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ORIGINAL ARTICLE

Prognostic value of preoperative soluble interleukin 2 receptor α as a novel immune biomarker in epithelial ovarian cancer

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Abstract

Purpose Epithelial ovarian cancer (EOC) is regarded as the deadliest gynecological cancer, and the demand for novel non-invasive prognostic biomarkers remains significant. This study aimed to investigate the prognostic value of preoperative blood biomarkers in EOC patients.

Methods In total, 73 patients who had undergone ovarian mass resection were enrolled. Serum concentration of biomarkers, including soluble interleukin 2 receptor α (sIL-2R), was measured 1–2 weeks before surgery. Independent prognostic factors for progression-free survival (PFS) were investigated with multivariate Cox regression analysis. A prognostic model was subsequently developed and evaluated by discrimination, calibration and clinical net benefit. Furthermore, transcriptome data of 376 EOC cases from The Cancer Genome Atlas (TCGA) were analyzed with ESTIMATE, CIBERSORT and Maftools algorithm to evaluate the correlation of IL2RA expression with tumor immune microenvironment and immunotherapeutic response.

Results High sIL-2R concentration was found to be the only significant prognostic blood biomarker for PFS by multivariate Cox regression analysis in our center. A prognostic nomogram was developed with satisfactory discrimination, calibration and clinical net benefit. In addition, higher IL2RA expression was significantly associated with higher immune scores, activated $CD4^+$ T cells, M2 macrophages and resting dendritic cells in TCGA data. Furthermore, IL2RA expression was closely related to TMB scores.

Conclusions sIL-2R is a potential prognostic immune biomarker for EOC patients, and a comprehensive prognostic model comprising sIL-2R with satisfactory discrimination and clinical appliance was developed. Therefore, we recommend routine sIL-2R testing in EOC patients.

Keywords Epithelial ovarian cancer · Immune biomarker · sIL-2R · Prognostic

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Abbreviations

AIC	Akaike's information criterion
BP	Biological Process
BMI	Body mass index
CA125	Cancer antigen 125
CC	Cellular Component
DCA	Decision curve analysis
EOC	Epithelial ovarian cancer
FIGO	International Federation of Gynecology and Obstetrics
GO	Gene Ontology
GSEA	Gene Set Enrichment Analysis
IL2	Interleukin 2
IL-2R	Interleukin 2 receptor
IL8	Interleukin 8
IL6	Interleukin 6
KEGG	Kyoto Encyclopedia of Genes and Genomes

K-M	Kaplan–Meier
LMR	Lymphocyte-to-monocyte ratio
MF	Molecular function
NLR	Neutrophil-to-lymphocyte ratio
PFS	Progression-free survival
PFI	Platinum-free interval
PLR	Platelet-to-lymphocyte ratio
sIL-2R	Soluble interleukin 2 receptor α
TCGA	The Cancer Genome Atlas
TILs	Tumor-infiltrating lymphocytes
TIME	Tumor immune microenvironment
TMB	Tumor mutational burden
TME	Tumor microenvironment
TNFα	Tumor necrosis factor α

Introduction

Epithelial ovarian cancer (EOC) is commonly regarded as the deadliest gynecological cancer, with a staggering annual death-to-incidence rate of 66.0% worldwide [1]. The lack of specific symptoms and reliable screening methods results in 75% of patients presenting with an advanced disease stage at first diagnosis [2–4]. The 5-year survival rate of advanced-stage EOC patients is 29%; however, the 5-year survival rate can reach 92% if EOC is diagnosed at an early stage [5]. Recurrence after initial treatment is another important cause of cancer-related mortality, as nearly 75% of patients with recurrence are unfortunately incurable [2]. Consequently, new treatment strategies are of great need for these patients. Among these, immunotherapy has attracted significant interest with a recently improved understanding of the molecular basis of the immune recognition and immune regulation of cancer cells [6]. However, pioneering studies have shown that only a minority of patients are responsive to immunotherapy, and the common feature of these responders can be generally characterized as high activity of preexisting antitumor immunity [7]. However, there is no consensus signature to estimate the immune activity in EOC and to stratify patients accordingly. Therefore, to better predict the survival of EOC patients, the exploration of novel noninvasive prognostic biomarkers and their potential impact on the tumor immune microenvironment (TIME) is warranted.

Blood biomarkers reflect multiple aspects of the tumor microenvironment (TME) and have been shown to be associated with disease progression and prognosis. At present, cancer antigen 125 (CA125) remains the most important biomarker for EOC [8]. Attempts have been made to develop a more effective algorithm with CA125, but further efforts are still required [9–11]. Inflammation has been demonstrated to be a key factor in tumorigenesis and tumor growth [12]. Several inflammatory blood biomarkers, including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR), have been widely investigated as prognostic markers for EOC patient survival [13–16]. An inflammatory stimulus activates the innate immune system by recruiting and activating innate immune cells, to release immunomodulatory cytokines, which include interleukin 6 (IL6), tumor necrosis factor α (TNFα) and interleukin 8 (IL8). These inflammatory cytokines have been reported to affect the tumorigenesis, progression and metastasis of ovarian cancer [17–21]. Interleukin 2 (IL2) is a well-characterized cytokine that activates T lymphocytes by binding to its specific membrane receptor (interleukin 2 receptor [IL-2R]).The IL-2R is differentially expressed as a monomer (IL-2Rα), dimer (IL-2Rβ and IL-2Rγ) or trimer (IL-2Rα, IL-2Rβ and IL-2Rγ) on the surface of distinct types of immune cells, with low affinity, intermediate affinity or high affinity for IL2, respectively [22]. The soluble IL-2Rα chain (sIL-2R), also known as CD25, is shed into the circulation upon immune activation and can be used as a biomarker for monitoring immune-mediated diseases [23]. Although the precise source and function of sIL-2R remain controversial, the pretreatment serum concentration of sIL-2R has been shown to reflect the tumor activity, prediction and prognosis of many types of cancer [24]. Indeed, elevated sIL-2R has been correlated with a poor prognosis in multiple malignancies, such as head and neck cancer, non-Hodgkin's lymphoma and hepatocellular carcinoma [25–27]. In patients with ovarian cancer, the concentration of sIL-2R in the serum and ascites has been reported to be higher than normal [28, 29]. However, the prognostic value of sIL-2R in ovarian cancer has not been investigated and the correlation of IL2RA mRNA expression with the TIME in EOC remains unclear.

Our study aimed to investigate the prognostic value of preoperative blood biomarkers, including the immune-related biomarker sIL-2R, in EOC patients. In addition, an optimal prognostic model was developed to facilitate clinical application. Furthermore, we analyzed transcriptome RNA-seq data to investigate the correlation of IL2RA with the immune response and tumor-infiltrating lymphocytes (TILs) in ovarian cancer cases from The Cancer Genome Atlas (TCGA) database. Overall, our findings revealed that sIL-2R could be a novel prognostic biomarker for progression-free survival (PFS) and a potential indicator for immune escape in EOC.

Materials and methods

Patients

A total of 73 patients who had undergone surgery for an epithelial ovarian mass, either a malignant epithelial ovarian mass (EOC, $n=52$) or a benign epithelial ovarian mass

($n = 21$), between January 2015 and December 2018 were included in this study. Patients with a history of another primary cancer, coexisting autoimmune diseases or active inflammation were excluded. Pathological examinations were performed by experienced pathologists following standard procedures. Specific blood biomarkers were measured in all patients. Relevant clinicopathological characteristics, including age, body mass index (BMI), residual tumor status after debulking surgery, histology, International Federation of Gynecology and Obstetrics (FIGO) stage, grade, total number of cycles of platinum-based chemotherapy and platinum status were documented and analyzed. The present study was approved by the Institutional Review Board of our hospital (SYSEC-KY-KS-2021–079), and informed consent was obtained from all enrolled patients.

Treatments and follow-up

The primary treatment for EOC patients was debulking surgery combined with 6–8 cycles of platinum-based chemotherapy. After primary treatment, patients were routinely followed up every 1–2 months for the first 6 months, every 3 months for the next 2 years and every 6 months thereafter. If recurrence was suspected, additional imaging examinations were performed to determine the recurrence status. PFS was defined as the time from diagnosis to the date of recurrence or the last follow-up. The time between the completion of platinum-based treatment and the detection of recurrence was defined as the platinum-free interval (PFI). A PFI no less than 6 months was considered platinum sensitive, while a PFI less than 6 months was considered platinum resistant.

Blood biomarkers measurement

Blood serum samples were collected 1–2 weeks before surgery or neoadjuvant chemotherapy. Serum CA125 concentrations were measured using an electrochemiluminescence immunoassay (Roche). The NLR, LMR and PLR were derived from complete blood counts (hemograms), and they were calculated as the absolute neutrophil counts/lymphocyte counts, absolute lymphocyte counts/monocyte counts and platelet counts/lymphocyte counts, respectively. Concentrations of sIL-2R, IL6, TNFα and IL8 were measured with an electrochemiluminescence immunoassay according to the manufacturer's manuals (Siemens).

Immunohistochemistry

All available formalin-fixed paraffin-embedded (FFPE) surgical specimens were retrieved in 5um section slides, which were subsequently deparaffinized with xylene and rehydrated through a graded alcohol series. Heat-induced epitope retrieval (HIER) was performed by immersing the slides at 98 ℃ for 20 min in EDTA (PH 9.0). Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide. The slides were then treated with 3% bovine serum albumin (BSA) for 30 min and exposed to primary antibody for IL2RA (1:100, ab128955) overnight at 4 ℃. The reactivity of IL2RA was revealed with a horseradish peroxidase (HRP)-labeled polymer-conjugated secondary antibody followed by diaminobenzidine (DAB, ab209101). All slides were scanned with a Pannoramic 250 Flash III digital scanner (3DHISTECH, Budapest, Hungary). IL2RA staining was evaluated and measured by two independent pathologists without the knowledge of relevant sIL-2R results.

Survival analysis

To determine the prognostic blood biomarkers and clinicopathological variables for EOC patients, multivariate Cox regression analyses with stepwise variable selection using the Akaike information criterion (AIC) were performed. Correlations between blood biomarkers and clinicopathological variables were analyzed. Furthermore, Kaplan–Meier (K–M) survival analysis with log-rank test was performed to compare survival between groups.

Development and evaluation of nomogram

A nomogram was developed to visualize the prognostic model and facilitate its clinical application. Harrell's concordance index (C-index) indicated the discrimination power of the model with a range of 0.5 (no discrimination)–1.0 (perfect match). Calibration of the model, referring to the agreement between the observed and predicted probabilities, was also visually assessed with a calibration plot. Decision curve analysis (DCA) was conducted to evaluate the clinical benefit of the prognostic model.

TCGA raw data

Transcriptome RNA-seq level 3 data of 379 EOC cases and the corresponding clinical data were downloaded from TCGA database (TCGA-OV, https://portal.gdc.cancer.gov/).

Calculation of immune and stromal scores

The ESTIMATE algorithm was used to calculate immune scores and stromal scores of each ovarian cancer sample by applying the downloaded TCGA-OV data [30]. The samples were divided into high score and low score groups based on the median cutoff values. The correlation of IL2RA expression with immune/stromal scores was assessed by a non-parametric test.

Gene set enrichment analysis (GSEA)

C2 gene sets from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database and C5 gene sets from the Gene Ontology (GO) Biological Process (BP), Cellular Component (CC) and Molecular Function (MF) v7.2 collections were downloaded from the Molecular Signatures Database. And GSEA was performed with GSEA-4.1.0 software (http://www.broadinstitute.org/gsea/) to identify significantly enriched pathways in the high and low IL2RA expression groups in TCGA-OV data. A nominal P value < 0.05 and a false discovery rate (FDR) q value < 0.05 were considered statistically significant.

TILs profile

The CIBERSORT computational method was applied to estimate the TILs abundance profile in TCGA-OV data [31]. The difference test and correlation test between IL2RA expression and TILs subsets were further analyzed.

Exploration of immunotherapeutic response

The tumor mutational burden (TMB) has been demonstrated to be closely related to immunotherapeutic response [32, 33]. And the tumor-specific mutated genes were evaluated and summed by the R package Maftools to calculate TMB. In this study, we explored the correlation between IL2RA and TMB in TCGA data with Wilcoxon rank-sum test, which may provide useful insight into the potential application of sIL-2R in immunotherapeutic response.

Statistical analysis

Comparisons between groups were performed with a Chi-square test for categorical variables and the Mann–Whitney U test for continuous ones. Correlation analysis was performed using Spearman rank correlation analysis.

All computations were carried out in SPSS 25.0 software (IBM Corp, Armonk, NY), GraphPad Prism 8.0 software (GraphPad Software, San Diego, California USA) and R 3.6.1. Two-sided P values < 0.05 were considered statistically significant.

Results

Analysis of this study

The analysis flow of our study is shown in Fig. 1. In summary, to identify the significant prognostic blood biomarkers in our hospital cohort, univariate and multivariate Cox regression analyses were performed. K–M survival analysis was also performed. The prognostic nomogram for PFS was constructed with significant blood biomarkers and clinicopathological variables. To further investigate the role of IL2RA in the TIME and immunotherapeutic response, transcriptome RNA-seq data of 376 EOC cases were downloaded from TCGA database and analyzed with CIBERSORT, ESTIMATE and Maftools algorithms. IL2RA mRNA expression levels were assessed, and subsequent series of analyses, including GSEA and difference and correlation analyses with TILs, were performed.

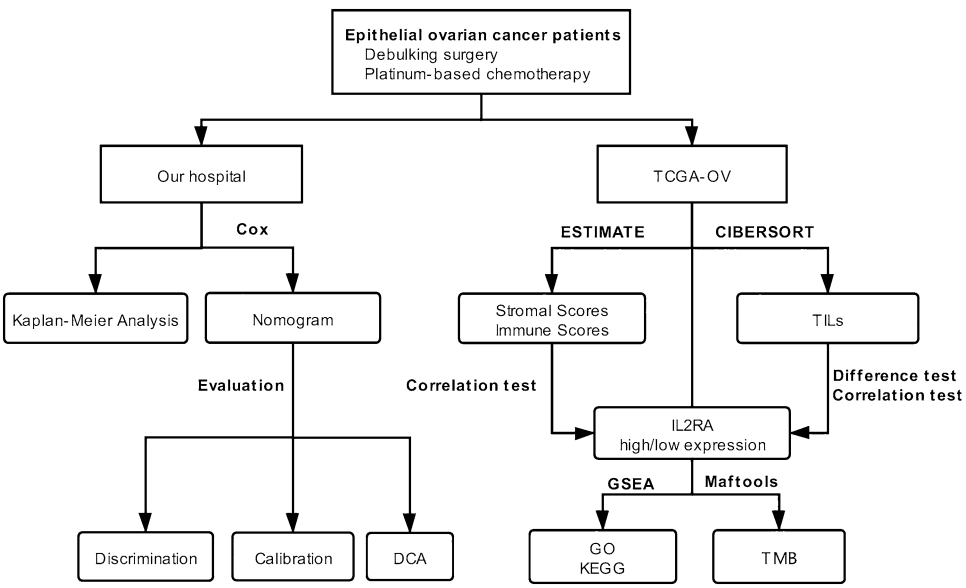


Fig. 1 Analysis workflow of this study. TCGA, The Cancer Genome Atlas; TIL, tumor-infiltrating lymphocyte; GSEA, gene set enrichment analysis; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DCA, decision curve analysis

Concentration of sIL-2R in EOC

The serum concentration of sIL-2R was significantly higher in patients with malignant ovarian cancer compared to patients with benign ovarian masses ($P < 0.001$, Fig. 2A). Patients in advanced-stage EOC had significantly higher sIL-2R concentration compared to patients in early stages ($P = 0.04$, Fig. 2B). To further investigate the correlation between concentration of sIL-2R and disease progression, the concentration of sIL-2R was classified into high or low groups based on the median cutoff value (Supplementary Table 1), and the relationships between the concentration of sIL-2R and clinicopathological variables in 52 EOC patients are demonstrated in Table 1. sIL-2R was significantly correlated with age and residual tumor size after debulking surgery (both $P < 0.05$, Table 1). There was no correlation between sIL-2R and BMI, histology, FIGO stage, grade, platinum status or total number of cycles of platinum-based chemotherapy.

To identify the correlation between IL2RA expression in tumor tissues and sIL-2R concentration in serum, IHC analysis of tumor sections demonstrated that IL2RA expressed in 50 (96.2%) patients (Supplementary Fig. 2A) and the intensity of IL2RA expression were significantly correlated with the sIL-2R concentration in serum (Supplementary Fig. 2B).

Prognostic value of sIL-2R

Blood biomarkers with a P value < 0.10 in the univariate analysis (Supplementary Table 2) were further included into the multivariate analysis, and high sIL-2R was the only statistically significant prognostic predictor for PFS among all blood biomarkers (HR: 2.57, 95% CI: 1.22–5.42, $P = 0.01$, Table 2). K-M survival analysis also demonstrated that

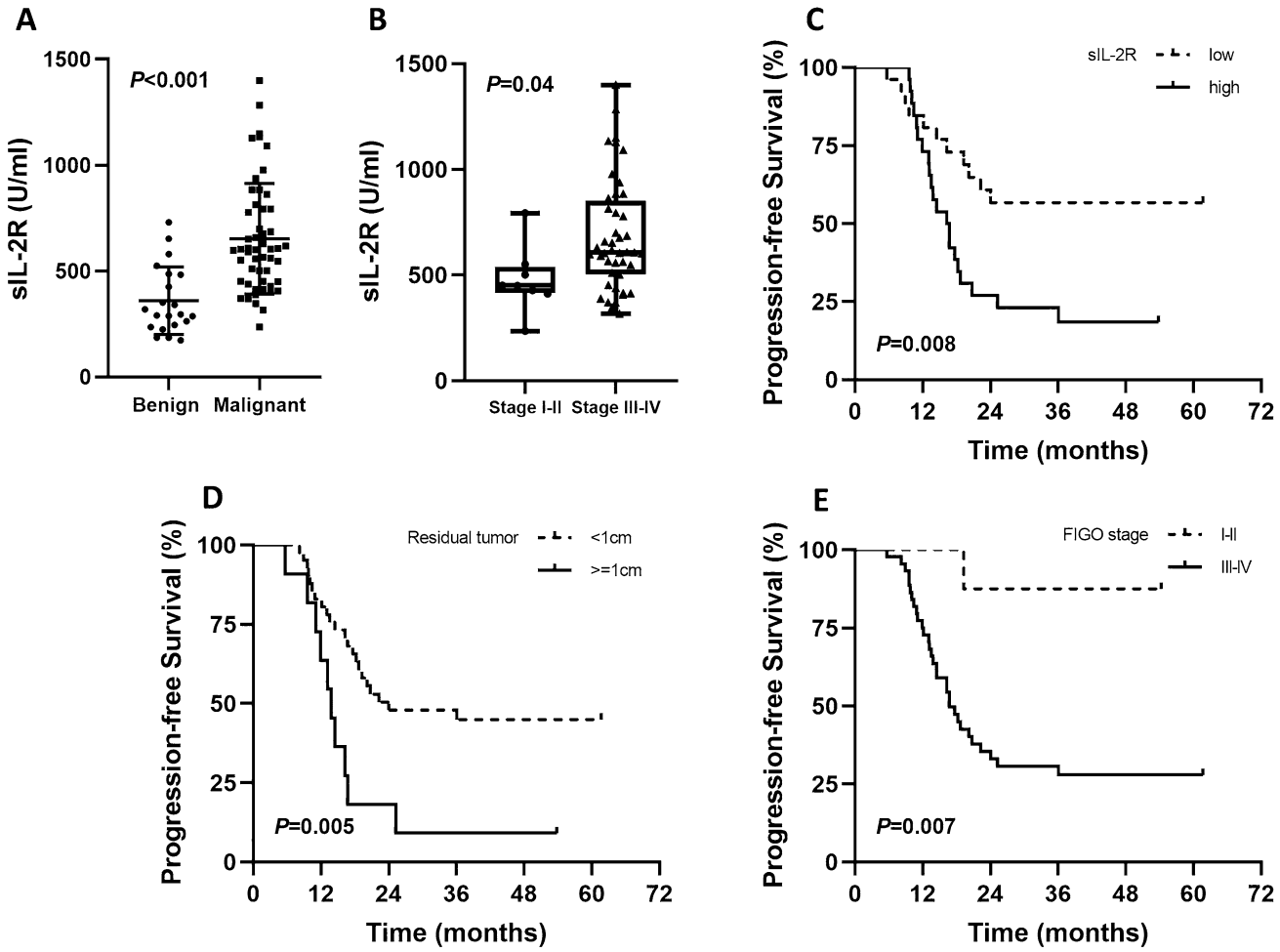


Fig. 2 Differentiated concentration of sIL-2R in samples and its correlation with tumor progression and prognosis. The concentration of sIL-2R was significantly higher in patients with EOC compared with patients with a benign ovarian mass (**A**) and in patients with advanced-stage EOC (**B**). Kaplan–Meier survival curves for progression-free survival in epithelial ovarian cancer patients with respect to sIL-2R (**C**), residual tumor size (**D**) and FIGO stage (**E**). The P value was calculated from log-rank test. sIL-2R, soluble interleukin 2 receptor α chain; FIGO, International Federation of Gynecology and Obstetrics; EOC, epithelial ovarian cancer

Table 1 Clinical characteristics of patients with epithelial ovarian cancer according to a sIL-2R threshold of 601.0 U/ml

Variables	sIL-2R-low, $n=26$	sIL-2R-high, $n=26$	P value
Age (years), median (IQR)	47.5 (40.5–77.8)	53.5 (48.8–63.3)	0.01
BMI (kg/m^2), median (IQR)	23.3 (20.8–24.9)	22.6 (20.3–26.8)	0.93
Residual tumor, *n* (%)			
< 1 cm	24 (92.3)	17 (65.4)	0.02
> = 1 cm	2 (7.7)	9 (34.6)	
Histology, *n* (%)			
Serous	21 (80.8)	21 (80.8)	> 0.999*
Non-serous[a]	5 (19.2)	5 (19.2)	
FIGO stage, *n* (%)			
I-II	7 (26.9)	1 (3.8)	0.05*
III-IV	19 (73.1)	25 (96.2)	
Grade, *n* (%)			
I-II	4 (15.4)	3 (11.5)	> 0.999*
III	22 (84.6)	23 (88.5)	
Platinum status, *n* (%)			
Resistant	3 (11.5)	5 (19.2)	0.70*
Sensitive	23 (88.5)	21 (80.8)	
Total cycles of chemotherapy, *n* (%)			
< 6	7 (26.9)	5 (19.2)	0.51
> = 6	19 (73.1)	21 (80.8)	

**P* value of Fisher's exact test for at least one expected count less than 5; a. non-serous included mucous type, clear cell type and endometrioid type

sIL-2R Soluble interleukin 2 receptor α chain; *IQR* Interquartile range; *BMI* body mass index; *FIGO* International Federation of Gynecology and Obstetrics

Table 2 Multivariate Cox regression analysis for progression-free survival

Variables	Multivariate HR (95%CI)	P value	Variable C-index	Model C-index
Residual tumor (< 1 cm vs. > = 1 cm)	2.20 (1.02–4.73)	0.04	0.590	0.684
FIGO stage (I-II vs. III-IV)	8.00 (1.07–59.60)	0.04	0.600	
sIL-2R (unit/ml) (< 601.0 vs. > = 601.0)	2.57 (1.22–5.42)	0.01	0.603	

HR Hazard ratio; *CI* confidence interval; *FIGO* International Federation of Gynecology and Obstetrics; *sIL-2R*, soluble interleukin 2 receptor α chain

patients with high sIL-2R had worse PFS than those with low sIL-2R ($P=0.008$, Fig. 2C). The correlation between sIL-2R and clinicopathological variables was less than 0.50 for all comparisons, indicating that sIL-2R had more prognostic power other than the conventional clinicopathological variables (Supplementary Fig. 1).

On the other hand, independent prognostic clinicopathological variables were further identified through univariate and multivariate Cox regression analyses. In the univariate Cox regression analysis, residual tumor and FIGO stage were found to be statistically significant prognostic factors (all $P<0.05$, Supplementary Table 2), and histology was potentially significant ($P=0.06$). Furthermore, residual tumor (HR: 2.20, 95% CI: 1.02–4.73, $P=0.04$, Table 2) and FIGO stage (HR: 8.00, 95% CI: 1.07–59.60, $P=0.04$, Table 2) were significantly associated with PFS in the multivariate analysis. Similarly, in the K-M survival analysis, residual tumor > = 1 cm and advanced stage were also significantly associated with poor PFS ($P=0.005$, Fig. 2D; $P=0.007$, Fig. 2E).

Development and evaluation of the prognostic nomogram

A prognostic nomogram incorporating both clinical variables and blood biomarkers was developed (Fig. 3A). The prognostic model with only sIL-2R achieved a C-index of 0.603, while the addition of prognostic clinical variables led to a significant increase in the C-index (0.684; $P=0.004$, Table 2). Moreover, calibration plots demonstrated

satisfactory agreement between the nomogram-predicted PFS and actual survival (Fig. 3B, C). DCA showed a higher net benefit than the "intervention for all" and "intervention for none" when the threshold probability for one-year PFS ranged from 3 to 38% or when the threshold probability for 2-year PFS ranged from 14 to 77% (Fig. 3D, E).

Correlation of IL2RA mRNA expression with TIME and immunotherapeutic response in EOC

To explore the relationship between IL2RA expression and TME regarding immune or stromal components, immune scores and stromal scores were calculated with ESTIMATE algorithms, and samples were classified into high or low groups based on the median cutoff values. IL2RA expression was significantly higher in patients with high immune scores compared to patients with low immune scores (Fig. 4A). Additionally, IL2RA expression was much higher in patients with high stromal scores than those low stromal scores (Fig. 4A).

To explore the underlying molecular mechanisms, GSEA was implemented in the IL2RA high-expression and low-expression groups. The top 10 pathways of GO terms (BP, CC and MF) and KEGG pathways in the IL2RA high-expression group are shown in Fig. 4B, and all satisfied the following requirements: normalized enrichment score (NES) > 2.0, nominal $P < 0.0001$ and FDR $q < 0.0001$. The GO enrichment analysis indicated that the genes in the IL2RA high expression group were mainly enriched in immune-related GO terms. The representative significantly upregulated BP gene sets involved in the immune response included activation of immune response, adaptive immune response, lymphocyte activation involved in immune response and regulation of innate immune response (Fig. 4C).

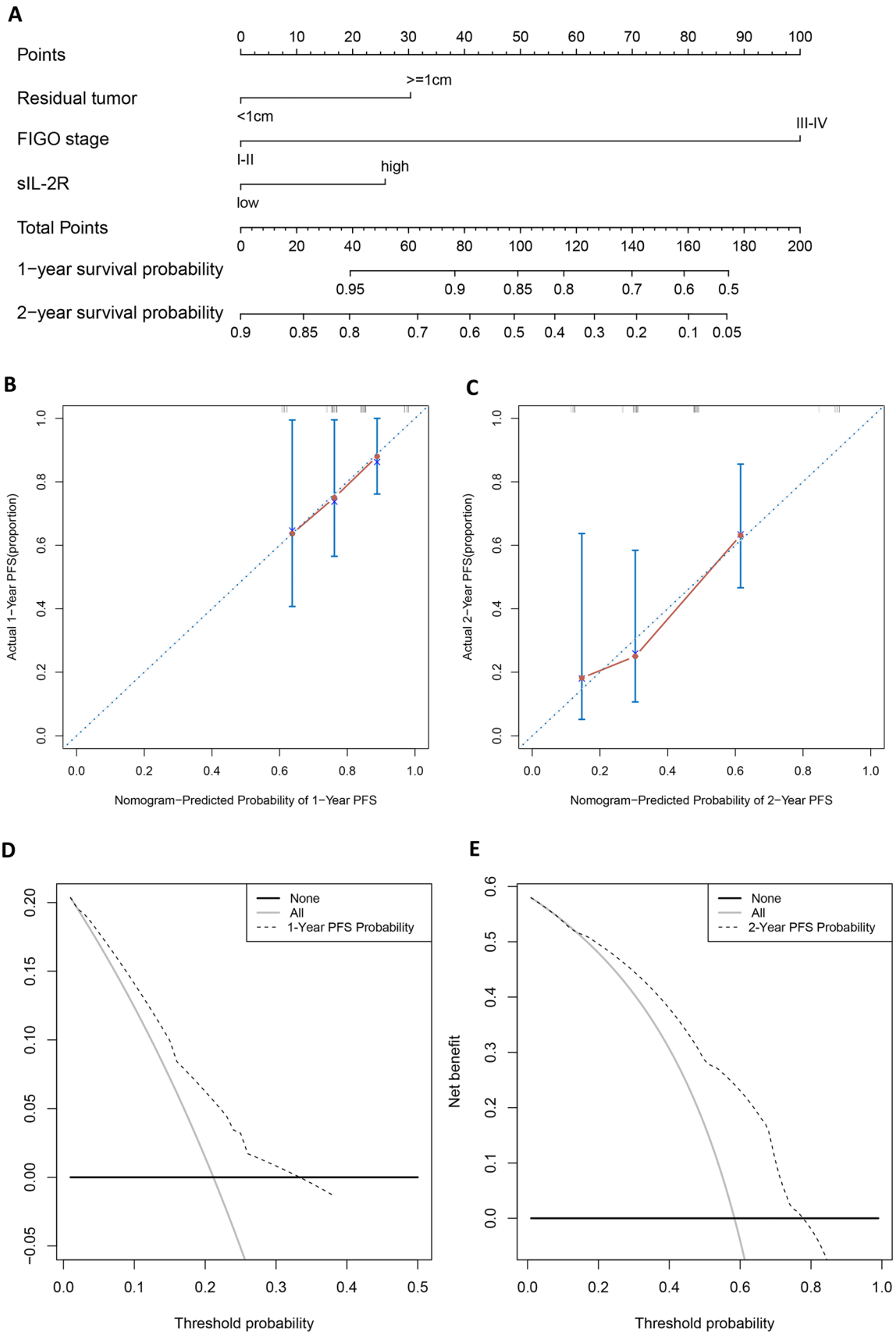
To further confirm the correlation of IL2RA expression with the TIME, the proportion of tumor-infiltrating immune subsets was analyzed using CIBERSORT algorithm, and 21 types of immune cell profiles in EOC samples were investigated (Supplementary Fig. 3). The intersection between the difference test (Fig. 4D) and correlation test (Fig. 4E) showed that a total of three kinds of TILs were correlated with the expression of IL2RA, including activated CD4 + T cells, M2 macrophages and resting dendritic cells. These results further supported that IL2RA expression correlated with tumor immune cell infiltration and had a significant impact on the TME.

Using the R package Maftools, the mutation data were analyzed and summarized. The mutations were stratified based on the IL2RA expression. The top 20 driver genes with the highest alteration frequency between the high- and low-expression subgroups are shown in Fig. 4F. We then calculated TMB scores based on TCGA somatic mutation data. The TMB in the high-expression group exceeded that in the low-expression group, showing that IL2RA expression had a high correlation with TMB (Fig. 4G).

Discussion

Considerable efforts have been made to develop more accurate and effective tools for early screening and survival prediction in EOC patients. Blood biomarkers are promising noninvasive and convenient tools that reflect multiple aspects of the TME, which is closely associated with tumorigenesis and prognosis. Previous studies have reported that various inflammatory factors, including NLR, LMR, PLR, IL6, TNFα and IL8, are associated with ovarian cancer patients survival [14–19]. sIL-2R has been reported to be a statistically independent immune biomarker associated with activated innate immune function in various types of cancer but not ovarian cancer [25–27, 34]. However, no consensus has been reached, and current methods are limited in actual clinical practice; thus, novel predictors are urgently needed. In the present study, independent prognostic predictors for PFS, including blood biomarkers and clinicopathological variables, were investigated in 52 patients with EOC. sIL-2R was the only statistically significant prognostic blood biomarker for PFS in the multivariate Cox regression analysis (HR: 2.57, 95% CI: 1.22–5.42, $P = 0.01$, Table 2). In other words, the prognostic power of sIL-2R outweighed that of the conventional biomarker CA125 and inflammation-related blood biomarkers, including the NLR, LMR, PLR, IL6, TNFα and IL8 [15–19]. Moreover, a prognostic model incorporating sIL-2R, residual tumor status and FIGO stage with satisfactory discrimination, calibration and clinical benefit was developed (C-index = 0.684, Table 2 and Fig. 3). Our results not only further validated the prognostic value of the residual tumor status and FIGO stage [2, 4], but also demonstrated sIL-2R to be a novel prognostic factor in EOC patients.

Recently, immunotherapy has demonstrated promising results and attracted significant attention, with fast-track approvals in numerous cancer types but not in EOC [7]. A major barrier to successful immunotherapy for ovarian cancer patients is the immunosuppressive TME [6]. The TME, which contains a repertoire of immune cells, stromal cells, endothelial cells and cancer-associated fibroblasts, has been established as a hallmark of cancer and thus a promising field to explore predictors of the immunotherapy response [35]. Emerging evidence suggests that favorable tumor immune cell infiltration has a strong correlation with the antitumor response and results in a positive survival prognosis in EOC patients [6, 36]. Immune checkpoint receptors, including programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), suppress tumor immune elimination and promote immune evasion

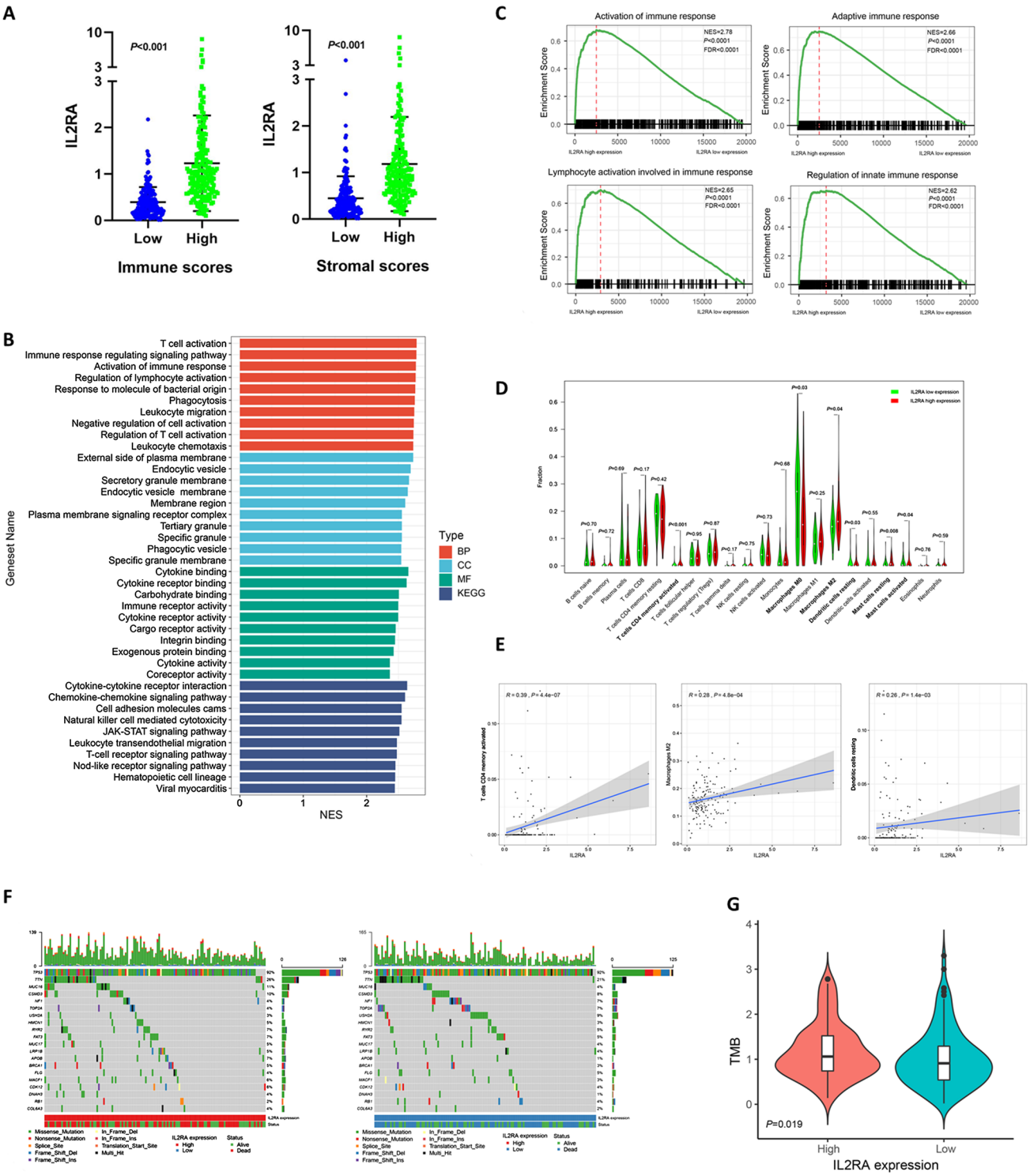

A
Points
0 10 20 30 40 50 60 70 80 90 100
Residual tumor
>=1cm
<1cm
FIGO stage
III-IV
I-II
sIL-2R
high
low
Total Points
0 20 40 60 80 100 120 140 160 180 200
1−year survival probability
0.95 0.9 0.85 0.8 0.7 0.6 0.5
2−year survival probability
0.9 0.85 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.05
B
Actual 1−Year PFS(proportion)
Nomogram−Predicted Probability of 1−Year PFS
C
Actual 2−Year PFS(proportion)
Nomogram−Predicted Probability of 2−Year PFS
D
None
All
1-Year PFS Probability
Net benefit
Threshold probability
E
None
All
2-Year PFS Probability
Net benefit
Threshold probability

◂**Fig. 3** Prognostic nomogram (**A**), calibration curve (**B** and **C**) and DCA curve (**D** and **E**) of PFS for EOC. **A** A vertical line between each variable and point scale can be drawn to determine the points for each variable. The predicted survival rate was calculated according to the total points by drawing a vertical line from the total points scale to the 1- and 2-year survival scales. **B** and **C** Nomogram-predicted survival is plotted on the x-axis; actual survival is plotted on the y-axis. A plot along the 45-degree line indicates a perfect model in which the predicted probabilities are identical to the actual outcomes. **D** and **E** The y-axis measures the net benefit, which is calculated by summing the benefit (true positives) and subtracting the harms (false positives). The dashed line indicates the prognostic model, and the two other lines indicate the "intervention for all" (light gray line) and "intervention for none" (black line). A model is considered of clinical value if it has a higher net benefit than other models and simple strategies at any given threshold. EOC, epithelial ovarian cancer; sIL-2R, soluble interleukin 2 receptor α chain; FIGO, International Federation of Gynecology and Obstetrics; DCA, decision curve analysis

[37]. Phase II studies of anti-PD1/PD-L1 and CTLA-4 therapy in ovarian cancer have shown that tumor immune infiltration was predictive of the therapeutic response [6, 37]. What's more, TMB, the number of somatic mutations per DNA megabase (Mb), has emerged as a novel biomarker of response to immunotherapy and is related to the emergence of neoantigens that trigger antitumor immunity [38]. The positive relationship between TMB and response to CTLA-4 and PD-1 inhibition has been demonstrated in melanoma and non-small cell lung cancer [32, 33]. There is growing evidence that ovarian cancers with a higher somatic mutation burden also respond better to cytotoxic chemotherapy [39]. However, noninvasive predictive blood biomarkers for immunotherapy in EOC patients remain unknown. Thus, predictive blood biomarkers for different types of immunotherapies are urgently needed.

Since we found that IL2RA expression in tumor tissues had a high correlation with sIL-2R in serum, the transcriptome data of IL2RA in EOC cases from TCGA database were analyzed to further explore the role of sIL-2R in the TIME. We found that IL2RA expression was positively correlated with immune scores and stromal scores in EOC samples (Fig. 4A). Immune scores have various prognostic effects in different cancers and remain controversial in EOC [40, 41]. Our analysis of the TCGA-OV dataset indicated that immune scores had no significant association with PFS or OS. A large-scale study has proposed that immune scores were significantly correlated with immune infiltration in patients with advanced EOC and concluded that immune scores could be predictive of the response to chemotherapy and immunotherapy [41]. On the other hand, numerous studies have reported the prognostic value of TILs in EOC [36]. M2 macrophages, activated $CD4^+$ T cells and resting dendritic cells have been demonstrated to be associated with poor PFS or OS [42]. In our study, CIBERSORT analysis of TILs profile elucidated that IL2RA expression was correlated with CD4 + T cells, M2 macrophages and resting dendritic cells in EOC samples (Fig. 4D, E). In addition, the high-expression group TMB was significantly higher than that in the low-expression group TMB. According to the above results, we infer that the IL2RA expression may predict immune status for oncotherapy in EOC. As demonstrated in our result, the IL2RA expression in tumor tissues and sIL-2R levels in serum were closely related; therefore, the prognostic impact of sIL-2R is likely derived from IL2RA expression and its close relation with the TIME. Previous study has demonstrated that sIL-2R may function as a decoy receptor, reducing the bio-availability of IL-2 [23]. Several studies have investigated the potential of sIL-2R on in vitro immune reactions [23]. Zorn et al. showed that in vitro sIL-2R is capable of neutralizing IL-2 therefore suppressing tumor immune responses [43]. Furthermore, recombinant sIL-2R has been reported to suppress T cell proliferation in vitro [44]. The dose-dependent inhibition of immune responses supports the hypothesis that sIL-2R functions as an IL-2 antagonist. Moreover, the well-established immune suppressor Treg has been shown to act via the preferential shedding of sIL-2R as decoy receptor [45]. Based on our initial results, we recommend the detection and quantitation of blood sIL-2R as a noninvasive and useful means of assessing the TIME in EOC.

To the best of our knowledge, our study is the first to identify sIL-2R as a significant independent prognostic factor for PFS in EOC patients. Furthermore, sIL-2R was demonstrated to be a better prognostic factor than conventional biomarkers, including the tumor biomarker CA125 and inflammatory markers NLR, LMR, PLR, IL6, TNFα and IL8. However, the limitations of the present study should be acknowledged. First, the sample size in the retrospective study was relatively small. Therefore, additional prospective studies with larger patient numbers are warranted to provide more definitive evidence and further validate our findings. Second, no patients in our study have received any form of immunotherapy, which makes it difficult to confirm the direct relationship between sIL-2R and immunotherapy response. And the exact mechanisms by which sIL-2R regulates tumor immune cell infiltration require further investigation. Third, the functional relationship between IL2RA mRNA expression and the serum sIL-2R concentration needs to be further validated.



Conclusion

In conclusion, we demonstrated sIL-2R to be a novel prognostic biomarker that contributes to the poor prognosis of EOC patients. Furthermore, a comprehensive prognostic model with satisfactory discrimination and clinical appliance was developed and calibrated for EOC patients. sIL-2R may also play a vital role in the EOC immune response by regulating immune cell infiltration. Therefore, we recommend routine evaluation of sIL-2R in EOC patient survival prediction.

◂**Fig. 4** Estimation of the tumor immune microenvironment and cancer immunotherapy response using IL2RA expression levels in the TCGA dataset. **A** IL2RA expression correlated with immune scores and stromal scores. The immune and stromal scores were stratified by the median cutoff value. **B** Top 10 GSEA pathways associated with high IL2RA expression based on GO (BP, CC and MF) and KEGG pathway. The X-axis shows the normalized enrichment score, and all the pathways shown represent those with significant *P* values ($P < 0.0001$) and FDRs (FDR < 0.0001). **C** GSEA enrichment plot for activation of immune response, adaptive immune response, lymphocyte activation involved in immune response and regulation of innate immune response. **D** Violin plot showing that IL2RA was significantly highly expressed in $CD4^+$ T cells, M2 macrophages, resting dendritic cells and resting mast cells and weakly expressed in M0 macrophages and activated mast cells ($P < 0.05$). The Wilcoxon rank-sum test was used as the significance test. **E** A scatter plot showing that IL2RA expression had a significant positive correlation with active $CD4^+$ T cells, M2 macrophages and resting dendritic cells ($P < 0.05$), and the Pearson correlation test was used as the correlation test. **F** Waterfall plot displays mutation information of the genes with high mutation frequencies in the IL2RA high- and IL2RA low-expression groups. **G** TMB difference in the IL2RA high- and IL2RA low-expression patients. BP, biological process; CC, cellular component; MF, molecular function; NES, normalized enrichment score; FDR, false discovery rate. TMB, tumor mutation burden

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Availability of data and material The datasets generated for this study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval The present study was approved by the Institutional Review Board of Sun Yat-sen Memorial hospital (SYSEC-KY-KS-2021–079).

Informed consent Informed consent was obtained from all enrolled patients. Patients signed informed consent regarding publishing their data and photographs.

Consent to participate Informed consent was obtained from all enrolled patients.

Consent for publication Patients signed informed consent regarding publishing their data and photographs.

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