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Original Article

Comprehensive analysis of the prognostic value and immune function of the *IDO1* gene in gynecological cancers

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Abstract: Gynecologic cancer is a serious global healthcare issue with high rates of mortality and morbidity. In recent years, tumor immunity and immunotherapy have attracted extensive attention for treatment of gynecological cancers. Indoleamine 2, 3-dioxygenase 1 (IDO1) plays a critical role in cancer immune escape, and its inhibition has been explored for immune-targeted therapies for many malignancies. However, knowledge about IDO1 involvement in the pathogenesis of gynecological cancers and its therapeutic potential is still evolving. In the current study, we integrated bioinformatics analysis of the prognostic value and immune function of IDO1 in gynecologic malignancies using Oncomine, GEPIA, HPA, TIMER, TISIDB, SurvExpress and Metascape database. Comprehensive analysis revealed that the transcription levels of IDO1 were significantly overexpressed in patients with gynecologic cancers, and IDO1-co-expressed gene signatures may be useful potential prognostic markers for gynecologic cancers. Furthermore, increased IDO1 expression correlated with immune infiltration cells, immune marker sets, and immunomodulators in gynecological cancers. These findings suggest that IDO1 plays an important role in immune infiltration and could potentially be an immunotherapeutic target for gynecological cancers. However, future large-scale and comprehensive research is required to validate our results.

Keywords: IDO1, gynecological cancers, prognostic value, immune function

Introduction

Gynecologic tumors are a type of malignancy that occurs in the female reproductive system, which mainly include cervical, ovarian and endometrial cancer [1]. In 2019, an estimated 109,000 new gynecologic cancers were diagnosed and there were 33,100 gynecologic cancer-related deaths in women in the United States alone [2]. Among the gynecologic cancers, cervical cancer still has a high mortality rate in low-income countries, although it can be effectively prevented by human papillomavirus (HPV) vaccines and early screening [3]. Ovarian cancer remains the 5th leading cause of cancer death and the deadliest type of gynecologic cancer, and endometrial cancer is the 6th leading cause of cancer death in women [2, 4, 5]. Despite the advent of advancements in surgical procedures, auxiliary chemotherapy, and molecular targeted drugs, most patients with advanced gynecologic cancers experience relapse, and long-term survival remains poor [3-5]. Thus, it is imperative to identify relevant prognostic factors and more effective molecular targets in order to improve the prognosis of gynecologic cancers.

Within the last decade, there has been growing evidence that the immune system plays a crucial role in the development, metastasis, and recurrence of malignancies, and immune-targeted therapies have been approved by the Food and Drug Administration (FDA) for treating different types of tumors [6, 7]. Several immunotherapy strategies have been attempted for treatment of gynecologic cancers, including therapeutic vaccines, cytokines, immune

modulators, adoptive transfer of endogenous or genetically modified T cells, and immune checkpoint inhibitors [8, 9]. In particular, currently, over ten immune checkpoint antibodies targeting CTLA-4 and PD-1/PD-L1 have received regulatory approval worldwide, and thousands are being investigated in active clinical trials [10-12]. However, the clinical experience with immune-targeted therapies in gynecologic cancers remains limited, and a low response and adverse reactions in patients with specific genotype tumors limit their further clinical application [11].

Indoleamine 2, 3-dioxygenase 1 (IDO1) is a theme-containing enzyme catalyzing tryptophan into kynurenine in the kynurenine pathway [13]. Over-activation of the kynurenine pathway results in cell cycle arrest and death of immune cells, which mediates immunosuppression and neovascularization in the tumor microenvironment [14, 15]. Growing evidence shows that IDO1 is overexpressed and associated with clinical prognosis in the vast majority of solid tumors [16], such as anal [17], breast [18], cervical [19], colorectal [20, 21], esophageal [22], and lung cancer [23]. More importantly, several molecular drugs for targeting IDO1 have been assessed in multiple clinical trials and have produced encouraging results [13, 14, 24]. However, the prognostic value, immune function, and clinical application of IDO1 have not been fully elucidated in gynecologic cancers. In the current study, we extended the knowledge base related to IDO1 using a variety of large databases, with the purpose of performing an integrated bioinformatics analysis of the prognostic value and immune function of IDO1 in the three most common gynecologic malignancies: cervical, ovarian, and uterine cancer.

Materials and methods

Ethics approval and consent to participate

This study was approved by the Academic Committee of the People's Hospital of China Three Gorges University, and conducted according to the principles expressed in the Declaration of Helsinki. All the datasets were retrieved from the publishing literature, so it was confirmed that all written informed consent was obtained.

Oncomine database analysis

The mRNA expression of IDO1 in various types of cancers and genes co-expressed with IDOI in gynecological cancers was analyzed within the Oncomine gene expression array datasets (www.oncomine.org) [25, 26]. Herein, it was employed to reveal the transcriptional profile of IDO1 in patient specimens from gynecologic oncology and healthy controls. The threshold was determined according to the following values: *P*-value: 0.01; fold change: 2.0; gene rank: 10%; and data type: mRNA.

GEPIA analysis

The online database gene expression profiling interactive analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) was used to investigate differential expression analysis, profiling according to pathological stages, patient survival analysis, and correlation analysis, based on RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the Cancer Genome Atlas (TCGA) and the GTEx projects [27, 28].

Immunohistochemistry analysis

The human protein atlas (HPA) database (https://www.proteinatlas.org) was used to compare protein expression of IDO1 between human normal and gynecological cancer tissues by immunohistochemistry (Scar bar = 200 μm) [29-31].

TIMER database analysis

TIMER (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types [32, 33]. The expression of IOD1 in gynecological cancers and the correlation of IDO1 expression with six immune infiltrates (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and DCs) was estimated via the gene module in TIMER. In addition, correlations between IDO1 expression and immune marker sets of tumor-infiltrating immune cells were explored via correlation modules in TIMER. The gene markers of tumor-infiltrating immune cell markers are referenced in prior studies [34, 35]. The correlation module generated the expression scatter plots between a pair of user-defined genes in a given cancer type, together

with the Spearman's correlation and the estimated statistical significance. The gene expression level was displayed with $\log_2$ RSEM.

TISIDB analysis

TISIDB is a web portal for tumor and immune system interaction, which integrates multiple heterogeneous data types (http://cis.hku.hk/TISIDB/) [36]. In this study, TISIDB was used to explore the correlation between abundance of immunomodulators (immunoinhibitor, immunostimulatory, and MHC molecules) and exprression, copy number, or methylation of IDO1. In addition, the correlation between IDO1 expression and clinical features (OS, stage, and grade) in gynecological cancers was also analyzed.

Metascape analysis

Metascape (http://metascape.org) is an effective, efficient, and user-friendly gene-list analysis tool for experimental biologists to comprehensively analyze and interpret OMICs-based studies in the big data era [37]. It provides an automated meta-analysis tool to understand common and unique pathways within a group of orthogonal target-discovery studies. In this study, Metascape was used to conduct pathway and process enrichment analysis of co-expressed genes with IDOI in cervical, ovarian, and endometrial cancers. Only terms with *P* value < 0.01, minimum count 3, and enrichment factor > 1.5 were considered significant. The most statistically significant term within a cluster was chosen as the one representing the cluster.

SurvExpress analysis

SurvExpress (http://bioinformatica.mty.itesm.mx/SurvExpress) is a web-based tool providing survival multivariate analysis and risk assessment based on gene expression [38]. In our analysis, SurvExpress was used to provide survival analysis and risk assessment for IDO1 co-expressed gene signatures in cervical squamous cell carcinoma (CESC), ovarian serous cystadenocarcinoma (OV) and uterine corpus endometrial carcinoma (UCEC). Patients of the indicated datasets were stratified according to the median value of the prognostic index. High and low risk groups were divided based on the maximized risk algorithm. The log-rank test was used to statistically evaluate the equality of survival curves.

Statistical analysis

The results generated in Oncomine are displayed with *P*-values, fold changes, and ranks. Survival curves were generated by the Kaplan-Meier plots and GEPIA, and the results of survival analysis are displayed with hazard ratio (HR) and *P* or Cox *P*-values from a log-rank test. The correlation of gene expression was evaluated by Spearman's correlation with *P*-values < 0.05 considered statistically significant. The correlation strength for the absolute value of correlation coefficient was defined as follows: 0.00-0.19; "very weak", 0.20-0.39; "weak", 0.40-0.59; "moderate", 0.60-0.79; "strong", and 0.80-1.00; "very strong".

Results

The expression levels of IDO1 in gynecological cancers

To determine differences in the mRNA expression of IDO1 between tumor and normal tissues in gynecological cancers, we performed a comprehensive analysis using the Oncomine, TIMER, and GEPIA databases. As shown in **Figure 1A**, ONCOMINE analysis revealed that the transcription levels of IDO1 were significantly overexpressed in patients with cervical cancer tissues in three datasets [39-41], and the transcription levels of IDO1 in ovarian cancer tissues were significantly higher than those in the normal samples in one dataset [42]. TIMER analysis demonstrated that the IDO1 expression was higher in most solid tumors, including cervical, ovarian, and endometrial cancer (**Figure 1B**). Using the GEPIA analysis, the results also indicated that the expression levels of IDO1 were higher in gynecological cancer tissues than in normal tissues (**Figure 1C** and **1D**). We also sub-group-analyzed the expression of IDO1 with tumor stage or grade for gynecological cancers. The results indicated that IDO1 expression did not significantly differ among gynecological cancers using GEPIA and TISIDB (**Figure 2A-I**).

To further investigate the protein expression level of IDO1 in gynecological cancers, we performed immunohistochemistry analysis of the protein expression of IDO1 using the HPA. As

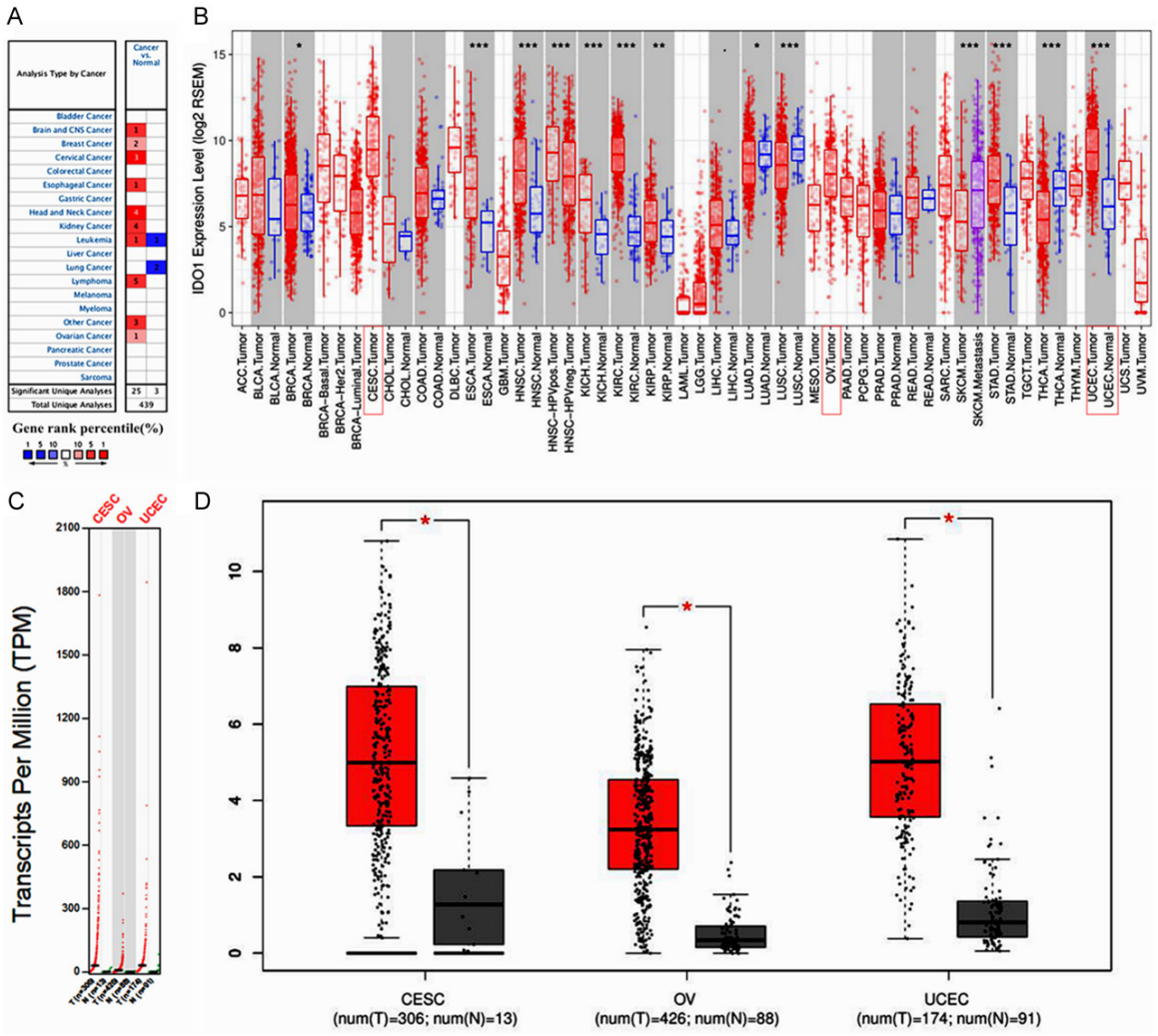


Figure 1. The expression levels of IDO1 in gynecological cancers (Oncomine, TIMER and GEPIA database). A. The mRNA expression of IDO1 of different cancers compared with normal tissues in the Oncomine database. B. IDO1 mRNA expression in different types of solid tumors and in corresponding normal tissues by TIMER (*P < 0.05, **P < 0.01, ***P < 0.001). C. Scatter diagram of IDO1 mRNA expression in gynecological cancers compared to normal tissues using GEPIA. D. Box plot of IDO1 mRNA expression in gynecological cancers compared to normal tissues using GEPIA (*P < 0.05).

shown in **Figure 3A-C**, the results showed that IDO1 protein expression also was upregulated in cervical, ovarian, and endometrial cancers compared with corresponding normal tissues. Simultaneously, we performed a pan-cancer analysis of the protein expression of IDO1 using the HPA, which presented the protein expression of IDO1 in 12 different tumor types. The results indicated that most malignant tissues were negative for IDO1. Nevertheless, single cases of several malignancies showed strong cytoplasmic staining, such as colorectal, ovarian, cervical, endometrial, stomach, and pancreatic cancers. Positivity was most abundantly seen in cervical (50.0%), endometrial (33.3%), and ovarian (18.2%) cancers (**Figure 3D**).

The prognostic value of IDO1 in gynecological cancers

The prognostic value of IDO1 mRNA expression in patients with gynecological cancers was analyzed by using the GEPIA and TISIDB database. The relationships between IDO1 expression and prognosis of different gynecological cancers are shown in **Figure 4A-F**. Regrettably, the results showed that DO1 expression levels

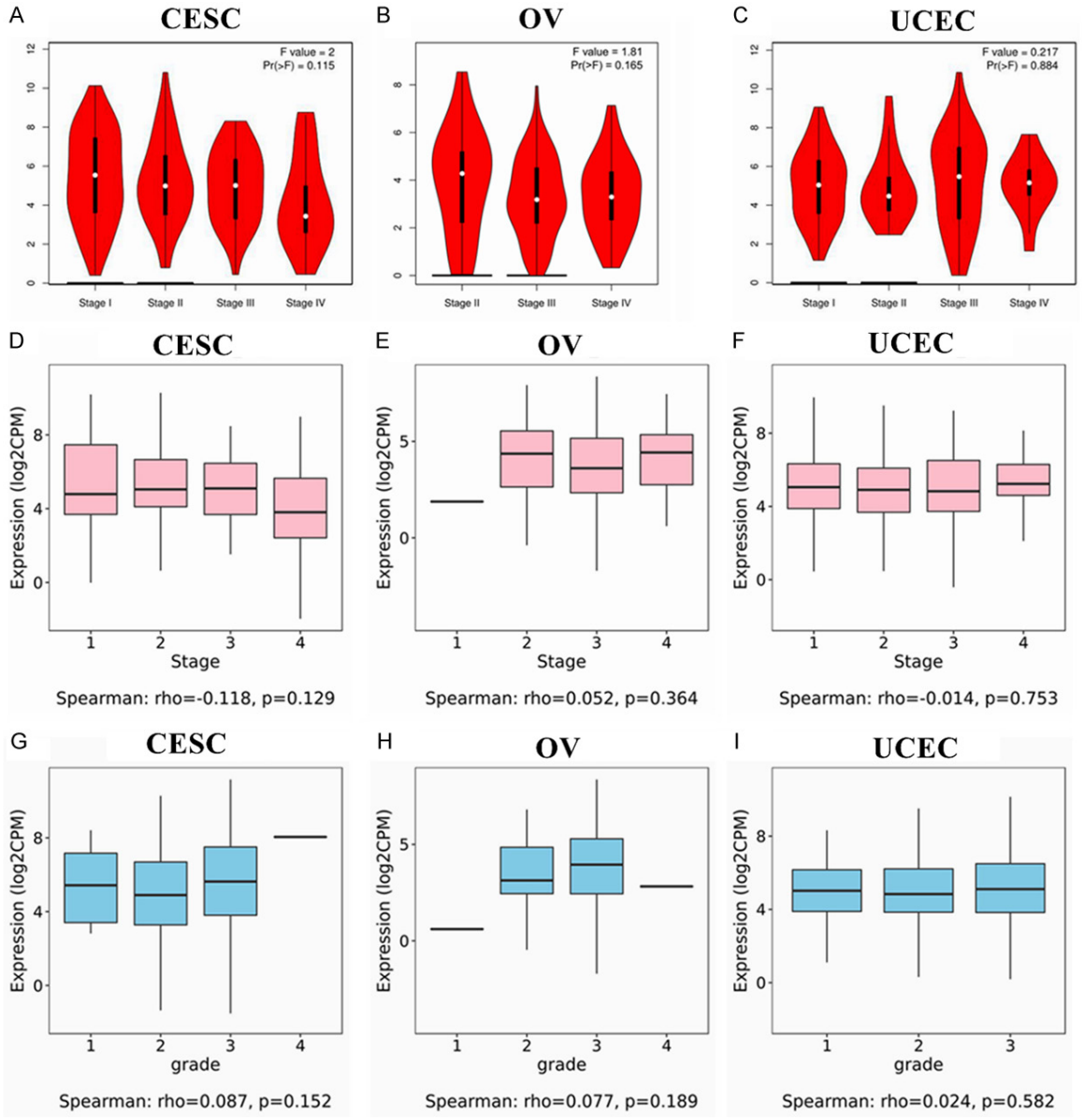


Figure 2. The expression levels of IDO1 in subgroups of patients with gynecological cancers stratified based on tumor stage or grade (GEPIA database and TISIDB database). A-C. Boxplot showing relative expression of IDO1 in normal individuals or in CESC, OV and UCEC patients in stages 1, 2, 3 or 4 using GEPIA, respectively. D-F. Boxplot showing relative expression of IDO1 in normal individuals or in CESC, OV and UCEC patients in stages 1, 2, 3 or 4 using TISIDB, respectively. G-I. Boxplot showing relative expression of IDO1 in normal individuals or in CESC, OV and UCEC patients in grade 1, 2, 3 or 4 using TISIDB, respectively (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

have little influence on overall survival (OS) in CESC and UCEC patients, and are only correlated with longer OS in OV patients using GEPIA.

Correlation analysis between IDO1 and immune infiltration in gynecological cancers

We assessed the correlation between IDO1 expressions with immune infiltration levels in gynecological cancers from TIMER. The results showed that IDO1 expression has significant correlations with tumor purity, B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells (DCs) in gynecological cancers. In CESC, the IDO1 expression level had significant positive correlations with infiltrating levels of CD8+ T cells ($r = 0.398$, $P = 7.67e-12$), CD4+ T cells ($r = 0.323$, $P = 3.68e-08$), neutrophils ($r = 0.678$, $P = 1.29e-38$), and DCs ($r = 0.503$, $P = 4.44e-19$) (**Figure 5A**). Similarly,

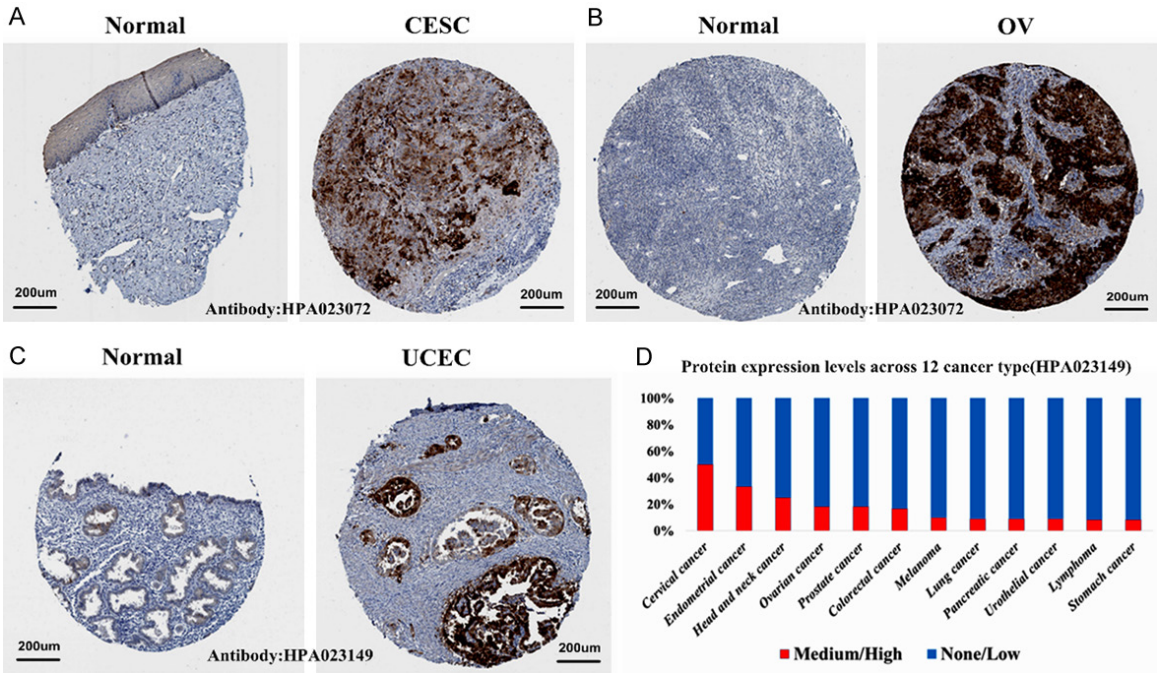


Figure 3. Immunohistochemistry analysis for IDO1 in gynecological cancers (HPA database). A-C. Protein expression level of IDO1 in CESC, OV and UCEC was significantly higher than corresponding controls using the HPA, respectively. D. Pan-cancer analysis of the protein expression of IDO1 using the HPA. Scale bars, 200 μm.

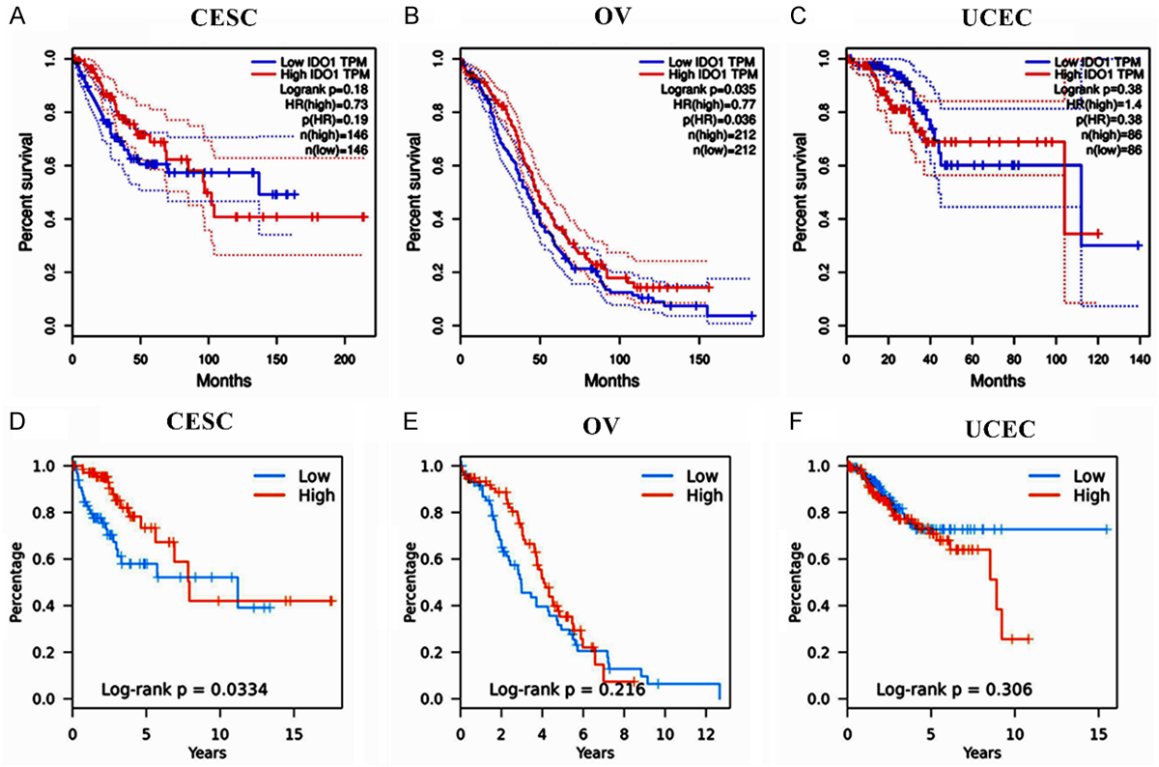


Figure 4. Kaplan-Meier survival curves comparing the high and low expression of IDO1 in gynecological cancers (GEPIA database and TISIDB database). A-C. Survival curves of OS based on the high and low expression of IDO1 in CESC, OV and UCEC patients using GEPIA, respectively. D-F. Survival curves of OS based on the high and low expression of IDO1 in CESC, OV and UCEC patients using TISIDB, respectively.

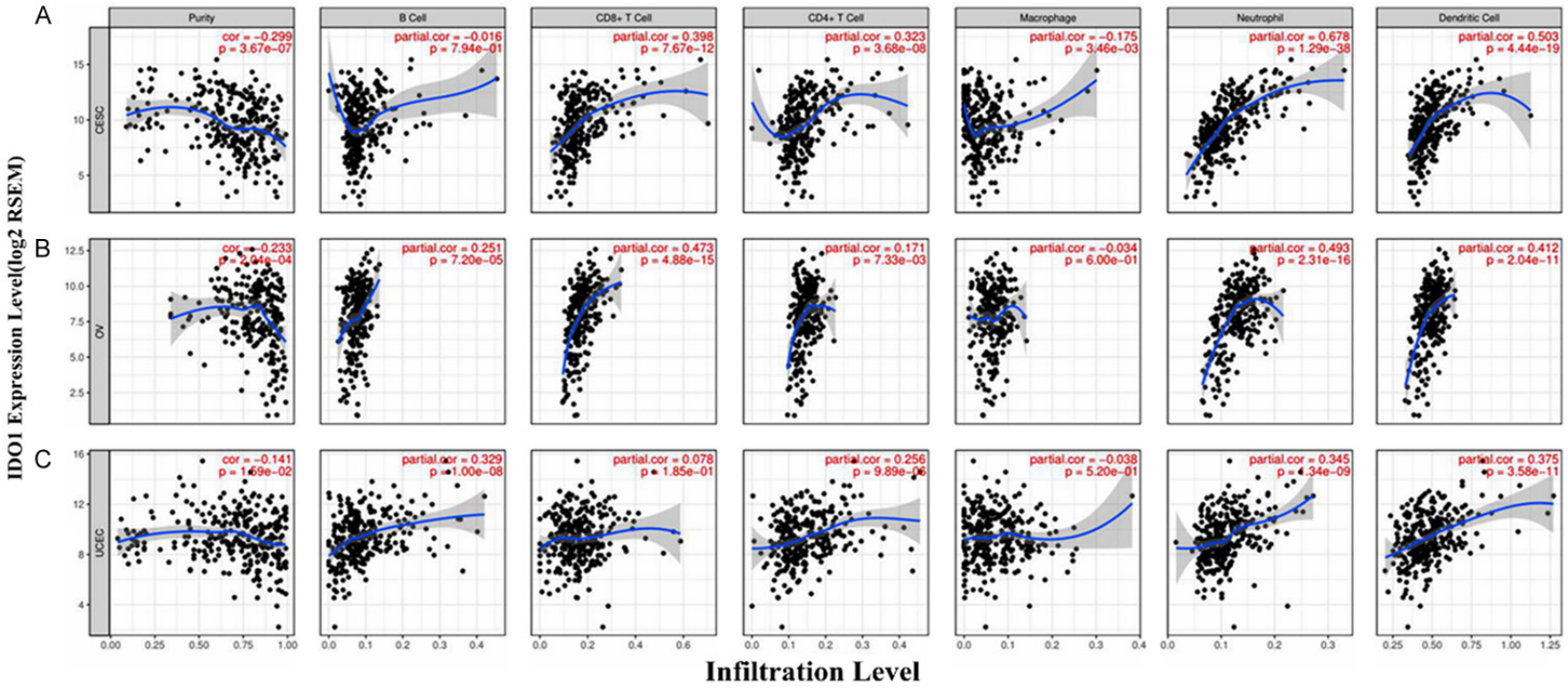


Figure 5. Correlation of IDO1 expression with immune infiltration level in gynecological cancers (TIMER database). A. Correlation of IDO1 expression with immune infiltration level in CESC. B. Correlation of IDO1 expression with immune infiltration level in OV. C. Correlation of IDO1 expression with immune infiltration level in UCEC.

there were positive correlations with infiltrating levels of B cells (*r* = 0.251, *P* = 7.20e-5), CD8+ T cells (*r* = 0.473, *P* = 4.88e-15), CD4+ T cells (*r* = 0.171, *P* = 7.33e-03), neutrophils (*r* = 0.493, *P* = 2.31e-16), and DCs (*r* = 0.412, *P* = 2.04e-11) in OV (**Figure 5B**), and the IDO1 expression level had significant positive correlations with infiltrating levels of B cells (r = 0.329, P = 1.00e-8), CD4+ T cells (*r* = 0.256, P = 9.89e-06), neutrophils (*r* = 0.345, *P* = 1.34e-09), and DCs (*r* = 0.4375, *P* = 3.58e-11) in UCEC (**Figure 5C**).

Correlation analysis between IDO1 and immune marker sets in gynecological cancers

To investigate the relationship between IDO1 and the diverse immune infiltrating cells, we continued to analyze the correlations between IDO1 expression and immune marker genes of different immune cells, including T cells (such as Th1, Th2, Th17, Treg, and exhausted T cells), CD8+ T cells, Tumor associated macrophages (TAMs), B cells, monocytes, M1 macrophages, M2 macrophages, neutrophils, natural killer cells, and DCs in gynecological cancers using the TIMER and GEPIA databases (**Tables 1**, S1 and Figure S1A-X). After the correlation adjustment by purity, the results revealed that the IDO1 expression level was significantly correlated with most immune marker sets of various immune cells in gynecological cancers. Specifically, we found that the expression levels of most marker sets of T cells, CD8+ T cells, TAMs, monocytes, M2 macrophages, natural killer cells, and DCs have strong correlations with IDO1 expression in CESC, OV, and UCEC. In addition, we further validated the correlation between IDO1 expression and the above markers of various immune cells in gynecological cancers using the GEPIA database. The correlation results between IDO1 and markers of various immune cells are similar to those found using GEPIA (Table S1).

Correlation analysis between IDO1 and immunomodulators in gynecological cancers

To further explore the potential immune mechanism of IDO1 in gynecological cancers, we further analyzed the correlation between the abundance of immunomodulators and expression, methylation, and copy number of IDO1 using TISIDB. As shown in Table S2 and **Figure 6A-I**, high IDO1 expression was significantly positively correlated with histocompatibility complexs (MHCs) and most immunoinhibitors, and negatively or weakly correlated with immunostimulator in gynecological cancers. IDO1 methylation was significantly associated with immunomodulators in OV, but this phenomenon was not significant for CESC and UCEC. Simultaneously, the copy number variation of IDO1 was not closely related to the immunomodulators in gynecological cancers.

Enrichment analysis of IDO1 co-expression genes and prognostic signature in gynecological cancers

Co-expression of IDO1 genes was analyzed using Oncomine in gynecological cancers. The 20 most significant gene sets correlated with IDO1 are shown in the heat map for CESC, UCEC, and OV, respectively (Table S3 and **Figure 7A-C**). The functions of the 20 most significant gene sets correlated with IDO1 underwent comprehensive enrichment analysis in Metascape. The gene set enriched for Reactome, KEGG, and GO biological processes is responsible mainly for cytokine signaling in the immune system, antigen processing, and presentation and activation of the immune response. In addition, the gene set was also involved in the positive regulation of I-kappaB kinase/NF-kappaB signaling, interferon alpha/beta signaling, regulation of the leukocyte apoptotic process, transcriptional misregulation in cancer, and post-translational protein phosphorylation. Comprehensive enrichment analysis and visualization of IDO1 co-expression genes in gynecological cancers are shown in **Figure 7D-G** and Table S4.

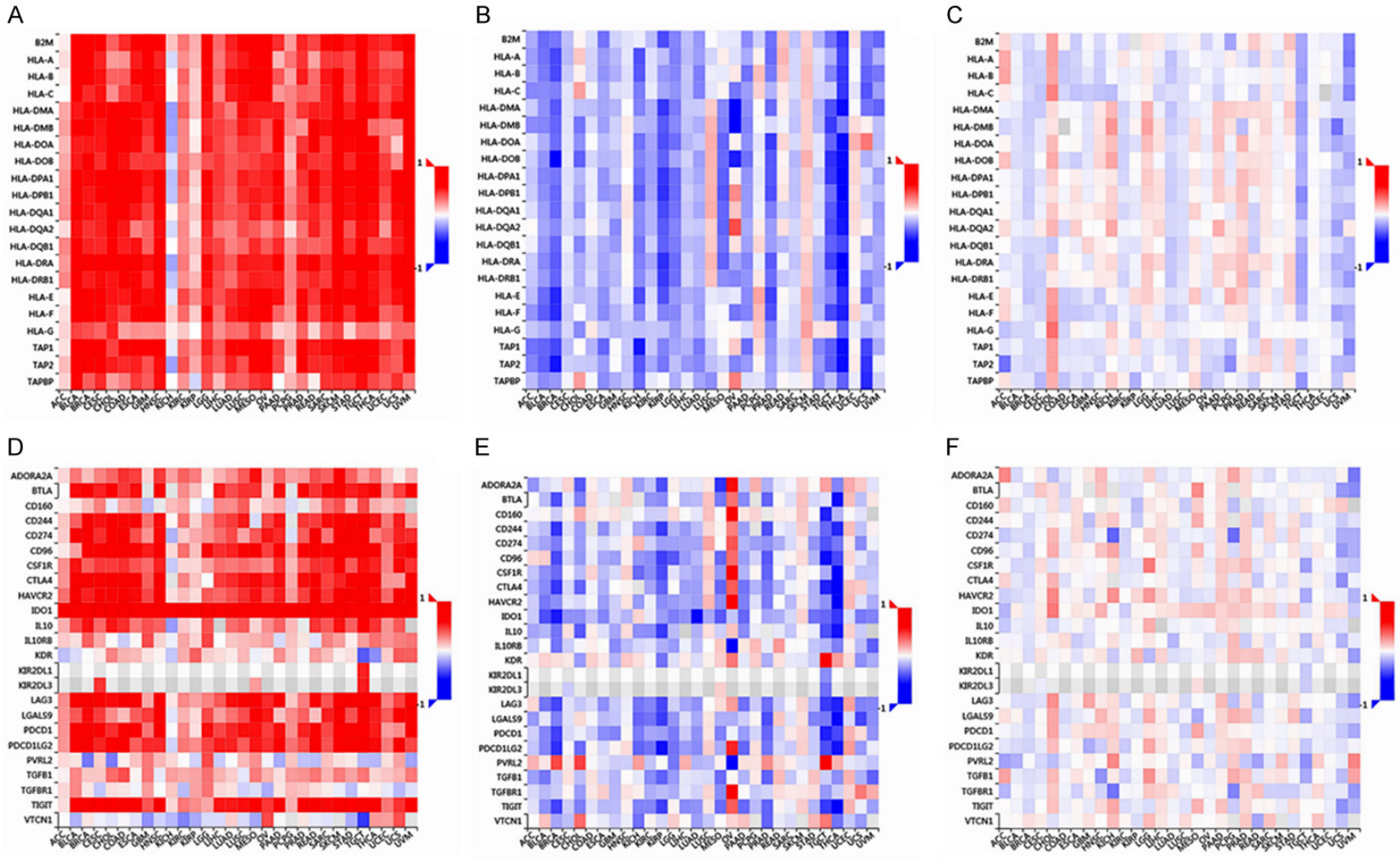
Given the increasing focus on the prognostic value of the IDO1 co-expression gene signature, we analyzed IDO1 gene co-expression using the TCGA dataset for CESC, OV, and UCEC by SurvExpress. The results are shown in **Figure 8A-C**. As shown, the patients were classified into predicted low and high-risk groups according to the Prognostic Index. The CESC, OV, and UCEC low and high risk groups were 96 and 95, 289 and 166, and 166 and 166, respectively. In addition, our results showed that most of the IDO1 co-expressed genes did not show significant differences in the low-risk and high-risk groups, and the differences in expression of IDO1 co-expressed genes in three gynecologic

Table 1. Correlation analysis between IDO1 and relate genes and markers of immune cells in TIMER. CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma

Description	Gene markers	CSEC				OV				USEC			
		None	*p*-value	Purity	*p*-value	None	*p*-value	Purity	*p*-value	None	*p*-value	Purity	*p*-value
T cell	CD3D	0.647	**0.00E+00**	0.606	**3.27e-29**	0.52	**2.2e-22**	0.499	**4.56e-17**	0.288	**7.05e-12**	0.288	**5.09e-07**
	CD3E	0.665	**0.00E+00**	0.625	**1.78e-31**	0.526	**0.00E+00**	0.525	**4.87e-19**	0.315	**5.04e-14**	0.294	**3.08e-07**
	CD2	0.653	**0.00E+00**	0.606	**3.24e-29**	0.534	**1e-23**	0.529	**2.52e-19**	0.32	**2.11e-14**	0.318	**2.52e-08**
Th1	STAT4	0.495	**2.43e-20**	0.42	**2.95e-13**	0.47	**4.59e-18**	0.427	**1.78e-12**	0.281	**2.26e-11**	0.253	**1.16e-05**
	STAT1	0.691	**0e-00**	0.665	**9.06e-37**	0.469	**0.00E+00**	0.459	**2.10e-14**	0.383	**0.00E+00**	0.333	**5.22e-09**
	IFN-γ	0.659	**1.44e-39**	0.625	**2.11e-31**	0.512	**1.25e-21**	0.455	**4.22e-14**	0.288	**6.93e-12**	0.294	**2.96e-07**
	TNF-α	0.187	**1e-03**	0.142	**1.82e-02**	0.27	**2e-06**	0.206	**1.06e-03**	0.213	**5.42e-07**	0.197	**7.17e-04**
	T-bet	0.661	**0.00E+00**	0.615	**2.90e-30**	0.534	**1.09e-23**	0.553	**2.22e-21**	0.293	**3e-12**	0.245	**2.23e-05**
Th2	GATA3	0.252	**8.75e-06**	0.217	**2.65e-04**	0.258	**5.7e-06**	0.179	**4.58e-03**	0.218	**2.66e-07**	0.238	**3.94e-05**
	STAT6	0.189	**9.21e-04**	0.155	**9.87e-03**	0.125	**2.96e-02**	0.096	**1.29e-01**	0.16	**1.77e-04**	0.131	**2.48e-02**
	STAT5A	0.411	**6.24e-14**	0.412	**9.48e-13**	0.129	**2.45e-02**	0.11	8.44e-02	0.249	**4.48e-09**	0.224	**1.11e-04**
	IL13	0.218	**1.25e-04**	0.132	**2.75e-02**	0.181	**1.59e-03**	0.19	**2.55e-03**	0.117	**6.24e-03**	0.116	**4.65e-02**
Th17	STAT3	0.202	**3.82e-04**	0.17	**4.65e-03**	0.126	**2.88e-02**	0.05	**4.33e-01**	0.275	**8.44e-11**	0.232	**6.01e-05**
	IL17A	0.139	**1.49e-02**	0.125	**3.79e-02**	0.131	**2.29e-02**	0.102	1.07e-01	0.042	3.28e-01	0.076	1.94e-01
Treg	FOXP3	0.526	**0.00E+00**	0.455	**1.54e-15**	0.455	**0.00E+00**	0.408	**2.17e-11**	0.232	**4.22e-08**	0.233	**5.63e-05**
	CCR8	0.408	**1.03e-13**	0.333	**1.41e-08**	0.334	**2.39e-09**	0.268	**1.83e-05**	0.175	**3.98e-05**	0.174	**2.81e-03**
	STAT5B	0.013	8.17e-01	0.018	7.59e-01	-0.164	**4.18e-03**	-0.185	**3.42e-03**	0.087	**4.35e-02**	0.011	8.53e-01
	TGFβ	0.135	**1.86e-02**	0.033	5.84e-01	0.173	**2.54e-03**	0.05	4.32e-01	0.141	**1.01e-03**	0.105	**7.15e-02**
T cell exhaustion	PD-1	0.631	**0.00E+00**	0.589	**3.13e-27**	0.462	**1.87e-17**	0.421	**4.07e-12**	0.24	**1.33e-08**	0.231	**6.51e-05**
	CTLA4	0.624	**0.00E+00**	0.575	**8.34e-26**	0.524	**9.96e-23**	0.501	**3.12e-17**	0.252	**2.47e-09**	0.259	**7.30e-06**
	LAG3	0.647	**0.00E+00**	0.604	**6.72e-29**	0.509	**0.00E+00**	0.502	**2.68e-17**	0.304	**3.87e-03**	0.301	**1.47e-07**
	TIM-3	0.611	**0.00E+00**	0.567	**5.28e-25**	0.436	**0.00E+00**	0.391	**1.61e-10**	0.365	**1.17e-18**	0.346	**1.22e-09**
	GZMB	0.62	**0.00E+00**	0.58	**2.83e-26**	0.652	**4.59e-38**	0.663	**6.13e-33**	0.307	**2.97e-13**	0.3	**1.59e-07**
CD8+ T cell	CD8A	0.68	**0.00E+00**	0.649	**1.42e-34**	0.466	**0.00E+00**	0.416	**8.05e-12**	0.329	**3.16e-15**	0.32	**2.04e-08**
	CD8B	0.507	**0.00E+00**	0.463	**3.85e-16**	0.338	**2e-09**	0.266	**2.15e-05**	0.243	**9.73e-09**	0.207	**3.54e-04**
TAMs	CCL2	0.27	**1.82e-06**	0.168	**5.09e-03**	0.421	**5.6e-15**	0.388	**2.15e-10**	0.253	**2.39e-09**	0.256	**9.35e-06**
	CD68	0.313	**2.74e-08**	0.245	**3.82e-05**	0.406	**2.07e-13**	0.363	**3.70e-09**	0.281	**3.17e-11**	0.26	**6.78e-06**
	IL10	0.368	**3.03e-11**	0.285	**1.44e-06**	0.268	**2.37e-06**	0.174	**5.95e-03**	0.261	**6.58e-10**	0.229	**7.82e-05**
B cell	CD19	0.363	**5.7e-11**	0.254	**1.87e-05**	0.097	**9.16e-02**	0.064	**3.15e-01**	0.205	**1.45e-06**	0.199	**6.26e-04**
	CD79A	0.339	**1.42e-09**	0.203	**6.58e-04**	0.282	**5.95e-07**	0.173	**6.10e-03**	0.276	**5.57e-11**	0.268	**3.33e-06**
Monocyte	CD86	0.568	**0.00E+00**	0.509	**1.10e-19**	0.454	**0.00E+00**	0.411	**1.52e-11**	0.362	**2.47e-18**	0.36	**2.11e-10**
	CD115	0.48	**0.00E+00**	0.39	**1.72e-11**	0.279	**8.68e-07**	0.204	**1.20e-03**	0.299	**1.38e-12**	0.296	**2.44e-07**
M1 Macrophage	INOS	0.065	2.54e-01	0.046	4.47e-01	-0.049	3.94e-01	-0.026	3.27e-01	0.282	**1.96e-11**	0.245	**2.30e-05**
	IRF5	0.131	**2.2e-02**	0.113	**6.05e-02**	0.248	**1.35e-05**	0.24	**1.30e-04**	0.282	**2.41e-11**	0.235	**4.73e-05**
	COX2	-0.011	8.47e-01	-0.065	2.82e-01	-0.014	8.14e-01	-0.065	3.04e-01	0.131	**2.11e-03**	0.175	**2.61e-03**

M2 Macrophage	CD163	0.429	**0.00E+00**	0.354	**1.35e-09**	0.326	**7.54e-09**	0.273	**1.25e-05**	0.286	**1.31e-11**	0.268	**3.14e-06**
	VSIG4	0.36	**1.2e-10**	0.291	**7.95e-07**	0.314	**2.84e-08**	0.256	**4.21e-05**	0.297	**1.5e-12**	0.278	**1.36e-06**
	MS4A4A	0.473	**0.00E+00**	0.404	**2.73e-12**	0.364	**9.12e-11**	0.328	**1.13e-07**	0.288	**9.69e-12**	0.256	**9.45e-06**
Neutrophils	CD66b	0.006	9.16e-01	0.02	7.46e-01	-0.02	7.28e-01	0.039	5.39e-01	0.012	7.72e-01	-0.014	8.15e-01
	CD11b	0.392	**1.55e-12**	0.334	**1.19e-08**	0.279	**8.61e-07**	0.214	**6.68e-04**	0.237	**2.22e-08**	0.246	**2.07e-05**
	CCR7	0.392	**1.41e-12**	0.317	**6.75e-08**	0.365	**7.21e-11**	0.328	**1.18e-07**	0.279	**4.32e-11**	0.267	**3.45e-06**
Natural killer cell	KIR2DL1	0.356	**1.37e-10**	0.311	**1.28e-07**	0.27	**1.85e-06**	0.244	**1.03e-04**	0.231	**4.76e-08**	0.126	**3.14e-02**
	KIR2DL3	0.482	**3.09e-19**	0.438	**1.94e-14**	0.372	**2.16e-11**	0.364	**3.13e-09**	0.229	**6.55e-08**	0.175	**2.72e-03**
	KIR2DL4	0.554	**5.28e-26**	0.526	**3.83e-21**	0.626	**2.52e-34**	0.609	**1.05e-26**	0.418	**1.88e-24**	0.337	**3.43e-09**
	KIR3DL1	0.421	**1.34e-14**	0.348	**2.68e-09**	0.275	**1.22e-06**	0.264	**2.37e-05**	0.245	**7.03e-09**	0.203	**4.80e-04**
	KIR3DL2	0.477	**9.19e-19**	0.422	**2.27e-13**	0.272	**1.47e-06**	0.236	**1.69e-04**	0.3	**8.52e-13**	0.275	**1.83e-06**
	KIR3DL3	0.339	**1.21e-09**	0.272	**4.54e-06**	0.253	**8.25e-06**	0.222	**4.17e-04**	0.141	**1e-03**	0.086	**1.42e-01**
	KIR2DS4	0.389	**1.71e-12**	0.358	**8.16e-10**	0.32	**1.24e-08**	0.3	**1.44e-06**	0.252	**2.37e-09**	0.231	**6.70e-05**
Dendritic cell	HLA-DPB1	0.607	**0.00E+00**	0.57	**3.01e-25**	0.511	**0.00E+00**	0.477	**1.40e-15**	0.431	**0.00E+00**	0.395	**2.19e-12**
	HLA-DQB1	0.512	**0.00E+00**	0.486	**7.53e-18**	0.386	**4.87e-12**	0.3	**1.40e-06**	0.438	**0.00E+00**	0.369	**6.60e-11**
	HLA-DRA	0.658	**0.00E+00**	0.631	**4.05e-32**	0.528	**0.00E+00**	0.482	**6.74e-16**	0.518	**0.00E+00**	0.471	**1.52e-17**
	HLA-DPA1	0.642	**0.00E+00**	0.614	**4.17e-30**	0.526	**0.00E+00**	0.481	**7.93e-16**	0.483	**0.00E+00**	0.431	**1.10e-14**
	BDCA-1	0.25	**9.44e-06**	0.182	**2.36e-03**	0.204	**3.46e-04**	0.125	**4.90e-02**	0.483	**0.00E+00**	0.182	**1.73e-03**
	BDCA-4	0.055	**3.34e-01**	-0.027	**6.56e-01**	0.062	**2.78e-01**	-0.01	**8.69e-01**	0.177	**3.16e-05**	0.119	**4.19e-02**
	CD11c	0.462	**0.00E+00**	0.368	**2.62e-10**	0.348	**6.17e-10**	0.304	**1.05e-06**	0.344	**1.5e-16**	0.32	**2.21e-08**

OV: Ovarian serous cystadenocarcinoma; UCEC: Uterine corpus endometrial carcinoma; Cor: R value of Spearman's correlation; Purity: correlation adjusted by purity. The bold values indicate that the results are statistically significant ($P < 0.05$).


A
B
C
D
E
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B2M
HLA-A
HLA-B
HLA-C
HLA-DMA
HLA-DMB
HLA-DOA
HLA-DOB
HLA-DPA1
HLA-DPB1
HLA-DQA1
HLA-DQA2
HLA-DQB1
HLA-DRA
HLA-DRB1
HLA-E
HLA-F
HLA-G
TAP1
TAP2
TAPBP
ADORA2A
BTLA
CD160
CD244
CD274
CD96
CSF1R
CTLA4
HAVCR2
IDO1
IL10
IL10RB
KDR
KIR2DL1
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LAG3
LGALS9
PDCD1
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PVRL2
TGFB1
TGFBR1
TIGIT
VTCN1

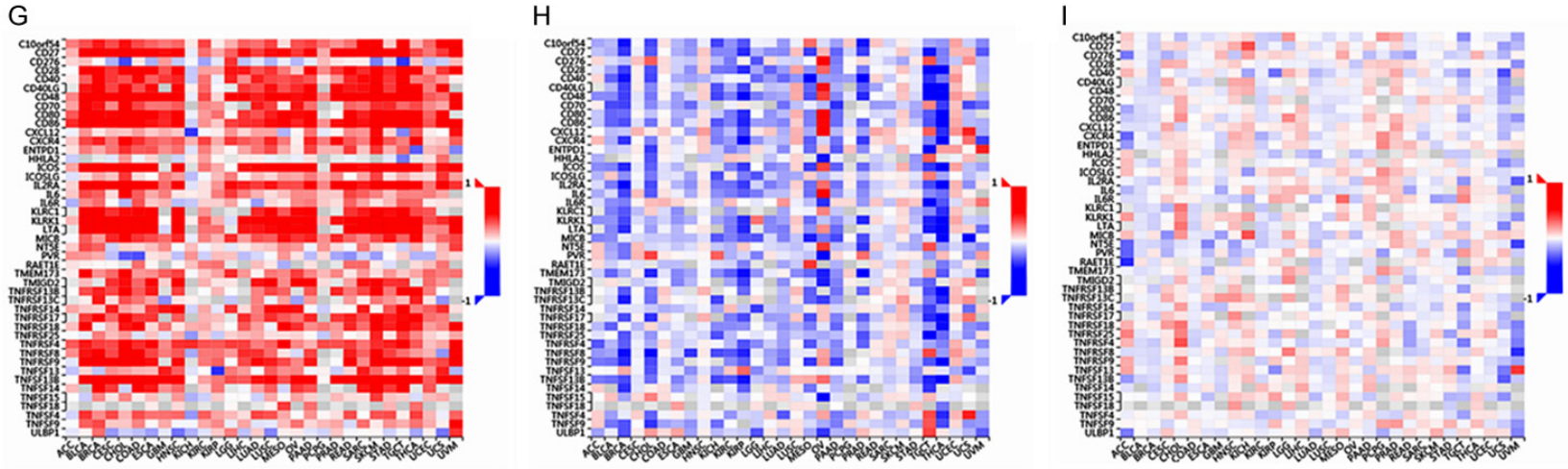
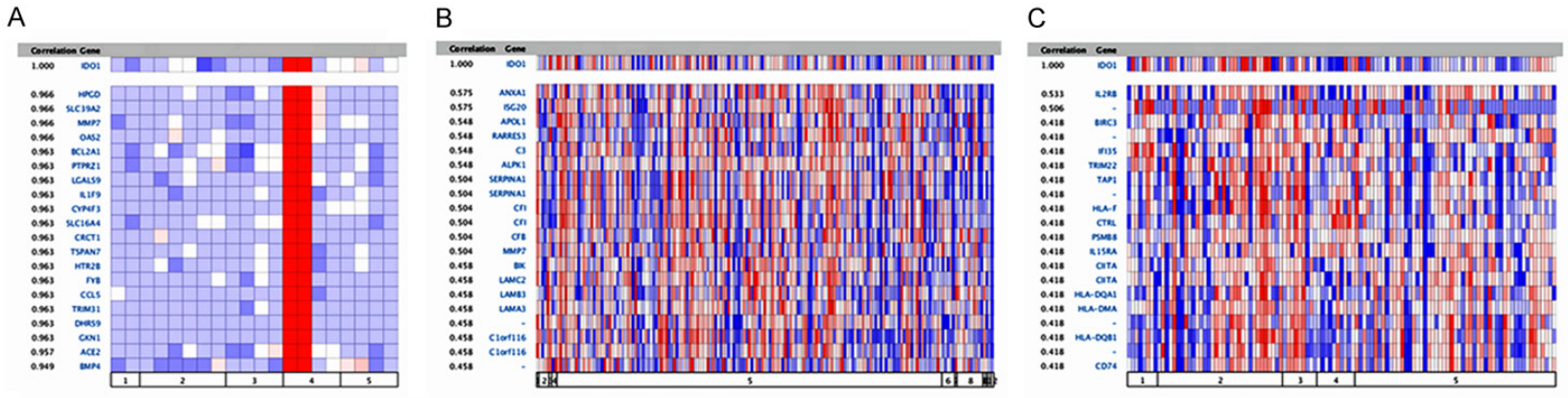


Figure 6. Correlation analysis between IDO1 and immunomodulators in gynecological cancers (TISIDB database). A-C. The heat map of correlation of IDO1 expression, methylation and copy number variation with immunoinhibitors in CESC. D-F. The heat map of correlation of IDO1 expression, methylation and copy number variation with immunoinhibitors in OV. G-I. The heat map of correlation of IDO1 expression, methylation and copy number variation with immunoinhibitors in UCEC.



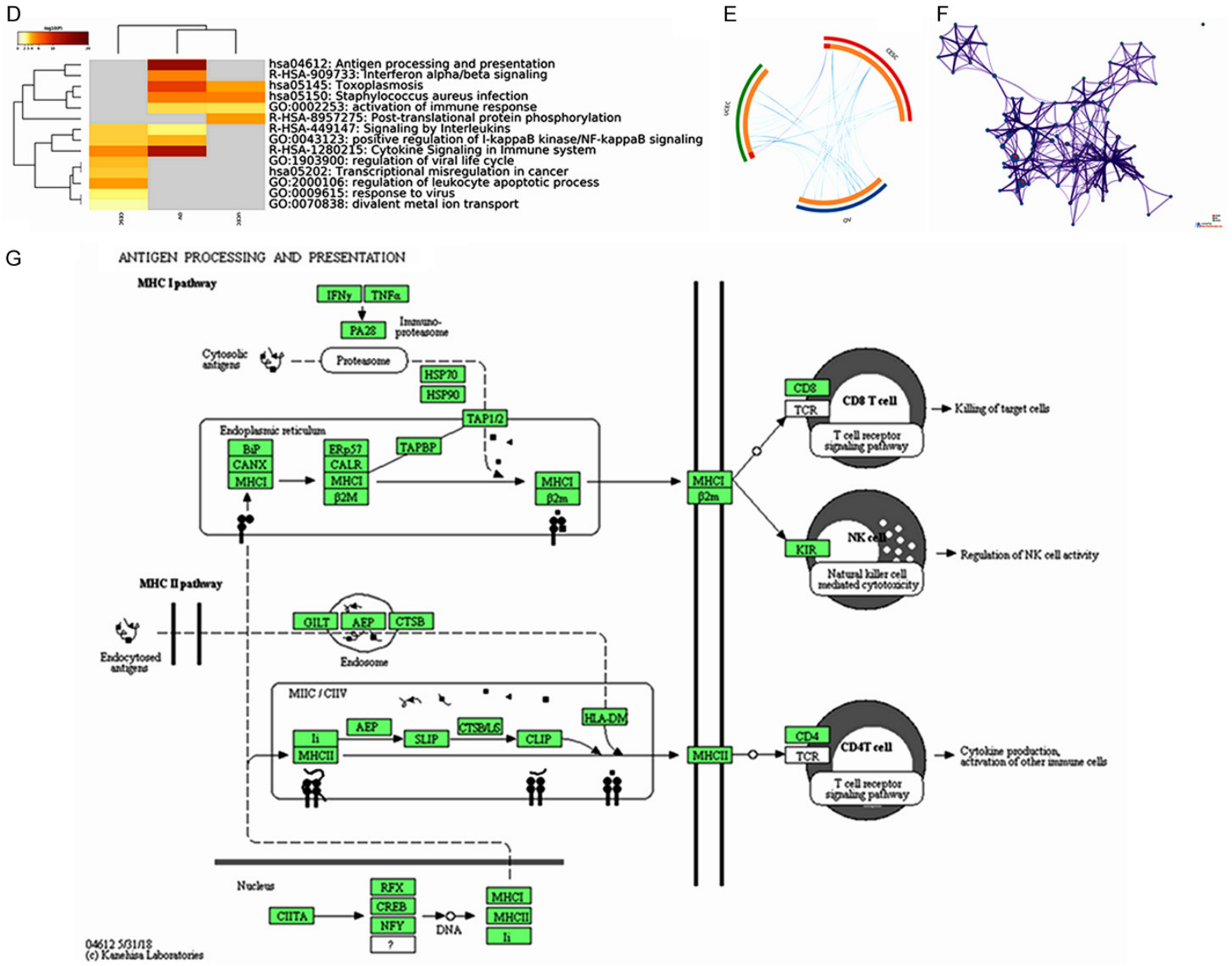

D
hsa04612: Antigen processing and presentation
R-HSA-909733: Interferon alpha/beta signaling
hsa05145: Toxoplasmosis
hsa05150: Staphylococcus aureus infection
GO:0002253: activation of immune response
R-HSA-8957275: Post-translational protein phosphorylation
R-HSA-449147: Signaling by Interleukins
GO:0043123: positive regulation of I-kappaB kinase/NF-kappaB signaling
R-HSA-1280215: Cytokine Signaling in Immune system
GO:1903900: regulation of viral life cycle
hsa05202: Transcriptional misregulation in cancer
GO:2000106: regulation of leukocyte apoptotic process
GO:0009615: response to virus
GO:0070838: divalent metal ion transport
E
F
G
ANTIGEN PROCESSING AND PRESENTATION
MHC I pathway
IFNγ
TNFα
PA28
Immuno-proteasome
Cytosolic antigens
Proteasome
HSP70
HSP90
TAP1/2
Endoplasmic reticulum
BiP
CANX
MHCI
ERp57
CALR
MHCI
β2M
TAPBP
MHCI
β2m
MHCI
β2m
CD8
TCR
CD8 T cell
T cell receptor signaling pathway
Killing of target cells
KIR
NK cell
Natural killer cell mediated cytotoxicity
Regulation of NK cell activity
MHC II pathway
Endocytosed antigens
GILT
AEP
CTSB
Endosome
MIIC / CIIV
Ii
MHCII
AEP
SLIP
CTSB/L/S
CLIP
HLA-DM
MHCII
CD4
TCR
CD4T cell
T cell receptor signaling pathway
Cytokine production, activation of other immune cells
Nucleus
CIITA
RFX
CREB
NFY
?
DNA
MHCI
MHCII
Ii
04612 5/31/18
(c) Kanehisa Laboratories

Figure 7. Enrichment analysis IDO1 co-expression genes in gynecological cancers (Oncomine and Matascape database). A-C. The heat map of the 20 significant gene sets correlated with IDO1 for CESC, UCEC and OV, respectively. D. Comprehensive enrichment analysis IDO1 co-expression genes in gynecological cancers. E. Circos visualization of Comprehensive enrichment analysis IDO1 co-expression genes in gynecological cancers. F. Network of enriched terms colored by the type of gynecological cancers. G. Antigen processing and presentation pathway regulated by the IDO1 in gynecological cancers.

tumors were similar (**Figure 8D-F**). Remarkably, Kaplan-Meier survival curves showed that the high-risk group displayed a significant poor OS outcome compared to the low-risk group in CESC (HR = 2.33, 95% CI = 1.24-4.38), OV (HR = 1.51, 95% CI = 1.20-1.90), and UCEC (HR = 2.41, 95% CI = 1.06-5.49) (**Figure 8G-I**). These results suggested that these IDO1 co-expressed gene signatures may serve as an important prognostic marker, which can be used to distinguish among patients in the high-risk group and predict prognosis in gynecological cancers.

Discussion

In 2003, Uyttenhove et al. found that IDO1 was overexpressed in the vast majority of human cancer tissues and that tumors expressing IDO1 could resist immune rejection [43]. The following year, many studies subsequently demonstrated that IDO1 promotes immune escape and encourages pathogenic inflammatory processes, which play an important role in the development and metastasis of tumors [14, 24, 44]. Moreover, the expression and activity of IDO1 has usually been associated with negative prognostic factors and worse outcome measures in the field of oncology [16]. More importantly, there has been great attention given to IDO1 for its use in cancer immunotherapy, and several IDO1-inhibitors are currently being tested *in vitro* and in clinical trials [10-12]. Although no IDO1 inhibitor has been approved by the FDA until now, several strategies for targeting IDO1 have been assessed in multiple clinical trials and have produced encouraging results such as 1-MT (indoximod and NLG8189), second-generation IDO1 inhibitors (INCB024360 and NLG919), selective IDO1 inhibitors (BMS-986205 and PF-06840-003), and IDO1-targeting vaccines [24, 45]. Regrettably, the underlying functions and mechanisms of IDO1 in gynecological cancer progression and immunology are still unclear. Thus, our study comprehensively evaluated the potential role and immune function of IDO1 in gynecologic cancers. The findings in our study could potentially be used to develop new immunotargets and strategies for gynecologic cancers.

In this study, we examined the expression levels of IDO1 using the Oncomine, TIMER, and GEPIA databases. These three online databases all revealed that the transcription levels of IDO1 are significantly overexpressed in patients with gynecologic cancers. Simultaneously, we performed a pan-cancer analysis of the protein expression of IDO1 using the HPA, and the results indicated that most malignant tissues of gynecologic cancers showed strong cytoplasmic staining. This difference in the expression of IDO1 in gynecologic tumors has also been confirmed by several studies. For instance, Hascitha J et al. showed that IDO1 mRNA levels were up-regulated in cervix cancer tissue compared to in normal cervix tissue [46]. Kristeleit R et al. found that IDO1 expression was observed in 94% of ovarian cancer, primary peritoneal carcinoma, and fallopian tube cancer samples [47]. Regrettably, the subgroup analysis indicated that the expression of IDO1 did not significantly differ among different tumor stages or grades of gynecological cancers, and our survival analysis showed that the DO1 expression levels have little influence on the clinical outcomes of gynecological cancer patients. The above findings may be due to the small sample sizes for different tumor stages or grades and different cutoff values used for survival analysis; future large-scale studies are required to validate our results. Most strikingly, the IDO1 co-expressed gene signatures may be used as a predictive biomarker panel, which can be further applied to distinguish patients in the high-risk group and predict prognosis in gynecological cancers.

We further analyzed the immune infiltration and function of IDO1 in gynecological cancers. Our findings suggested that IDO1 expression has significant correlation with tumor purity, B cell infiltration, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, DCs, and their immune marker genes, especially T cells and their subgroups, in gynecological cancers. Simultane-

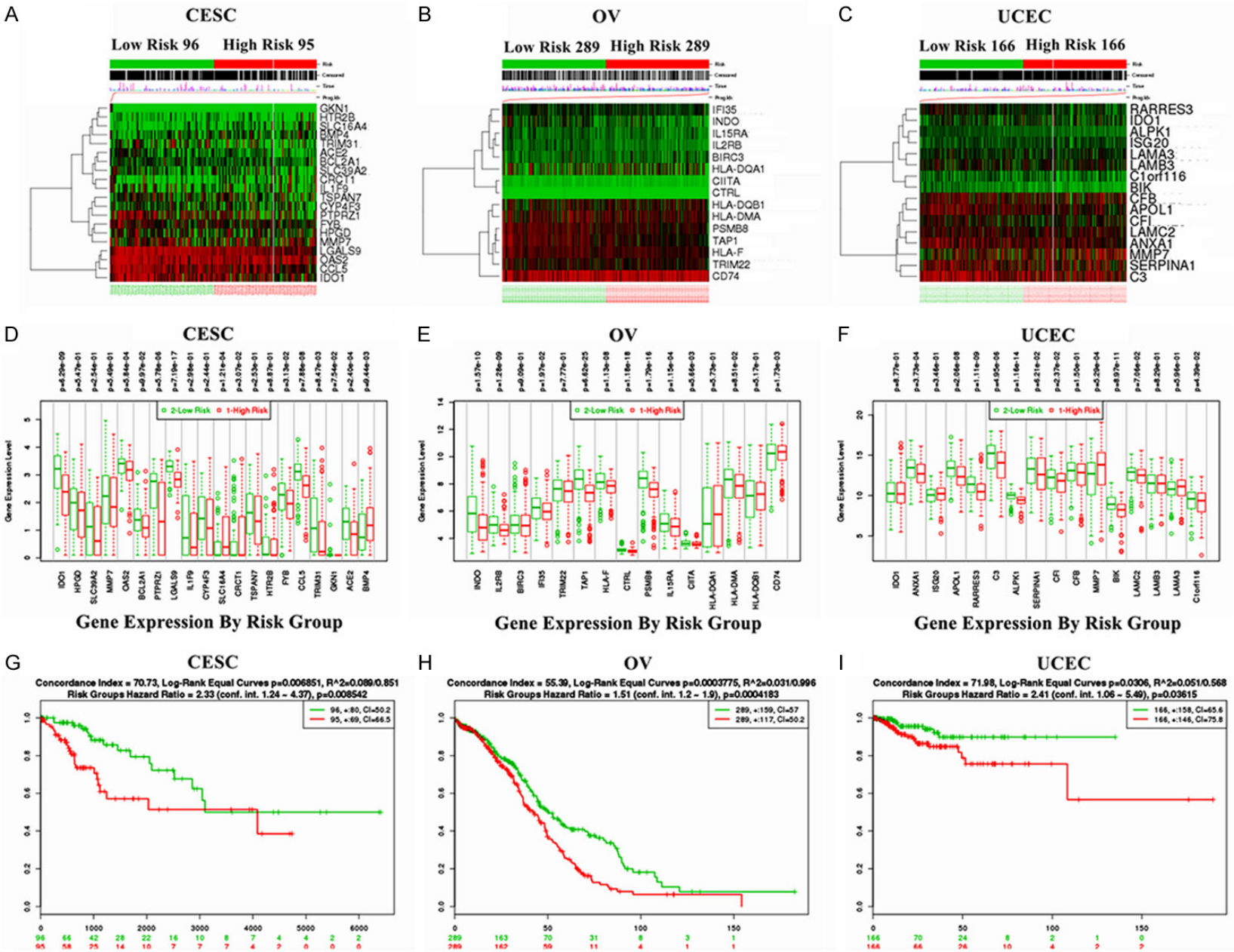

A
CESC
Low Risk 96 High Risk 95
GKN1
HTR2B
SLC16A4
BMP4
TRIM31
ACE2
BCL2A1
SLC39A2
CRCT1
IL1F9
TSPAN7
CYP4F3
PTPRZ1
FYB
HPGD
MMP7
LGALS9
OAS2
CCL5
IDO1
B
OV
Low Risk 289 High Risk 289
IFI35
INDO
IL15RA
IL2RB
BIRC3
HLA-DQA1
CIITA
CTRL
HLA-DQB1
HLA-DMA
PSMB8
TAP1
HLA-F
TRIM22
CD74
C
UCEC
Low Risk 166 High Risk 166
RARRES3
IDO1
ALPK1
ISG20
LAMA3
LAMB3
C1orf116
BIK
CFB
APOL1
CFI
LAMC2
ANXA1
MMP7
SERPINA1
C3
D
CESC
2-Low Risk 1-High Risk
Gene Expression Level
IDO1 HPGD SLC39A2 MMP7 OAS2 BCL2A1 PTPRZ1 LGALS9 IL1F9 CYP4F3 SLC16A4 CRCT1 TSPAN7 HTR2B FYB CCL5 TRIM31 GKN1 ACE2 BMP4
Gene Expression By Risk Group
E
OV
2-Low Risk 1-High Risk
Gene Expression Level
INDO IL2RB BIRC3 IFI35 TRIM22 TAP1 HLA-F CTRL PSMB8 IL15RA CIITA HLA-DQA1 HLA-DMA HLA-DQB1 CD74
Gene Expression By Risk Group
F
UCEC
2-Low Risk 1-High Risk
Gene Expression Level
IDO1 ANXA1 ISG20 APOL1 RARRES3 C3 ALPK1 SERPINA1 CFI CFB MMP7 BIK LAMC2 LAMB3 LAMA3 C1orf116
Gene Expression By Risk Group
G
CESC
Concordance Index = 70.73, Log-Rank Equal Curves p=0.006851, R^2=0.089/0.851
Risk Groups Hazard Ratio = 2.33 (conf. int. 1.24 ~ 4.37), p=0.008542
96, +:80, CI=50.2
95, +:69, CI=66.5
H
OV
Concordance Index = 55.39, Log-Rank Equal Curves p=0.0003775, R^2=0.031/0.996
Risk Groups Hazard Ratio = 1.51 (conf. int. 1.2 ~ 1.9), p=0.0004183
289, +:159, CI=57
289, +:117, CI=50.2
I
UCEC
Concordance Index = 71.98, Log-Rank Equal Curves p=0.0306, R^2=0.051/0.568
Risk Groups Hazard Ratio = 2.41 (conf. int. 1.06 ~ 5.49), p=0.03615
166, +:158, CI=65.6
166, +:146, CI=75.8

Figure 8. IDO1 co-expression genes signature in gynecological cancers (SurvExpress database). A-C. The heat map of the IDO1 co-expression genes high and low risk group for CESC, UCEC and OV, respectively. D-F. The gene expression level of IDO1 co-expression genes were detected in high risk and low risk group for CESC, UCEC and OV, respectively. G-I. Kaplan-Meier survival curves of IDO1 co-expression genes were explored in high risk and low risk group for CESC, UCEC and OV, respectively.

ously, the IDO1 co-expressed genes are mainly enriched in the immune process in three common gynecological cancers, such as cytokine signaling in the immune system, antigen processing, presentation and activation of immune response, and positive regulation of I-kappaB kinase/NF-kappaB signaling. These results further confirmed that high IDO1 expression may play an important role in T cell-mediating immunosuppression, immune tolerance, and immune escape in the gynecological cancer microenvironment [14].

In addition, recent studies have demonstrated great promise for targeting immunosuppression in cancer, including clinical trials aimed at inhibiting PD-1, PD-L1, and CTLA-4 in patients with advanced cancer [10-12]. Similar to other immune checkpoints, IDO1 was also suggested to be an important target for innovative therapeutic strategies for immunotherapeutic intervention [10, 11]. Although several strategies for targeting IDO1 are currently in clinical trials and are being evaluated for their efficacy against a wide range of cancers, only the following studies have reported the use of novel IDO1 inhibitors for gynecological cancers [48]. One phase I study found that the combination of navoximod (GDC-0919) and atezolizumab demonstrated acceptable safety, tolerability, and pharmacokinetics for patients with advanced cancer, including cervical and ovarian cancer [49]. Another phase I/IIa study indicated that combining BMS-986205 with nivolumab is safe and boosts response rates among patients with cervical and bladder cancers [50]. One randomized, open-label, phase 2 study of the IDO1 inhibitor epacadostat (INCB024360) versus tamoxifen as therapy for biochemically recurrent (CA-125 relapse)-only epithelial ovarian cancer found no significant difference in efficacy between epacadostat and tamoxifen, and epacadostat was generally well-tolerated [47]. Mei J et al. found that as a selective IDO1 inhibitor, epacadostat (INCB024360) in combination with pembrolizumab generally was well-tolerated and had encouraging antitumor activity in multiple advanced solid tumors, including endometrial cancer [51]. Interestingly, we found that high IDO1 expression was significantly positively correlated with MHCs and most immunoinhibitors, and negatively or weakly correlated with immunostimulator in gynecological cancers. Taken together, the existing clinical trial results and our findings strongly suggest that IDO1 inhibition is a potential therapeutic tool for gynecological cancer treatment.

Conclusion

In conclusion, our work demonstrates that the transcription levels of IDO1 were significantly overexpressed in patients with gynecologic cancers, and IDO1 co-expressed gene signatures may be useful potential prognostic markers for gynecologic cancers. Furthermore, increased IDO1 expression correlates with immune infiltration cells, immune marker sets, and immunomodulators in gynecological cancers. Taken together, IDO1 plays an important role in the immune infiltration and will potentially be an immunotherapeutic target for gynecological cancer treatment. As noted in the discussion, the detailed mechanisms remain unclear, and future studies are needed to confirm our results and thus promote clinical targeted therapy using IDO1 for gynecological cancer treatment.

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Disclosure of conflict of interest

None.

Abbreviations

IDO1, Indoleamine 2, 3-dioxygenase 1; FDA, Food and Drug Administration; CESC, Cervical

squamous cell carcinoma and endocervical adenocarcinoma; OV, Ovarian serous cystadenocarcinoma; UCEC, Uterine corpus endometrial carcinoma; TCGA, The Cancer Genome Atlas; GEPIA, Gene Expression Profiling Interactive Analysis; TAMs, Tumor associated macrophages; HPA, Human Protein Atlas; HR, Hazard ratio; MHCs, Histocompatibility complexs; DCs, Dendritic cells; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; OS, Overall survival; PFS, progression-free survival.

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Table S1. Correlation analysis between IDO1 and relate genes and markers of immune cells in GEPIA

Description	Gene markers	CSEC				OV				USEC			
		Tumor		Norma		Tumor		Norma		Tumor		Norma	
		R	*p*-value	R	*p*-value	R	*p*-value	R	*p*-value	R	*p*-value	R	*p*-value
T cell	CD3D	0.54	**0.00E+00**	0.99	1.10E-01	0.3	**2.9e-10**	0.31	**2.90E-03**	0.16	**3.60E-02**	0.16	6.00E-01
	CD3E	0.56	**0.00E+00**	0.31	3.00E-01	0.32	**1.5e-11**	0.34	**1.00E-03**	0.26	**4.90E-04**	0.31	3.00E-01
	CD2	0.6	**0.00E+00**	0.96	1.70E-01	0.34	**5.1e-13**	0.29	**5.40E-03**	0.21	**6.30E-03**	0.11	7.20E-01
Th1	STAT4	0.47	**0.00E+00**	0.98	1.10E-01	0.17	**4.70E-04**	0.27	**1.20E-02**	0.13	8.10E-02	0.62	**2.40E-02**
	STAT1	0.49	**0.00E+00**	-0.77	4.40E-01	0.25	**2.6e-07**	0.42	**4.5e-05**	0.32	**1.7e-05**	0.42	1.50E-01
	IFN-γ	0.63	**0.00E+00**	-0.16	9.00E-01	0.35	**8.7e-14**	0.069	5.20E-01	0.27	**2.70E-04**	0.44	1.30E-01
	TNF-α	-0.013	**8.20E-01**	0.43	7.20E-01	0.069	1.60E-01	-0.1	3.60E-01	0.17	**2.90E-02**	0.61	**2.80E-02**
	T-bet	0.58	**0.00E+00**	0.5	6.60E-01	0.35	**2e-13**	0.27	**1.00E-02**	0.2	**7.70E-03**	0.063	8.40E-01
Th2	GATA3	0.0021	9.70E-01	1	**2.20E-02**	-0.0035	9.40E-01	-0.026	8.10E-01	0.089	2.40E-01	-0.1	7.50E-01
	STAT6	0.1	6.90E-02	-0.91	2.70E-01	0.083	8.60E-02	0.13	2.30E-01	0.13	9.00E-02	0.13	6.60E-01
	STAT5A	0.23	**5.7e-05**	-0.46	7.00E-01	0.16	**7.20E-04**	-0.15	1.70E-01	0.24	**1.70E-03**	0.62	**2.50E-02**
	IL13	0.089	1.20E-01	1	**3.70E-02**	0.099	**4.10E-02**	-0.1	3.50E-01	-0.0017	9.80E-01	0.93	**5e-06**
Th17	STAT3	0.061	2.90E-01	0.42	7.20E-01	0.11	**2.30E-02**	-0.04	7.10E-01	0.24	**1.50E-03**	0.78	**1.60E-03**
	IL17A	0.32	**6.8e-09**	0.98	1.30E-01	0.53	**0.00E+00**	0.023	8.30E-01	-0.019	8.00E-01	-0.11	7.20E-01
Treg	FOXP3	0.47	**0.00E+00**	0.86	3.40E-01	0.29	**2e-09**	-0.08	4.60E-01	0.14	6.00E-02	0.61	**2.70E-02**
	CCR8	0.39	**1.2e-12**	NA	NA	0.029	5.50E-01	0.017	8.70E-01	0.15	**4.40E-02**	0.94	**2.2e-06**
	STAT5B	-0.038	5.10E-01	-1	**2.90E-02**	‘-6e-05	**1.00E+00**	0.0071	9.50E-01	0.12	1.30E-01	0.14	6.60E-01
	TGFβ	0.038	5.10E-01	0.69	5.10E-01	0.12	**1.60E-02**	0.15	1.70E-01	0.11	1.30E-01	0.49	9.20E-02
T cell exhaustion	PD-1	0.46	**0.00E+00**	0.95	2.10E-01	0.32	**2.4e-11**	0.16	1.30E-01	0.19	**1.00E-02**	0.32	2.80E-01
	CTLA4	0.53	**0.00E+00**	0.99	8.40E-02	0.3	**1.9e-10**	0.22	**4.10E-02**	0.22	**3.70E-03**	0.5	8.20E-02
	LAG3	0.49	**0.00E+00**	-0.41	7.30E-01	0.37	**2.2e-15**	0.17	1.00E-01	0.19	**1.30E-02**	-0.035	9.10E-01
	TIM-3	0.53	**0.00E+00**	0.74	4.70E-01	0.23	**2e-06**	0.21	**5.50E-02**	0.25	**1.10E-03**	0.57	**4.40E-02**
	GZMB	0.41	**4.5e-14**	0.86	3.40E-01	0.32	**1.1e-11**	0.17	1.10E-01	0.24	**1.20E-03**	0.84	**3.60E-04**
CD8+ T cell	CD8A	0.56	**0.00E+00**	0.92	2.50E-01	0.3	**4.3e-10**	0.36	**5.90E-04**	0.25	**7.30E-04**	0.64	**1.80E-02**
	CD8B	0.11	**4.90E-02**	1	**4.20E-02**	-0.0036	9.40E-01	0.43	**3.3e-05**	0.07	3.60E-01	0.26	3.90E-01
TAM	CCL2	0.13	2.10E-01	1	**2.70E-02**	0.24	**8e-07**	0.039	7.20E-01	0.11	1.40E-01	0.88	**6.1e-05**
	CD68	0.3	**9.9e-08**	0.27	8.20E-01	0.19	**7.4e-05**	0.17	1.10E-01	0.13	7.80E-02	0.29	3.40E-01
	IL10	0.29	**2.6e-07**	0.51	6.60E-01	0.055	2.60E-01	0.082	4.50E-01	-0.0068	9.30E-01	-0.078	8.00E-01
B cell	CD19	0.43	**2e-15**	3.8e-05	**1.00E+00**	0.029	5.50E-01	0.12	2.50E-01	0.029	7.00E-01	-0.31	3.10E-01
	CD79A	0.32	**8.8e-09**	1	5.60E-03	0.18	**2.60E-04**	0.083	4.40E-01	0.13	7.80E-02	0.032	9.20E-01
Monocyte	CD86	0.53	**0.00E+00**	0.94	2.30E-01	0.24	**6.3e-07**	0.19	8.30E-02	0.2	**7.80E-03**	0.46	1.10E-01
	CD115	0.35	**2.7e-10**	-0.15	9.10E-01	0.12	**1.50E-02**	0.11	3.30E-01	0.15	5.20E-02	-0.19	5.30E-01
M1 Macrophage	INOS	0.15	1.00E-02	0.94	2.20E-01	0.046	3.50E-01	-0.1	3.30E-01	0.54	**1.5e-14**	0.89	**5.2e-05**
	IRF5	0.063	2.70E-01	-0.18	8.80E-01	0.11	**2.80E-02**	0.14	1.90E-01	0.15	**4.60E-02**	0.021	9.50E-01
	COX2	-0.069	2.30E-01	1	**2.70E-02**	-0.018	7.10E-01	0.1	3.40E-01	0.13	9.10E-02	0.58	3.80E-02

M2 Macrophage	CD163	0.29	**2.4e-07**	0.95	2.00E-01	0.15	**1.50E-03**	0.093	3.90E-01	0.12	1.30E-01	-0.14	6.50E-01
	VSIG4	0.29	**3.4e-07**	-0.072	9.50E-01	0.11	**2.30E-02**	0.11	3.30E-01	0.14	7.00E-02	-0.23	4.50E-01
	MS4A4A	0.42	**1.4e-14**	0.41	7.30E-01	0.16	**9.90E-04**	0.16	1.30E-01	0.09	2.40E-01	-0.19	5.30E-01
Neutrophils	CD66b	-0.035	5.40E-01	0.46	6.90E-01	-0.0022	9.60E-01	-0.0027	9.80E-01	-0.032	6.80E-01	NA	NA
	CD11b	0.14	1.70E-02	-0.4	7.40E-01	0.11	1.80E-02	0.11	3.10E-01	0.15	**4.70E-02**	-0.12	7.00E-01
	CCR7	0.28	**7.9e-07**	1	**1.30E-02**	0.15	**2.00E-03**	0.072	5.00E-01	0.14	6.30E-02	0.12	6.90E-01
Natural killer cell	KIR2DL1	0.089	1.20E-01	-0.61	5.80E-01	0.13	**9.10E-03**	0.12	2.70E-01	-0.0045	9.50E-01	0.75	3.40E-03
	KIR2DL3	0.066	2.50E-01	-0.87	3.30E-01	0.015	7.60E-01	0.17	1.20E-01	0.025	7.40E-01	0.95	5.1e-07
	KIR2DL4	0.22	**1.30E-04**	0.84	3.60E-01	0.3	**1.6e-10**	0.26	**1.40E-02**	0.42	**1.1e-08**	0.88	6.5e-05
	KIR3DL1	0.054	3.40E-01	-0.27	8.30E-01	0.14	**4.50E-03**	0.088	4.20E-01	0.00087	9.90E-01	0.82	6.30E-04
	KIR3DL2	0.052	3.60E-01	0.52	6.50E-01	0.14	**4.30E-03**	0.024	8.30E-01	0.13	9.30E-02	0.67	1.20E-02
	KIR3DL3	0.14	1.40E-02	NA	NA	0.096	**4.70E-02**	0.0026	9.80E-01	0.49	**5e-12**	0.42	1.50E-01
	KIR2DS4	0.0056	9.20E-01	-0.044	9.70E-01	0.081	9.30E-02	0.14	1.90E-01	0.065	3.90E-01	0.89	5.8e-05
Dendritic cell	HLA-DPB1	0.5	**0.00E+00**	1	**1.50E-02**	0.26	**5.3e-08**	0.29	**7.00E-03**	0.38	**2.2e-07**	0.029	9.30E-01
	HLA-DQB1	0.4	**2.8e-13**	0.99	9.00E-02	0.23	**1.8e-06**	0.37	**3.30E-04**	0.13	7.80E-02	0.71	6.30E-03
	HLA-DRA	0.48	0.00E+00	0.99	9.10E-02	0.28	**3.6e-09**	0.3	**4.30E-03**	0.34	**3.9e-06**	0.37	2.20E-01
	HLA-DPA1	0.51	0.00E+00	0.87	3.20E-01	0.28	**3.1e-09**	0.18	9.70E-02	0.28	**2.30E-04**	0.21	4.90E-01
	BDCA-1	0.15	**7.90E-03**	-0.13	9.10E-01	0.024	6.20E-01	0.057	6.00E-01	-0.02	8.00E-01	-0.19	5.40E-01
	BDCA-4	-0.097	9.10E-02	-0.99	1.10E-01	0.032	5.10E-01	0.08	4.60E-01	0.18	**1.50E-02**	0.31	3.00E-01
	CD11c	0.31	**1.9e-08**	0.85	3.60E-01	0.21	**1e-05**	0.4	**1.30E-04**	0.29	**8.1e-05**	0.77	2.30E-03

CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; OV: Ovarian serous cystadenocarcinoma; UCEC: Uterine corpus endometrial carcinoma. The bold values indicate that the results are statistically significant ($P < 0.05$).

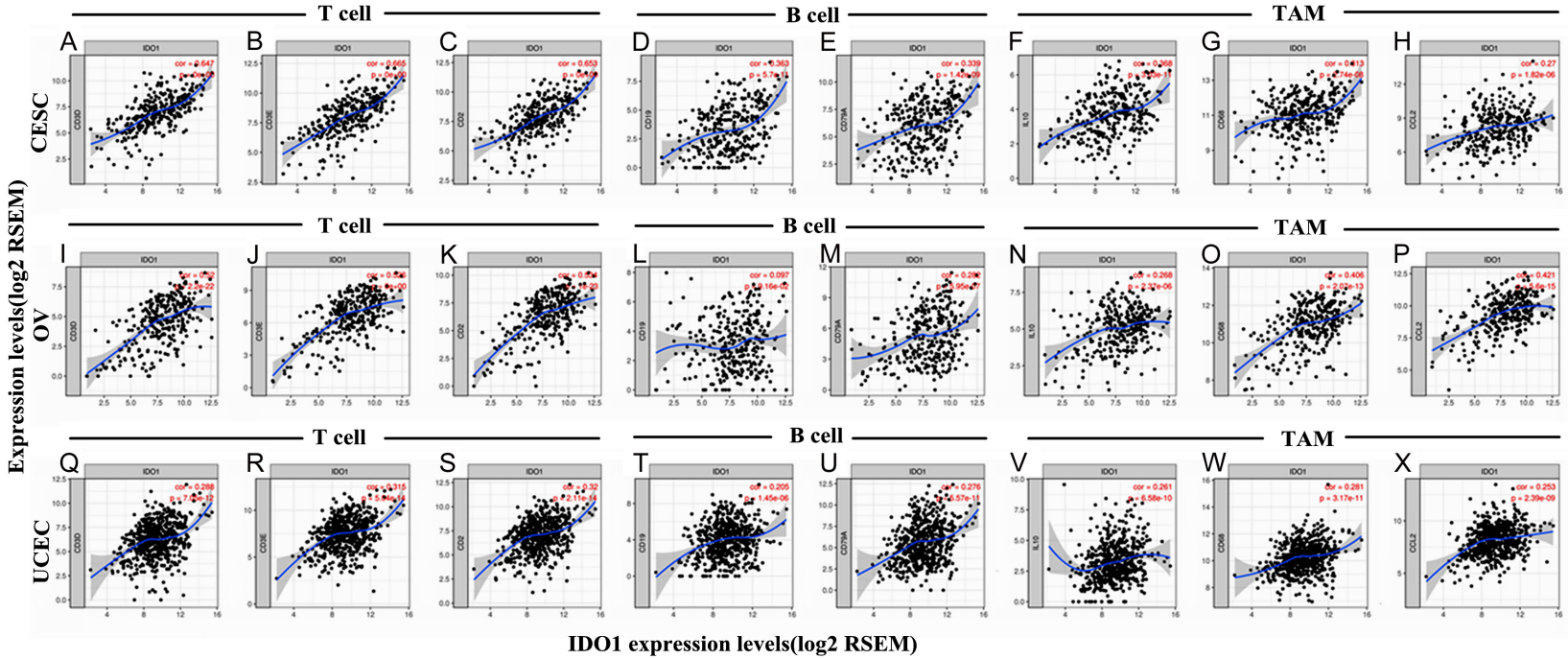

Figure S1. IDO1 expression correlated with gene markers of T cell, B cell and TAMs in gynecological cancers (GEPIA database). A-H. Scatterplots of correlations between IDO1 expression and gene markers of T cell, B cell and TAMs in CESC. I-P. Scatterplots of correlations between IDO1 expression and gene markers of T cell, B cell and TAMs in OV. Q-X. Scatterplots of correlations between IDO1 expression and gene markers of T cell, B cell and TAMs in UCEC.

Table S2. Correlation analysis between IDO1 and immunomodulators in gynecological cancers in TISIDB

	CSEC			OV			UCEC		
Immunoinhibitor	expression	methylation	copy number	expression	methylation	copy number	expression	methylation	copy number
ADORA2A	0.293	0.095	0.044	0.183	**0.583**	-0.015	0.143	0.197	-0.056
BTLA	**0.523**	-0.048	0.129	0.121	0.333	0.006	0.271	0.151	-0.046
CD160	0.303	0.027	-0.037	0.150	**0.650**	-0.012	0.136	0.011	0.083
CD244	**0.567**	-0.066	-0.031	**0.439**	0.300	0.016	0.316	0.149	-0.036
CD274	**0.504**	-0.033	-0.010	**0.558**	0.400	-0.055	0.381	0.054	-0.047
CD96	**0.641**	-0.093	0.006	**0.459**	0.350	-0.031	0.300	0.091	-0.012
CSF1R	**0.473**	-0.019	0.078	0.270	**0.700**	-0.048	0.291	0.043	-0.027
CTLA4	**0.620**	-0.094	0.026	**0.522**	0.383	-0.029	0.246	0.145	-0.053
HAVCR2	**0.605**	-0.059	0.029	**0.434**	**0.617**	-0.054	0.356	0.166	-0.040
IL10	0.360	-0.257	0.050	0.268	-0.133	-0.025	0.250	0.106	-0.065
IL10RB	0.200	-0.115	-0.015	0.219	**-0.617**	-0.076	0.250	0.034	-0.019
KDR	0.050	0.050	0.065	0.031	0.317	-0.043	0.119	0.082	0.009
KIR2DL1	-	-	-	-	-	-	-	-	-
KIR2DL3	0.482	-0.116	-0.041	-	-	-	-	-	-
LAG3	**0.644**	-0.068	-0.067	**0.513**	0.233	-0.033	0.297	0.216	-0.002
LGALS9	**0.462**	-0.070	0.013	**0.552**	-0.183	-0.012	0.394	-0.087	-0.048
PDCD1	**0.626**	-0.082	0.015	**0.466**	-0.033	-0.079	0.236	0.174	-0.040
PDCD1LG2	**0.526**	-0.018	-0.044	**0.499**	**0.517**	-0.047	0.314	0.236	-0.046
PVRL2	0.115	0.054	-0.094	-0.023	**-0.683**	0.034	0.074	-0.048	-0.011
TGFB1	0.130	0.066	-0.063	0.177	0.017	-0.059	0.134	0.173	-0.024
TGFBR1	-0.133	0.024	-0.035	-0.187	0.633	-0.029	0.044	0.098	-0.047
TIGIT	**0.692**	-0.113	0.004	0.478	0.383	-0.016	0.323	0.174	-0.040
VTCN1	-0.031	-0.148	0.042	0.435	0.100	0.006	0.232	-0.126	-0.053
Immunostimulator	expression	methylation	copy number	expression	methylation	copy number	expression	methylation	copy number
C10orf54	0.367	-0.008	0.012	0.352	-0.067	-0.062	0.311	0.185	-0.043
CD27	**0.526**	-0.036	0.073	**0.412**	0.150	-0.017	0.287	0.147	0.015
CD276	-0.157	0.120	-0.156	-0.142	**0.733**	0.014	-0.132	-0.116	-0.066
CD28	**0.412**	0.034	0.159	0.114	0.317	-0.110	0.187	0.128	0.013
CD40	**0.411**	0.035	0.007	**0.404**	-0.020	-0.055	0.296	0.211	-0.053
CD40LG	0.388	0.041	0.016	**0.441**	**0.500**	-0.022	0.262	0.139	-0.019
CD48	**0.603**	-0.052	0.075	**0.497**	**0.400**	-0.026	0.338	0.203	-0.055
CD70	0.248	-0.041	-0.058	**0.482**	-0.133	-0.031	0.244	0.126	-0.052
CD80	**0.518**	0.014	-0.001	**0.505**	**0.750**	-0.013	0.322	0.147	-0.019

CD86	**0.562**	-0.064	0.029	**0.452**	**0.517**	-0.041	0.354	0.173	-0.029
CXCL12	0.129	0.148	0.172	0.015	**0.700**	-0.004	0.034	0.219	0.018
CXCR4	0.285	0.009	0.085	0.030	-0.083	0.021	0.081	-0.022	-0.026
ENTPD1	0.282	0.040	0.061	**0.490**	0.117	0.016	0.143	0.197	-0.003
HHLA2	-0.555	0.017	0.015	-0.034	**-0.500**	-0.116	-0.064	-0.028	0.061
ICOS	**0.589**	-0.045	0.039	**0.542**	-0.383	-0.003	0.271	0.185	-0.022
ICOSLG	0.124	-0.011	0.064	0.177	-0.150	0.055	0.061	0.008	-0.076
IL2RA	**0.532**	-0.034	0.042	0.369	0.300	-0.108	0.273	0.153	-0.012
IL6	0.076	0.124	0.016	0.241	**0.600**	-0.045	0.265	0.130	0.048
IL6R	-0.007	0.023	0.103	0.241	-0.433	-0.011	0.041	-0.070	0.027
KLRC1	**0.426**	-0.134	-0.146	**0.518**	0.250	-0.012	0.349	0.123	-0.016
KLRK1	**0.635**	-0.072	-0.007	**0.550**	-0.117	0.020	0.363	0.177	-0.034
LTA	**0.547**	-0.015	0.017	**0.533**	0.233	-0.042	0.238	0.117	-0.013
MICB	**0.447**	-0.077	-0.228	**0.425**	-0.283	-0.127	0.178	-0.037	0.096
NT5E	-0.096	0.131	-0.135	0.213	**0.417**	-0.004	-0.012	-0.086	-0.002
PVR	-0.126	0.145	-0.154	0.060	-0.817	-0.028	-0.150	0.007	0.021
RAET1E	0.010	-0.053	0.044	-	-	-	0.133	-0.123	0.096
TMEM173	0.304	0.034	0.016	0.395	-0.467	0.016	0.312	-0.048	-0.101
TMIGD2	**0.429**	-0.107	-0.073	**0.528**	-0.467	-0.014	0.299	0.096	-0.031
TNFRSF13B	0.375	0.010	0.106	-	-	-	0.262	0.128	0.022
TNFRSF13C	0.067	-0.017	0.159	-0.194	-0.400	0.024	0.050	0.144	-0.042
TNFRSF14	**0.466**	-0.050	-0.035	0.497	0.117	-0.112	0.328	0.030	-0.031
TNFRSF17	0.308	0.027	0.123	0.287	0.117	0.008	0.293	0.101	-0.007
TNFRSF18	0.156	-0.137	0.199	**0.426**	-0.050	-0.071	0.195	-0.019	0.072
TNFRSF25	0.138	-0.061	0.044	0.091	-0.167	-0.015	0.090	-0.077	0.155
TNFRSF4	0.371	-0.029	0.055	0.243	-0.117	-0.084	0.126	0.156	-0.011
TNFRSF8	0.377	-0.031	0.018	-0.070	0.183	-0.001	0.152	0.206	-0.024
TNFRSF9	**0.576**	-0.018	-0.037	0.249	0.217	-0.069	0.319	0.136	-0.069
TNFRSF13	0.227	-0.024	0.059	0.253	-0.033	0.023	0.209	-0.170	-0.054
TNFRSF13B	**0.614**	-0.056	0.045	**0.564**	0.300	-0.040	0.331	0.164	-0.034
TNFRSF14	**0.409**	-0.048	-0.024	**0.407**	-0.033	-0.005	0.345	-0.045	0.046
TNFRSF15	0.051	0.075	0.016	0.190	-0.550	-0.046	0.139	-0.055	-0.009
TNFRSF18	-	-	-	-	-	-	-	-	-
TNFRSF4	0.369	0.094	0.044	-0.008	0.183	0.013	0.146	0.147	0.053
TNFRSF9	0.110	-0.004	-0.084	0.286	-0.167	0.038	0.163	0.079	-0.051
ULBP1	0.293	0.038	0.115	0.049	-0.417	-0.013	0.032	-0.060	0.084

MHC molecule	expression	methylation	copy number	expression	methylation	copy number	expression	methylation	copy number
B2M	**0.622**	-0.147	-0.030	**0.631**	-0.050	-0.096	**0.500**	0.075	-0.004
HLA-A	**0.519**	-0.162	-0.097	**0.608**	-0.067	-0.033	0.381	-0.001	0.008
HLA-B	**0.558**	-0.123	-0.108	**0.644**	0.050	-0.019	**0.447**	-0.006	0.006
HLA-C	**0.555**	-0.064	-0.133	**0.611**	-0.317	-0.042	**0.401**	-0.064	-
HLA-DMA	**0.591**	-0.067	-0.117	**0.524**	-0.833	-0.028	**0.457**	-0.012	-0.035
HLA-DMB	**0.551**	-0.061	-0.049	**0.401**	-0.683	-0.029	0.307	0.082	-0.127
HLA-DOA	**0.544**	-0.056	-0.047	0.379	0.033	-0.047	0.352	0.175	-0.119
HLA-DOB	**0.494**	-0.048	-0.008	**0.498**	-0.533	-0.025	**0.450**	0.086	-0.001
HLA-DPA1	**0.639**	-0.070	-0.066	**0.521**	0.033	-0.044	**0.478**	0.095	-0.047
HLA-DPB1	**0.601**	-0.061	-0.004	**0.507**	0.267	-0.025	**0.428**	0.067	-0.054
HLA-DQA1	**0.533**	-0.082	-0.034	**0.440**	0.133	-0.018	0.377	0.009	-0.048
HLA-DQA2	**0.523**	-0.086	0.043	0.283	0.417	0.008	0.235	0.073	-0.073
HLA-DQB1	**0.504**	-0.035	-0.009	0.384	0.067	-0.043	**0.437**	0.026	-0.038
HLA-DRA	**0.657**	-0.072	-0.088	**0.527**	-0.200	-0.039	**0.513**	0.027	-0.066
HLA-DRB1	**0.571**	-0.580	-0.113	**0.512**	-0.283	-0.049	**0.478**	-0.003	-0.005
HLA-E	**0.625**	-0.096	-0.101	**0.625**	-0.050	-0.007	**0.462**	0.043	-0.006
HLA-F	**0.604**	-0.123	-0.099	**0.672**	-0.317	-0.045	0.362	0.099	-0.026
HLA-G	0.365	-0.092	-0.026	0.320	0.017	0.008	0.184	-0.046	0.011
TAP1	**0.701**	-0.149	-0.089	**0.716**	0.117	-0.082	**0.488**	0.025	-0.004
TAP2	**0.610**	-0.107	-0.083	**0.638**	-0.283	-0.093	**0.426**	0.046	0.003
TAPBP	**0.499**	-0.025	-0.161	**0.498**	0.300	-0.135	0.367	-0.044	0.015

CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; OV: Ovarian serous cystadenocarcinoma; UCEC: Uterine corpus endometrial carcinoma. The bold values indicate that the result is a moderate and above correlation (|Cor| > 0.40).

Table S3. The IDO1 co-expression genes in gynecological cancers in Oncomine

Original_id	CESC	OV	UCEC	Gene ID	Type	Tax ID	Description
MMP7	1	0	1	4316	Gene_ID	H. sapiens	matrix metallopeptidase 7
ISG20	0	0	1	3669	Gene_ID	H. sapiens	interferon stimulated exonuclease gene 20
IFI35	0	1	0	3430	Gene_ID	H. sapiens	interferon induced protein 35
LAMB3	0	0	1	3914	Gene_ID	H. sapiens	laminin subunit beta 3
LGALS9	1	0	0	3965	Gene_ID	H. sapiens	galectin 9
C1orf116	0	0	1	79098	Gene_ID	H. sapiens	chromosome 1 open reading frame 116
RARRES3	0	0	1	5920	Gene_ID	H. sapiens	phospholipase A and acyltransferase 4
PSMB8	0	1	0	5696	Gene_ID	H. sapiens	proteasome subunit beta 8
TRIM22	0	1	0	10346	Gene_ID	H. sapiens	tripartite motif containing 22
BIK	0	0	1	638	Gene_ID	H. sapiens	BCL2 interacting killer

LAMC2	0	0	1	3918	Gene_ID	H. sapiens	laminin subunit gamma 2
OAS2	1	0	0	4939	Gene_ID	H. sapiens	2'-5'-oligoadenylate synthetase 2
CCL5	1	0	0	6352	Gene_ID	H. sapiens	C-C motif chemokine ligand 5
HLA-DQA1	0	1	0	3117	Gene_ID	H. sapiens	major histocompatibility complex, class II, DQ alpha 1
HLA-DQB1	0	1	0	3119	Gene_ID	H. sapiens	major histocompatibility complex, class II, DQ beta 1
SLC39A2	1	0	0	29986	Gene_ID	H. sapiens	solute carrier family 39 member 2
BMP4	1	0	0	652	Gene_ID	H. sapiens	bone morphogenetic protein 4
TSPAN7	1	0	0	7102	Gene_ID	H. sapiens	tetraspanin 7
HPGD	1	0	0	3248	Gene_ID	H. sapiens	15-hydroxyprostaglandin dehydrogenase
SERPINA1	0	0	1	5265	Gene_ID	H. sapiens	serpin family A member 1
CFI	0	0	1	3426	Gene_ID	H. sapiens	complement factor I
SLC16A4	1	0	0	9122	Gene_ID	H. sapiens	solute carrier family 16 member 4
ANXA1	0	0	1	301	Gene_ID	H. sapiens	annexin A1
ACE2	1	0	0	59272	Gene_ID	H. sapiens	angiotensin I converting enzyme 2
HLA-DMA	0	1	0	3108	Gene_ID	H. sapiens	major histocompatibility complex, class II, DM alpha
CTRL	0	1	0	1506	Gene_ID	H. sapiens	chymotrypsin like
CRCT1	1	0	0	54544	Gene_ID	H. sapiens	cysteine rich C-terminal 1
IL2RB	0	1	0	3560	Gene_ID	H. sapiens	interleukin 2 receptor subunit beta
ALPK1	0	0	1	80216	Gene_ID	H. sapiens	alpha kinase 1
CIITA	0	1	0	4261	Gene_ID	H. sapiens	class II major histocompatibility complex transactivator
FYB	1	0	0	2533	Gene_ID	H. sapiens	FYN binding protein 1
LAMA3	0	0	1	3909	Gene_ID	H. sapiens	laminin subunit alpha 3
CD74	0	1	0	972	Gene_ID	H. sapiens	CD74 molecule
TAP1	0	1	0	6890	Gene_ID	H. sapiens	transporter 1, ATP binding cassette subfamily B member
CFB	0	0	1	629	Gene_ID	H. sapiens	complement factor B
HLA-F	0	1	0	3134	Gene_ID	H. sapiens	major histocompatibility complex, class I, F
BCL2A1	1	0	0	597	Gene_ID	H. sapiens	BCL2 related protein A1
GKN1	1	0	0	56287	Gene_ID	H. sapiens	gastrokine 1
C3	0	0	1	718	Gene_ID	H. sapiens	complement C3
IL1F9	1	0	0	56300	Gene_ID	H. sapiens	interleukin 36 gamma
PTPRZ1	1	0	0	5803	Gene_ID	H. sapiens	protein tyrosine phosphatase receptor type Z1
APOL1	0	0	1	8542	Gene_ID	H. sapiens	apolipoprotein L1
IL15RA	0	1	0	3601	Gene_ID	H. sapiens	interleukin 15 receptor subunit alpha
CYP4F3	1	0	0	4051	Gene_ID	H. sapiens	cytochrome P450 family 4 subfamily F member 3
TRIM31	1	0	0	11074	Gene_ID	H. sapiens	tripartite motif containing 31
HTR2B	1	0	0	3357	Gene_ID	H. sapiens	5-hydroxytryptamine receptor 2B
BIRC3	0	1	0	330	Gene_ID	H. sapiens	baculoviral IAP repeat containing 3

CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; OV: Ovarian serous cystadenocarcinoma; UCEC: Uterine corpus endometrial carcinoma.

Table S4. Top 20 enriched pathways of IDO1 co-expression genes in gynecological cancers in Metascape

GO	Category	Description	Count	%	Log10(P)	Log10(q)
hsa05321	KEGG Pathway	Inflammatory bowel disease (IBD)	17	30.36	-30.73	-26.41
GO:0042110	GO Biological Processes	T cell activation	26	46.43	-29.19	-25.18
GO:0001817	GO Biological Processes	regulation of cytokine production	27	48.21	-25.87	-22.25
hsa04612	KEGG Pathway	Antigen processing and presentation	15	26.79	-24.91	-21.44
hsa04640	KEGG Pathway	Hematopoietic cell lineage	14	25	-21.27	-18.31
M36	Canonical Pathways	PID IL27 PATHWAY	10	17.86	-20.01	-17.12
M290	Canonical Pathways	PID IL12 STAT4 PATHWAY	10	17.86	-18.77	-15.91
R-HSA-6785807	Reactome Gene Sets	Interleukin-4 and Interleukin-13 signaling	13	23.21	-18.66	-15.84
GO:0002697	GO Biological Processes	regulation of immune effector process	19	33.93	-18.45	-15.63
GO:0001906	GO Biological Processes	cell killing	12	21.43	-14.31	-11.78
GO:0045580	GO Biological Processes	regulation of T cell differentiation	10	17.86	-12.03	-9.64
GO:0002237	GO Biological Processes	response to molecule of bacterial origin	12	21.43	-10.74	-8.47
GO:0002507	GO Biological Processes	tolerance induction	6	10.71	-10.58	-8.33
hsa05142	KEGG Pathway	Chagas disease (American trypanosomiasis)	8	14.29	-10.04	-7.82
GO:0050900	GO Biological Processes	leukocyte migration	13	23.21	-10.01	-7.81
GO:0042092	GO Biological Processes	type 2 immune response	6	10.71	-9.74	-7.56
GO:1901214	GO Biological Processes	regulation of neuron death	10	17.86	-8.56	-6.52
GO:0033077	GO Biological Processes	T cell differentiation in thymus	6	10.71	-7.82	-5.86
GO:0030879	GO Biological Processes	mammary gland development	7	12.5	-7.38	-5.44
GO:0010942	GO Biological Processes	positive regulation of cell death	12	21.43	-7.08	-5.17

CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; OV: Ovarian serous cystadenocarcinoma; UCEC: Uterine corpus endometrial carcinoma.