemotherapy significantly increased the immune score of tumors, suggesting that chemotherapeutic agents can stimulate an immune signaling profile in cancer cells.

Materials and Methods

Cohort datasets of ovarian cancer

16 public cohort datasets with transcriptome data of high-grade, late-stage serous ovarian cancer were obtained through database searches of PubMed, ArrayExpress, TCGA and GEO (Table 1), and processed as described in our previous study (16). In this study, we further added a recently released cohort dataset of 80 primary ovarian cancers (17). All the datasets have at least 40 transcriptome-profiled primary samples of advanced serous ovarian cancer (grade 2 or 3 and FIGO stage III or IV) with overall survival time-to-event data. We restricted our analysis to the primary, advanced serous ovarian tumors with survival data. To avoid systematic bias across datasets, we analyzed each dataset independently and then integrated using meta-analysis for the evaluation of an overall prognostic value of genes. The response to chemotherapy in ovarian cancer cohorts was determined by corresponding previous studies (10,17). Chemotherapy status was defined as sensitive if there is no evidence of disease progression within 6 months of the end of primary treatment, and was defined as resistant if there is evidence of disease progression within ~6 months from the end of primary treatment.
Immune cell marker genes

A compendium of 782 marker genes related to 28 tumor-infiltrating immune cell types was obtained from the previous study (18). These genes are representative of specific immune cells and are not expressed in cancer cells or in normal tissues. Gene set enrichment analysis (GSEA) using these marker genes has been proved to be effective to evaluate the infiltration of immune cells (18).

Immune score

The prognostic evaluation of immune marker genes and antigen-presenting genes was determined by Cox proportional hazards regression analysis in the 16 cohort datasets of ovarian cancer. An overall hazard ratio (HR) and the variance of HR were estimated by meta-analysis using fixed-effects model. In total, 76 genes were identified to be significantly prognostic of favorable outcome (Supplementary Table 1; Cox proportional hazard model, p < 0.02, FDR < 0.1). For each of the 76 prognostic genes, the HR and the standard estimates (SE) of HR were used to determine the weight of a marker gene in immune score. Then, the immune score of a sample is given by:

\[ S = \sum_{i=1}^{76} \frac{1 - HR_i}{se(HR_i)} \times x_i, \]

where \( x_i \) is the normalized expression of gene \( i \) in that sample. The Z-score normalized immune score was used in our analyses.

Genomic data of TCGA

Level 3 data of TCGA in this study, including clinical information, gene expression data (U133A array and RNAseq data), somatic mutation data (MAF file) and copy number variation (CNV) data were downloaded from Broad GDAC FIREHOSE (http://gdac.broadinstitute.org). Neo-antigens of ovarian cancer were downloaded from the database of The Cancer Immunome Atlas (TCIA) (18). Copy number gain or loss of genes, which was determined by GISTIC 2.0 software, were documented as “1, 2” and “-1, -2”, respectively. GISTIC values (-2, -1, 0, 1, 2) of genes were used to estimate the association between CNV and immune score using linear regression model. Samples with somatic mutation of BRCA1/2 were obtained from the MAF file of TCGA ovarian cancer, while samples with germline mutations of BRCA1/2 were obtained from the previous publication of TCGA group (10).

For pan-cancer survival analysis, RNAseq data of 9,264 tumor samples across 24 cancer types were downloaded from GEO (GSE62944). This dataset provides the expression values...
of genes processed using the same pipeline (19). Log2 transformed fragment per kilobase per million (FPKM) was used for analysis.

**Quantitative real-time PCR**

OVCAR-3 cells were obtained from ATCC on 23/05/2015 and were checked free of mycoplasma contamination by PCR and culture. OVCAR-3 cells were treated with vehicle, Cisplatin (20µM) for 36h or IFN-γ (100 IU; P5664, Beyotime) for 12h. Total RNA was extracted using TRIzol (Invitrogen, Life Technologies) according to the manufacturer’s instructions. RNA was reverse-transcribed into cDNA using PrimeScript RT Master Mix Kit (RR036Q, Takara). Quantitative real-time PCR (qRT-PCR) was performed using the Real-Time PCR Detection System (Applied Biosystems, Foster City, CA) with a FastStart Universal SYBR Green Master (Rox; Sigma) and a standard temperature protocol. Expression levels of target mRNA were normalized relative to levels of 18S RNA.

**Statistical analysis**

Standard statistical tests including Student T test, Wilcoxon rank sum test, Fisher exact test, Log-rank test and Cox proportional hazard regression were used for the analysis of clinical data and genomics data. Meta-analysis was performed using R package “metafor”. GSEA was performed using Bioconductor package “clusterProfiler”. Differential expression was determined using eBayes function of "Limma" package. All the analyses were performed in R 3.3.1.

**Results**

**Large-scale meta-analysis reveals the prognostic value of intratumoral immune gene expression**

To develop a prognostic scoring system indicative of immune infiltration, a compendium of 782 marker genes related to 28 tumor-infiltrating immune cell types was analyzed (18). These genes are representative of specific immune cell types and not expressed in cancer cells or normal tissues. The prognostic value of these genes, as well as the antigen-presenting genes, was evaluated in 16 publicly available cohorts of more than 2200 primary tumors of advanced serous ovarian cancer (Table 1). We then used a meta-analysis to leverage the 16 datasets for an overall prognostic evaluation for each immune marker genes. In total, 69 marker genes and 7 antigen-presenting genes were identified as favorable prognostic factors (Cox proportional hazard model, p < 0.02, FDR < 0.1). The number of favorable prognostic immune marker genes was significantly higher than expected by chance (empirical p <
0.0001 by random perturbations). The 76 favorable prognostic genes and their corresponding immune subpopulations are shown in a network (Figure 1A). The majority of them are from immune subpopulations of adaptive immunity, including Th1, Th2, activated B cell or T cell, effector memory T cell and γδ T cell. Several markers of subpopulations from innate immunity, such as NK cell, NK T cell, macrophage, MDSC, CD56 dim and neutrophil, are also included in the network.

The fact that a large fraction of marker genes of not only T cell but also other immune subpopulations are prognostic suggests a complicated tumor microenvironment as a reflection of host immunity. We thus developed an immune score based on the combined expression of these genes by taking their contributions to risk prediction into account. High immune score reflects an overall high expression of favorable prognostic genes. In TCGA dataset, we observed a significant correlation between our immune score and the fraction of immune cell infiltration estimated by an independent leukocyte-specific methylation signature (Pearson’s r = 0.55, Supplementary Figure 1A) (20). We also observed a strong correlation between immune score and the cytolytic activity of infiltrating T cells (Pearson’s r = 0.81, Supplementary Figure 1B) (21). Therefore, the immune score based on the expression of all the favorable prognostic marker genes reflects the intratumoral immune activity in ovarian cancer.

To further chart the immune pattern underlying the immune score, we assessed the infiltration of the immunome of 28 subpopulations using single-sample gene set enrichment analysis (ssGSEA) as previously described (18), and computed their correlation with the immune score (Figure 1B and Supplementary Table 2). Based on the score of ssGSEA, the prognostic value of different immune subpopulations was also evaluated within each dataset and was integrated using meta-analysis for an estimation of overall prognostic effect. The infiltration of most immune subpopulations was positively correlated to immune score and was associated with favorable prognosis in ovarian cancer datasets, especially the activated CD8+ T cell and activated CD4+ T cell (Supplementary Figure 2A). On the contrary, the plasmacytoid dendritic cell was negatively associated with immune score in many datasets, and was likely to be associated with a poor prognosis (Supplementary Figure 2B). The significantly favorable prognostic immune cells, ordered by their HR, are effector cells (activated CD4+ T, activated CD8+ T, activated B, effector memory CD4+ and effector memory CD8+), CD56 bright natural killer cells, T follicular helper cells and type 1 T helper
In general, the infiltration of subpopulations related to adaptive immunity has a stronger correlation with immune score and is more likely to be favorable prognostic factor than subpopulations related to innate immunity (Figure 1B). In fact, all the immune subpopulations of adaptive immunity except Treg cells are associated with favorable outcome of ovarian cancer.

**Loss of chemokine and IFN-γ pathway genes are associated with low immune score**

We hypothesized that the immunophenotype could be shaped by the genotypes of tumor. To test this, we looked for associations between gene alteration and the immune score in TCGA dataset using a regression-based method. However, we didn’t observe a significant association of immune score with the mutation of any gene; neither did we observe a significant correlation with the mutation burden or the burden of neo-antigens (Supplementary Figure 3). On the other hand, we found numerous genes for which the copy number status was significantly associated with immune score (n = 3800 at FDR < 0.05), which was consistent with the fact that ovarian cancer is a C-class tumor characterized by recurrent copy number alterations (22). We plotted the significance of associations between immune score and copy number status across the genome, which revealed several highly enriched chromosome regions (Figure 2A). On chromosomes 1, 2 and 20, genes are likely to be amplified and the amplification is associated with lower immune score (negative correlation), whereas on chromosome 4 and 9, genes are likely to have copy number deletion and the deletion is associated with lower immune score (positive correlation). Chromosome 4 and 9 are of particular interest, because these two regions peaked at the loci encoding chemokines and IFN-γ pathway genes. For example, chemokines including CXCL9, CXCL10 and CXCL11, which elicit chemoattraction for T cells and NK cells, are all located in 4q21-23. The expression of these chemokines, which could be induced by IFN-γ, is essential for immune infiltration (23). Recent studies have shown that the loss of IFN-γ pathway genes is a mechanism of repressing anti-tumor immunity and the resistance to CTLA-4 blockade (24). Here we suggest that the loss of chemokine genes should also be considered as an alternative mechanism since advanced ovarian cancer is characterized by the deletion of chromosome arm 4q (10).

Using qPCR and public available data (25), we confirmed that IFN-γ could induce the expression of CXCL9, CXCL10 and CXCL11 in ovarian cancer cells and ovarian epithelial cells (Figure 2B-C). Based on the copy number alteration of IFN-γ pathway genes and
chemokines, we found that ovarian tumors could be divided into three groups: group 1 with copy number loss of both IFN-γ pathway genes and chemokines, group 2 with copy number loss of either IFN-γ pathway genes or chemokines, and group 3 without copy number loss of most of these genes (Figure 2D). Consistently, we found that the group 1 showed the lowest immune score whereas the group 3 showed the highest immune score (Figure 2E). The association between the loss of IFN-γ pathway genes and immune score was also observed in TCGA melanoma dataset where the loss of these genes has been confirmed as a mechanism of immune resistance (Supplementary Figure 4) (24). In fact, a large fraction of chemokines and IFN-γ pathway genes are favorable prognostic factors in ovarian cancer according to our meta-analysis of the 16 cohort datasets (Figure 2D), suggesting that these genes are required for intratumoral immune activity. As expected, the expression of IRF-1 in tumors with the loss of IFN-γ pathway genes was significantly lower than other tumors (Supplementary Figure 4D). Consequently, loss of IFN-γ pathway genes dampened the correlation between IFN-γ and immune score across ovarian tumors (Figure 2F). These results suggest that the loss of chemokines and IFN-γ pathway genes impairs the regulation on immune infiltration and may be associated with immune resistance in EOC.

Chemotherapy induces chemokine expression and increases immune score

Intra-tumor microenvironment can be harshly changed by neoadjuvant chemotherapy (26-28). We therefore compared 25 pairs of post- vs. pre-chemotherapy ovarian tumors with each pair of tumors obtained from the same patient from a previous study (29), which revealed 825 upregulated and 917 downregulated genes (FDR < 0.05). The downregulated genes were highly enriched in cell cycle related functions, whereas the upregulated genes were mostly enriched for immunoreactive functions (Figure 3A), suggesting that chemotherapy has stimulated the infiltration of immunoreactive cells. The marker genes of adaptive immunity subpopulations were highly enriched in post-chemotherapeutic ovarian tumors, including CD8A, CD8B, GZMA and PRF1 among the top upregulated genes (Figure 3B). Consistently, we observed significantly increased immune score and cytolytic activity in post-chemotherapy tumors compared with their paired pre-chemotherapy tumors (Figure 3C). Moreover, tumors having a strong pre-existing immune activity are also likely to have a relatively stronger immune activity after chemotherapy than other tumors.

We next investigated whether chemotherapy agents could enhance the capability of tumor cells responding to host immunity. The expression of chemokines and antigen-presenting
genes are important for the recruitment of lymphocytes (30-32). In the 16 cohort datasets, we observed strong correlations between the expression of these genes and the immune score in ovarian cancer (Supplementary Figure 5). Using a publicly available dataset (33), we found that carboplatin could engender the overexpression of chemokine and antigen-presenting genes in EOC cells (Figure 3D). We also tested this in another EOC cell line (OVCAR-3), and observed similar overexpression of chemokine and antigen-presenting genes after treatment of cisplatin for 36 hours (Figure 3E). These results suggest that tumor cells treated with chemotherapy agents have an enhanced ability to foster the infiltration of immunoreactive cells.

We hypothesized that the clinical response to chemotherapy could be dependent on the anti-tumor immune response. Since there are few genomic data for post-therapeutic tumors, we tested the association between the response to chemotherapy and pre-existing immune activity. We classified patients into the high immune score group and the low immune score group in two of our collected datasets with available information about the response to chemotherapy (10,17). Consistent with the hypothesis, we found that patients in the high immune score group have a better response to chemotherapy than patients in the low immune score group (Figure 3F). In total, we found 98 chemo-sensitive patients and 47 chemo-resistant patients in the high immune score group, and 90 chemo-sensitive patients and 70 chemo-resistant patients in the low immune score group in the two datasets (Fisher exact test, p < 0.05). In addition, chemo-sensitive tumors were enriched of activated CD4+ T cells compared to chemo-resistant tumors (Supplementary Figure 6).

**Immune score is predictive of clinical outcome independent of BRCA mutation**

Patients with a high immune score displayed better overall survival (OS) than patients with a low immune score in most of collected EOC datasets (Figure 4A), as estimated by multivariate Cox proportional hazards model controlled for clinical factors including age, tumor stage and grade. Meta-analysis result suggested a significant favorable prognostic value in EOC patients (high vs. low immune score, HR = 0.71 [0.64, 0.80], p = 8.3E-9). Using pan-cancer RNA-seq data, we further examined the association of immune score with OS in 18 solid cancers, of which two were significant in addition to ovarian cancer: skin cutaneous melanoma (SKCM) and uterine corpus endometrial carcinoma (UCEC) (Figure 4B). Meta-analysis revealed a significant pan-cancer effect of immune score (high vs. low immune score, HR = 0.82 [0.74, 0.91], p = 1.3E-4). To further explore the prognostic value
of immune score, we divided the patients into 4 equal groups according to the immune score of samples. We observed that the OS of patients was determined by immune score, with a median OS of 2.98, 3.16, 3.73 and 4.32 years for the 4 groups of patients with increasing immune score (log-rank test, p < 0.003).

Recently, BRCA1/2 mutations have been shown to have a significant impact on TILs (34). Mutations of BRCA1/2 occur in a substantial fraction of advanced ovarian cancer samples (21%) and are predictive of favorable outcome due to an improved response to chemotherapy (10). We analyzed the difference of immune score between BRCA wild-type tumors and tumors with BRCA deficiency. The immune score was not significantly different in tumors with BRCA hypermethylation compared to wild-type tumors. However, it showed a significant increase in tumors with BRCA mutations accompanied with heterozygous deletion of BRCA (Figure 4D). Tumors with BRCA mutation were significantly enriched for infiltration of activated CD8+ and CD4+ T cells (Figure 4E). Survival analysis of groups, defined by BRCA mutation and immune score, showed that patients with BRCA1/2 mutation and high immune score displayed prolonged OS than patients with wild-type BRCA1/2, whereas patients with BRCA1/2 mutation but low immune score displayed no significant survival difference than wild-type patients (Figure 4F and Supplementary Figure 7). These results confirm that BRCA1/2 mutation is a determinant of tumor infiltrate, and is more likely to be predictive of clinical outcome when it has successfully stimulated the immune infiltration.

**Immune score predicts response to immunotherapy**

The identification of predictive markers is crucial for immunotherapy with checkpoint blockade as only a minority of patients is responsive to the therapy. We demonstrated that, in melanoma, the ovarian cancer derived immune score was prognostic (Figure 4B) and that loss of IFN-γ pathway genes resulted in significantly decreased immune score (Supplementary Figure 4), suggesting that the immune score also captured the pre-existing anti-tumor immune response in melanoma. Since pre-existing immune activity is a powerful marker in the setting of checkpoint blockade (8), we reasoned that the immune score might also be predictive of the response to immunotherapy. We first tested the prognostic value of immune score in the 4 immunotherapy datasets with transcriptome data released (9,35-37). These include 3 melanoma datasets received anti-CTLA-4 or anti-PD-1 therapy and an urothelial cancer datasets received anti-PD-L1 therapy. A meta-analysis of the 4 datasets
shows that the immune score is significantly associated with favorable survival in immunotherapy datasets (Figure 5A; HR = 0.73[0.57-0.93], p = 0.01). Significantly prolonged survival was observed for high vs. low immune score patients in the combined melanoma cohort (Figure 5B; Median OS 3.24 vs. 1.72 years, Log-rank p = 0.01). Totally, we found 28 (41.2%) responders of immunotherapy in the high score group of the 4 datasets, and only 19 (26.8%) responders in the low score group (p = 0.077 by Fisher exact test). Notably, the immune score enabled the classification of responders and non-responders with a predictive power comparable with the expression of the target of immunotherapy (Supplementary Figure 8).

Discussion

Using large-scale datasets, we systematically evaluated the prognostic values of immune infiltration in advanced EOC patients and proposed an immune score to investigate the genomic determinants of in situ immune activity and its correlation with the response to chemotherapy. Association with immune score identified the loss of chemokine genes and IFN-γ pathway genes that are significantly correlated with low immune infiltration and poor outcome of patients. The immune score is predictive of outcome and the response to chemotherapy and immunotherapy, suggesting that it provides an appropriate tool for pre-treatment stratification of patients and for post-trial evaluation.

The inhibition of immune infiltration has been increasingly identified as an important mechanism for tumor to evade from host immunity (38). TILs are often suppressed by selective neoantigen exposure (21), immune suppressive cytokines and the deletion of IFN-γ pathway genes (24). PD-1 blockage has been found to enhance T cell migration by elevating IFN-γ inducible chemokines in the tumor (39), indicating that the loss of chemokines and IFN-γ pathway genes would impair the response to immunotherapy. However, large-scale genomic analysis has identified that the chromosome region 4q and 9p, which contain chemokine genes and IFN-γ pathway genes respectively, are among the most significant regions with recurrent copy number loss in EOC, with a loss frequency of 67% and 66% respectively (10). Nevertheless, in most cases, it is likely to be the heterozygous loss, which means the expression of these genes could still be induced by appropriate stimulations. We found that platinum agents could induce the expression of chemokine genes and antigen-presenting genes (Figure 3D-E), and boosted the immune infiltration in post-chemotherapy tumors. Although conflicting data exist regarding the immunostimulatory vs.
immunorepressive capability of platinum agents (40,41), we found that they can increase anti-tumor immunity via up-regulation of MHC genes and T-cell-attractant chemokines in ovarian cancer, as evidenced by the increased cytolytic activity of post-chemotherapy tumors (Figure 3C). These results are consistent with the reported interplay between immunotherapy and chemotherapy (42,43), and suggest an opportunity for designing combinatorial approaches for EOC patients whose tumors have a heterozygous loss or normal copies of chemokine genes.

The importance of spontaneous antitumor immunity was highlighted by the comprehensive associations between TILs and patient’s survival in human solid cancers (44,45). For example, in colorectal cancer, it was recently reported that the poor survival of patients with microsatellite stability tumor is likely due to the low density of TILs (13). In EOC, numerous studies have reported the prognostic value of TILs (4). However, there is currently no consensus on how to best characterize the immune infiltrates for prognostic evaluation. Using a compendium of prognostic immune marker genes, we developed the immune score as a reflection of the complexity of tumor immunogenicity in order to provide the strongest prognostic value for EOC. We chose a compendium of immune marker genes over individual marker gene (i.e., CD8A) or the cytolytic activity (estimated using expression of GZMA and PRF1) (21,33), due to the fact that these commonly used marker genes, including CD8A, GZMA and PRF1, are not strongest prognostic factors among the marker genes that we have identified (Figure 1A).

The strong prognostic value of immune score and its association with TILs indicate that EOC could response to immunotherapy. Pilot studies have shown that immunotherapies that capitalize on pre-existing antitumor immune response are more likely to be successful (8). Therefore, reflective factors of tumor immunogenicity, such as neoaentigen load, the expression of MHC class I genes (18) and the normalization of intra-tumor blood vessels (23), are predictive for the response to immunotherapy. Notably, impressive clinical response to immunotherapy has been seen for tumors with exceptionally high mutation loads in melanoma and non-small cell lung cancer (46,47). However, EOC has a relatively low mutation load, and consequently low abundance of neoantigens in this disease (10). As a result, we failed to observe a significant correlation between mutation load and TILs in terms of CD8A expression or the cytolytic activity (Pearson correlation test, p >0.05). Therefore, novel signatures of pre-existing antitumor immune response need to be developed. We
assumed that an immune signature that is most prognostic in EOC could be most reflective for pre-existing antitumor immunity. The immune score is designed based on this assumption and indeed it is predictive of the outcome after immunotherapy (Figure 5). Although we don’t have a dataset yet to verify the immune score for EOC patients, the similarity of the genotype-immunophenotype relationship between melanoma and EOC suggesting a similar intra-tumor immune microenvironment between the two cancer types.

Patients with EOC have experienced little improvement with regard to the treatment and outcome during the past decades (48). Most patients diagnosed with advanced ovarian cancer received multiple cycles of platinum-based chemotherapy (49). So far, the major determinant of clinical outcome for these patients is still the response to chemotherapy, which highly depends on whether the tumors have DNA repair defects, including especially the BRCA1/2 mutations (10). However, here we found that both the response to chemotherapy and the survival benefit of BRCA1/2 mutations are significantly associated with tumor immunogenicity. High immune score is associated with improved response to chemotherapy and a higher frequency of BRCA1/2 mutations in EOC. In addition, patients with BRCA1/2 mutations and low immune score are less likely to have survival benefit comparing to the patients with high immune score (Figure 4F). There results indicate that assessment of tumor immunity via immune score provides an independent indicator of chemo-sensitivity. Moreover, the increased immune score after chemotherapy is consistent with the recent finding that chemotherapy enhances host immune response in metastatic ovarian tumors (28). These findings open up opportunities for new combinatorial therapies, since chemotherapy can stimulate host immune response that is associated with the effectiveness of immunotherapy, and in return, the immune response also improves chemo-sensitivity.

There are limitations in this study. First, the remarkable intra-tumor or intra-patient heterogeneous immune microenvironment of advanced serous ovarian cancer has not been considered. It has been recently demonstrated that the immune infiltration of different lesions of the same tumor can be highly distinct in ovarian cancer (50). Importantly, the tumor heterogeneity determines the effect of chemotherapy (51), as well as the effect of checkpoint blockade immunotherapy (52). Second, the immune score uses a serious of immune marker genes and thus is unspecific about the constitution of specific immune subpopulations. In addition, prospective studies should be performed to further evaluate the data before our findings could be more broadly applied. Nevertheless, it is a valuable signature because we
systematically assessed its association with immune infiltrates and evaluated its prognostic value by the comprehensive collection of ovarian cancer cohorts. Our critical findings are consistent with previous studies. The immune score should help to further define the prognosis of EOC patients, to facilitate the comparison of the results from various immune modulators and to stratify patients who will benefit more from suggested combinatorial therapies.

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Reference


Table 1. Cohort datasets of high-grade, late-stage serous ovarian cancer.

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\(^a\)FIGO stage III or IV; \(^b\)Metastases and borderline tumors excluded.

Figure 1 legends

Figure 1 | Prognostic value of immune marker genes and immune cells. (A) Prognostic immune marker genes and their corresponding cell types. Markers (second layer) associated to the same cell type (outermost layer) share the color of the node and are connected by dotted lines. The width of solid lines is proportional to the prognostic significance. (B) Bubble plot of correlation between immune score and infiltrating immune subpopulations across 16 cohort datasets of advanced ovarian cancer (sorted alphabetically for adaptive and innate immunity respectively). The size of the circles gives the significance of the correlation, while the color indicates the Pearson correlation coefficient. The color of border indicates favorable (green; HR < 1) or poor prognosis (orange; HR > 1). The rectangle indicates p < 0.05 determined by univariate Cox proportional hazard model. The overall prognostic effect is estimated by meta-analysis using fixed-effects model.

Figure 2 | Genomic associations with immune score. (A) The significance of association between immune score and copy number variation for all genic loci (cyan for negative correlation and red for positive correlation). Dotted lines represent significance cutoffs of 1% and 10% FDRs. Chromosome regions significant at the 1% FDR and associated
with potential driver genes are shown in the right side. (B) QPCR result showing the elevated expression of chemokines after 12 hours treatment of IFN-γ (100 IU/ml) in OVCAR-3 cells. (C) Heatmap showing the expression of chemokines with or without treatment of IFN-γ (500 IU/ml, 6h) in HOSE cells. N = 4 replicates are used in each group. (D) Oncoplot of the copy number status of IFN-γ pathway genes/type I interferons and chemokines. Copy number loss or gain was determined by GISTIC 2.0 and defined as score < 0 or >0 respectively. Green bar and grey bar indicate the samples with or without copy number loss of IFN-γ pathway genes and type I interferons, respectively. (E) Association between immune score and copy number status of IFN-γ pathway genes and chemokines. Samples of ovarian cancer are divided into 3 groups as shown in D, including those with loss of chemokines and IFN-γ pathway genes (group 1), those with loss of chemokines or IFN-γ pathway genes (group 2) and those with no copy loss of them (group 3). Significance is determined by Wilcoxon Rank Sum test. (F) The correlation between immune score and IFNG (encoding gene of IFN-γ). Samples of ovarian cancer are divided into two groups with or without copy number loss of IFN-γ pathway genes, as shown by the green and grey bar in D. Pearson correlation (r) and Spearman correlation (rho) are calculated.

Figure 3 | Chemotherapy stimulates immune signaling in ovarian cancer. (A) Top 15 enriched GO biological processes by the upregulated genes in post- vs. pre-chemotherapy tumors. Enrichment analysis was performed using DAVID bioinformatics resources 6.7. (B) GSEA of 431 marker genes of adaptive immunity reveals the association between immune response and chemotherapy. (C) Pairwise comparison of immune score and cytolytic activity (log2 transformed) in tumors post- and pre-chemotherapy. Significance is determined by pairwise T test. (D) Heatmap showing the expression of chemokines and antigen-presenting genes with or without treatment of Carboplatin in ovarian cancer cells. (E) QPCR result showing the elevated expression of chemokines and antigen-presenting genes after 36 hours treatment of Cisplatin (20μM) in OVCAR-3 cells. Significance is determined by Student T test. (F) Association between immune score and the response to chemotherapy in ovarian cancer patients.

Figure 4 | Prognostic evaluation of immune score. (A) Forest plot visualizing hazard ratios (HRs) of multivariate survival analyses of immune score (high versus low by median) in all cohort datasets. Significance is determined by Cox proportional hazard model. The red diamond shows the fixed-effects meta-analysis summary of HRs over 16 datasets (HR=0.71; 95%CI: 0.64-0.80, p = 8.3e-09). (B) Pan-cancer multivariate survival analyses of immune score in solid cancer types. The red diamond shows the fixed-effects meta-analysis summary of HRs over 18 cancer types (HR=0.82; 95%CI: 0.74-0.91, p = 0.0001). (C) Kaplan-Meier survival curves of TCGA ovarian cancer patients with different immune scores. (D) Association between immune score and BRCA1/2 status. Significance is determined by Wilcoxon Rank Sum test. (E) GSEA reveals the association of BRCA1/2 mutation status with the infiltrating CD8+ and CD4+ T cells. (F) Kaplan-Meier survival curves of TCGA ovarian cancer patients, divided by BRCA1/2 mutation status and immune score.

Figure 5 | Prognostic value of Immune score in immunotherapy datasets. (A) Forest plot visualizing hazard ratios (HRs) of immune score in immunotherapy datasets. The diamond shows the fixed-effects meta-analysis summary of HRs over the 4 datasets (p = 0.01). The last column shows the HR of immune score determined by univariate Cox
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