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CD74 is a potential biomarker predicting the response to immune checkpoint blockade

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Abstract

Background Immune checkpoint blockade (ICB) has been improving the patient outcome in multiple cancer types. However, not all patients respond to ICB. Biomarkers are needed for selecting appropriate patients to receive ICB. CD74 is an important chaperone that regulates antigen presentation for immune response. However, the relationship between CD74 expression and ICB response remains elusive.

Methods The unified normalized pan-cancer dataset was downloaded from the UCSC database. Wilcoxon Rank Sum Rank Tests were used to analyze the expression differences between normal and tumor samples in each tumor type. Then, the prognostic value of CD74 was determined using univariable Cox proportional hazards regression analysis. The STRING database was utilized to construct the protein-protein interaction (PPI) network of CD74 and the signal pathways were analyzed as well. The correlation of CD74 expression with immune cells and immune regulating genes was investigated in the TIMER database. The TIDE framework was utilized to evaluate the relationship between CD74 expression and the response to immunotherapy. Moreover, the localization of CD74 in the tumor immune microenvironment was verified using multiplex immunohistochemistry. Clinically annotated samples from 38 patients with esophageal cancer treated with neoadjuvant chemotherapy combined with ICB were analyzed for CD74 expression using immunohistochemistry.

Results In this study, we investigated the prognostic and predictive value of CD74 in different types of cancer. Compared with normal tissue, the expression of CD74 was higher in tumor tissue in various cancers. High expression of CD74 was associated with improved patient prognosis in the majority of cancers. CD74 and its interacting proteins were mainly enriched in the immune-related pathways. The expression of CD74 was significantly positively correlated with B cells, CD4 T-cells, CD8 T-cells, neutrophils, macrophages and dendritic cells. TIDE analysis showed that tumors with high CD74 expression may have better responses to immunotherapy and improved patient survival. In patients

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with esophageal cancer who had received ICB, higher intratumoral CD74 expression was associated with improved response to ICB.

Conclusions The findings of this study suggest that the high expression of CD74 may be a potential predictive biomarker of response to ICB.

Keywords Immunotherapy, CD74, Treatment response, Biomarker

Introduction

Cancer remains the leading cause of death and a major obstacle to increasing life expectancy in countries around the world [1]. In addition to surgery, radiotherapy, chemotherapy and targeted therapy, immunotherapy has become an important treatment option for cancer. Immune checkpoint blockade (ICB) therapy is the most widely used immunotherapy in solid tumors. Recent advances in ICB therapy illustrate powerful enhancement of the patient's anti-tumor immune responses [2]. However, due to tumor heterogeneity, only a small subset of patients respond to this treatment [3]. Therefore, biomarkers are urgently needed to predict the treatment response to ICB. Several biomarkers, including programmed death ligand 1 (PD-L1) expression [4], tumor mutational burden (TMB) [5], mismatch repair deficiency [6], and inflammatory gene expression [7], have been explored for ICB treatment efficacy prediction. However, none of these biomarkers are optimal. For example, recent studies have shown that due to intratumoral or intertumoral heterogeneity, using PD-L1 as a marker for efficacy assessment may miss the opportunity to benefit from anti-PD-L1 therapy in some patients [8, 9]. High TMB (TMB-H) has been associated with better treatment efficacy in previous studies. In recent studies, however, the benefit from ICB in TMB-H patients was significantly lower than that in low TMB patients in some cancer types [10]. The predictive value of other biomarkers has similar drawbacks which prevent them from selecting the right patients to receive the right treatment [11].

The tumor microenvironment (TME) plays key roles in determining the treatment responses to anticancer therapies, including ICB [12]. The antitumor effect of immune cells depends on the effective activation of CD8+T cells, in which antigen presentation is an key process [13]. CD74 is an essential membrane protein that associates with class II major histocompatibility complex (MHC II) and is an important chaperone that regulates antigen presentation for immune response. CD74 is highly expressed in immune cells such as B cells, monocytes, macrophages, Langerhans cells, dendritic cells, and thymic epithelial cells [14]. It plays an important role in antigen presentation by mediating MHC-II complex assembly and subcellular trafficking [15]. Moreover, the cytokine MIF mediates a variety of biological functions through its receptor CD74, such as inhibiting monocyte migration and promoting the secretion of pro-inflammatory cytokines. In addition, CD74 is involved in inflammatory diseases through various mechanisms, such as inhibiting amyloid-β processing and regulating CCL2 expression [16]. So far, the role of CD74 in tumor immunity and in predicting the response to ICB remains unclear.

In this study, we aim to investigate the relationship between CD74 expression and tumor immune responses using data of 30 solid tumor types obtained from The Cancer Genome Atlas (TCGA). We further explore the predictive value of CD74 for ICB using data of esophageal cancer patients receiving neoadjuvant immunotherapy.

Materials and methods

Differential expression analysis

The unified normalized pan-cancer dataset was downloaded from the university of California Santa Cruz (UCSC) database (https://xenabrowser.net/). Then, the expression data of ENSG00000019582 (CD74) gene in each sample were extracted, and log2(x+1) transformation was performed. The cancer types with less than three samples and blood cancers were excluded. Finally, the CD74 expression data of 30 cancer types were obtained. Wilcoxon Rank Sum Rank Tests were used to analyze the expression differences between normal and tumor samples in each tumor type, with a p-value of less than 0.05 being considered statistically significant. The statistical analysis was performed using R software (version 3.6.4).

Survival analysis

A high-quality dataset for survival analysis was obtained from the TCGA database [17]. Tumor alterations relevant for genomics-driven therapy follow-up data were obtained from UCSC (https://xenabrowser.net/datapages/). Then, the expression data of the above 30 cancer types and the overall survival (OS) data of corresponding patients were obtained. The prognostic value of CD74 was determined using univariable Cox proportional hazards regression analysis. OS was analyzed using the Kaplan-Meier method, with a log-rank test evaluating its difference. P-value of less than 0.05 was considered statistically significant. R survival package was used for the analysis.

Human protein-protein interaction analysis

The STRING database (https://cn.string-db.org/) was utilized to construct the protein-protein interaction (PPI) network of CD74. Gene oncology (GO) annotation and Kyoto encyclopedia of genes and genomes (KEGG) pathway analyses were performed on all nodes in the network. Heatmap was plotted by SRplot (http://www.bioinformatics.com.cn), an online platform for data analysis and visualization.

Correlation between CD74 expression and immune cells

The expression profile of CD74 was mapped to GeneSymbol, and the infiltration scores of B cells, CD4 T-cells, CD8 T-cells, neutrophil, macrophages, dendritic cells (DC) were assessed for each patient in each tumor based on gene expression data using the tumor immune estimation resource database (TIMER) (http://timer.cistrome.org/) [18]. The Corr.Test function in the R psych package (Version 2.1.6) was used to calculate the Pearson correlation coefficient of CD74 and immune cell infiltration scores in each tumor. P-values of less than 0.05 were considered statistically significant.

Correlation between CD74 expression and immune-related genes

The expression data of 38 label genes related to three types of immune pathways (8 genes related to T cell activation, 21 genes related to immune cell trafficking, and 9 genes related to antigen presentation) were extracted from the UCSC database. All the normal samples were filtered, and log2(x+1) transformation was performed. The Pearson correlation coefficient of CD74 expression with the 38 label genes were calculated, and p-values of less than 0.05 were considered statistically significant.

Predictive value of CD74 to immunotherapy using publicly available data

The Tumor Immune Dysfunction and Exclusion (TIDE) (tide.dfci.harvard.edu) is a computational framework developed to evaluate potential of tumor immune escape based on the gene expression profiles of cancer samples [19]. The TIDE database was used in the present study to analyze the relationship between CD74 expression and the cytotoxic T cell infiltration (CTL) in tumor tissues from patients with melanoma, bladder cancer, glioma, and clear cell renal cell carcinoma who received immunotherapy. The prognostic value of CD74 in these patients was analyzed as well.

The Mariathasan2018_PDL1 dataset contains whole transcriptome profiles of tumors from 368 patients with metastatic urothelial bladder cancer (mUC) treated with an anti–PD-L1 agent (atezolizumab) [20]. We used the R package IMvigor210CoreBiology (http://researchpub.gene.com/IMvigor210CoreBiology) to download the rawdata, which contains RNA-seq data of the primary tumor, objective response rates for the treatment, and survival data for 348 patients with metastatic urothelial carcinoma who received immunotherapy (anti-PD-L1). The patients were divided into CD74 high expression group and CD74 low expression group according to the optimal cutoff values as generated from the X-tile software [21]. The patient's response to immunotherapy according to CD74 expression level was presented in a histogram, which was drawn by GraphPad Prism 7 software. Kaplan-Meier curves were drawn using the R package Survminer, with a log-rank test evaluating the difference. P-value of less than 0.05 was considered statistically significant.

Validation of the predictive value of CD74 to immunotherapy

Patients and tissue samples

The subcellular localization of CD74 and CD80 was detected in the tumor tissues of two gastric cancer patients from Shantou Central Hospital. Data of a consecutive series of 38 patients with esophageal cancer who received neoadjuvant ICB therapy combined with chemotherapy between November 2019 and February 2021 at Shantou Central Hospital was used for the analysis. The formalin-fixed and paraffin-embedded (FFPE) tissue blocks of 38 surgically resected specimens and 15 matched biopsy specimens were obtained from the Division of Pathology, Shantou Central Hospital. The pathologic response of neoadjuvant therapy was evaluated using the mandard score, with a score of 1–2 defined as responder, and a score of 3–5 defined as non-responder.

Immunohistochemistry

FFPE samples were cut into 4-μm sections onto positively charged slides. After being deparaffinized in xylene and hydrated in ethanol, the slides in citrate buffer (pH 6.0) were subjected to heat-induced antigen retrieval in microwave for 15 min, and then treated with 3% hydrogen peroxide. The slides were washed with PBS and incubated with normal goat serum. The CD74 antibody (Clone D5N3I; catalog # 77274; CST) was used in 1:1000 dilution for staining, followed by the secondary antibody (catalog # PV9000; ZSBIO) incubation according to the manufacturer's protocol. The staining was visualized with 3,3′-diaminobenzidine (DAB; catalog #ZLI9018; ZSBIO) and the slides were lightly counterstained with hematoxylin. Slides were then dehydrated, cleared, and mounted. Appropriate positive and negative control samples were run concurrently (data not shown). Histological score for CD74 was determined by two pathologists blinded to clinicopathological and survival information of patients. Briefly, staining intensity of cells was transformed into histological index as follows: Negative (0)=no staining,

"+" (1+)=weak staining, "++" (2+)=moderate staining, "+++" (3+)=strong staining. The percentage of cells for each intensity of staining was assessed. The histological score was calculated by combining the intensity and percentage (formula used: 1 x percentage of cells with weak staining+2 x percentage of cells with moderate staining+3 x percentage of cells with strong staining).

Multiplex immunohistochemistry (mIHC)

The steps of tissue sectioning, dewaxing, rehydration, and antigen retrieval were the same as the above-mentioned immunohistochemical staining. Slides were incubated overnight at 4 °C with the CD74 antibody (LN-2; catalog # sc-6262; Santa Cruz) followed by Alexa Fluor® 488-AffiniPure Donkey Anti-Mouse IgG (H+L) (catalog # 715-545-150; Jackson ImmunoResearch Inc.) for 1 h at 24 °C in the dark. Then, the slides were incubated with the CD80 antibody (EPR1157(2); catalog # ab134120; Abcam) at 24 °C for 2 h, followed by Alexa Fluor® 647-AffiniPure Donkey Anti-Rabbit IgG (H+L) (catalog # 711-605-152; Jackson ImmunoResearch Inc.) for 1 h at 24 °C in the dark. The slides were mounted with anti-fade mounting medium containing DAPI (catalog # S2110; Solarbio) and photographed using an OLYMPUS BX53 microscope.

Statistical analysis

All the data of gene expression were normalized by log2(x+1) transformation. The differential expression of CD74 in pan-cancer was tested by Wilcoxon Rank Sum Rank Tests. The Kaplan-Meier curve and Cox proportional hazards model were used for survival analysis. The Pearson correlation coefficient was used to study the correlation between two variables. P value<0.05 was considered as significant. The visualization of the data is processed by R software (version 4.2.2).

Results

CD74 was overexpressed in pan-cancer tissues

Analysis of CD74 mRNA expression using data from the UCSC database revealed that the expression of CD74 was significantly upregulated in 21 kinds of cancers, including glioblastoma multiforme (GBM), glioma (GBMLGG), brain lower grade glioma (LGG), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), esophageal carcinoma (ESCA), stomach and esophageal carcinoma (STES), kidney renal papillary cell carcinoma (KIRP), pan-kidney cohort (KIPAN), colon adenocarcinoma (COAD), colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma (COADREAD), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), skin cutaneous melanoma (SKCM), thyroid carcinoma (THCA), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), testicular germ cell tumors (TGCT) and cholangiocarcinoma (CHOL). CD74 was significantly downregulated in lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ) and adrenocortical carcinoma (ACC) (Fig. 1A). In general, CD74 was overexpressed in tumor tissues compared with corresponding normal tissues in a majority of tumor types.

Correlation of CD74 expression level and OS in different cancer types

Survival analysis based on the Cox proportional hazards model revealed that high expression of CD74 was significantly correlated to improved OS in patients with BRCA, CESC, LUAD (lung Adenocarcinoma), SKCM and UCEC (uterine corpus endometrial carcinoma) (Fig. 1B-C), and was associated with poorer prognosis in patients with GBMLGG, LGG and KIPANB (Fig. 1B). Due to tumor heterogeneity, the relationship between CD74 expression and patient prognosis varies across different cancer types. Overall, in the 30 cancer types investigated, high CD74 expression was more often associated with a favorable patient prognosis.

CD74 and its PPI networks were involved in immune-related pathways

The PPI network of CD74 which was constructed from the STRING database contained 11 node proteins (Fig. 2A). These proteins are mainly distributed in MHC II protein complexes, endosomes and vesicles. The molecular functions of these proteins are mainly associated with MHC class II receptor activity, peptide antigen binding, immune receptor activity. The biological processes in which these proteins participated are mainly peptide antigen assembly with MHC class II protein complex, antigen processing and presentation of exogenous peptide antigen via MHC class II and adaptive immune response. KEGG enrichment analysis demonstrated similar results with the GO analysis (Fig. 2B). These results suggest that CD74 and its interacting proteins play a major role in antigen presentation and activation of immune responses.

CD74 expression positively correlated with anti-tumor immune cells and molecules

Immune cells, cytokines and chemokines in TME are closely related to tumor progression [22]. In this study, we analyzed the correlation between CD74 expression and the infiltration of six types of immune cells in 30 types of cancers, namely B cell, CD4 T-cell, CD8 T-cell, neutrophil, macrophage and DC. The expression of CD74 was positively correlated with B cell, CD4 T-cell, neutrophil, macrophage and DC to varying degrees (Fig. 3A).

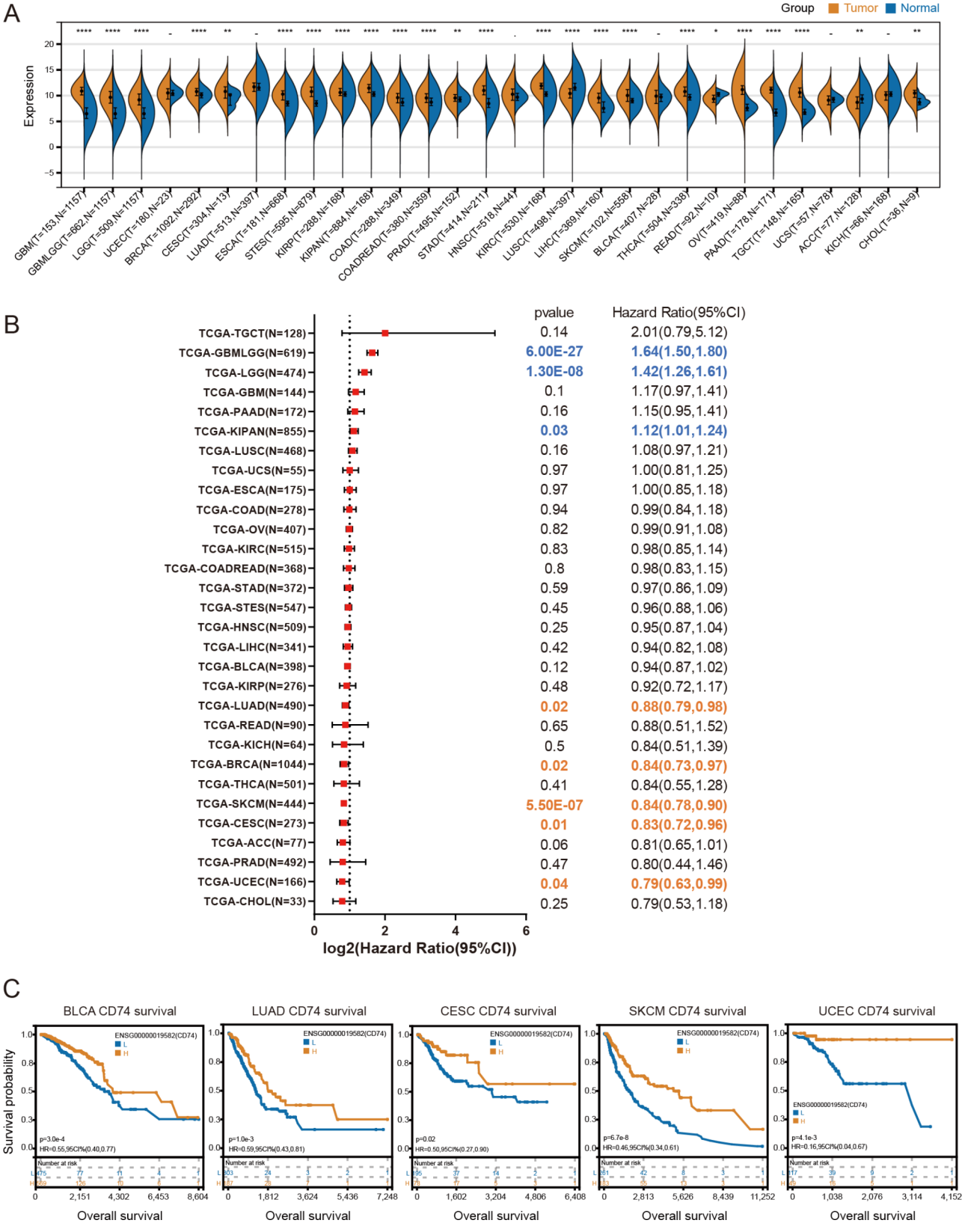

Fig. 1 CD74 expression level and its correlation with OS in different types of tumors. (**A**) Patterns of CD74 expression level between tumor and normal tissues in different cancer types. (**B**) Forest plot demonstrates the prognostic value of CD74 in OS among different cancer types. Data colored in blue and yellow represents significantly prognostic value of CD74 ($p < 0.05$). (**C**) Kaplan-Meier curves demonstrate that the OS in patients with high tumor CD74 expression are significantly improved compared to those with low tumor CD74 expression in multiple cancer types. OS, overall survival.

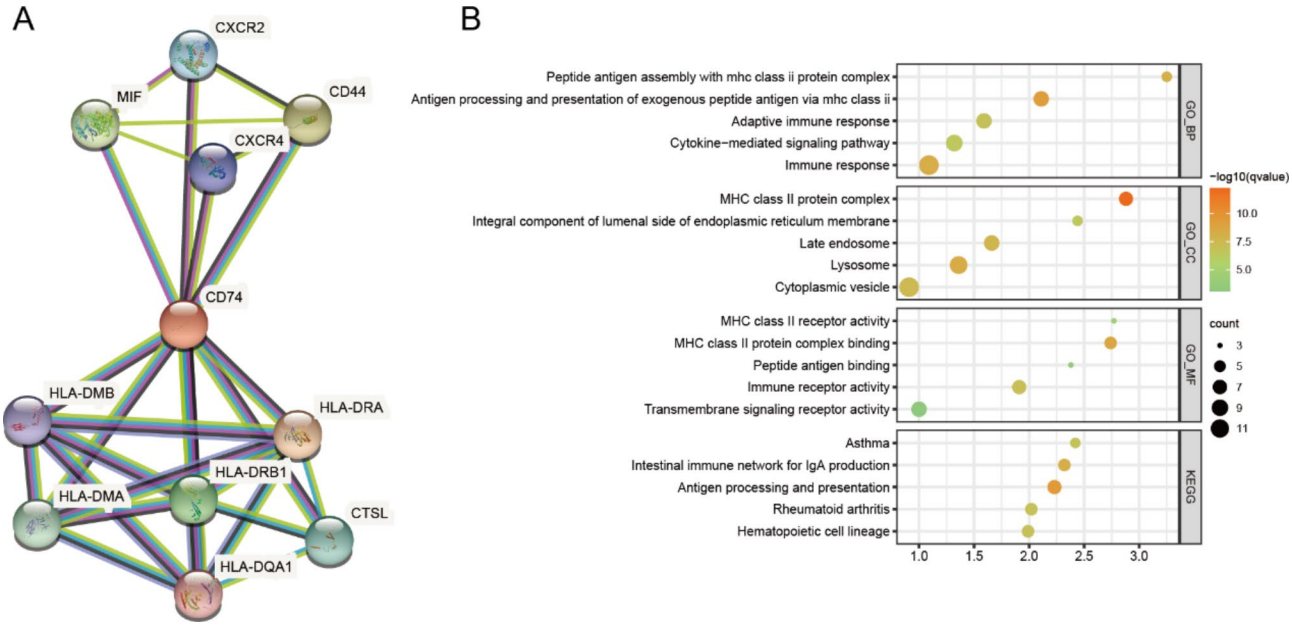

Fig. 2 PPI and signal pathway analysis of CD74. (**A**) PPI of CD74 and its interacting proteins using the STRING database. (**B**) GO annotation and KEGG pathway analysis of all nodes in the PPI network. GO, gene oncology; KEGG, Kyoto encyclopedia of genes and genomes; PPI, protein-protein interaction.

However, in THCA and GBM, CD74 expression was negatively correlated with CD8 T-cell. In three types of cancers (BRCA, ESCA and LUAD) with an indication for immunotherapy, CD74 expression was significantly positively correlated with the six types of immune cells. However, in ESCA, CD74 expression was significantly positively correlated with five types of immune cells, and the correlation with CD8 T-cells was not statistically significant (Fig. 3B).

Next, we assessed the correlations between the expression of CD74 and 38 label genes of 3 types of immune pathways. The results showed that in 30 types of cancers, CD74 expression was significantly positively correlated with the marker genes of T cell activation, immune cell trafficking, and antigen presentation (Fig. 3C). Specifically, CD74 showed moderate positive correlation with CD27 and CD28, which are co-activators of T cells and down-regulated in senescent T cells [23, 24]. CD74 was highly positively correlated with the expression of CXCR3, which has a key role in driving the trafficking of T helper 1 (TH1) cells, CD8 T-cells and natural killer (NK) cells into the TME, and is responsible for recruiting and maintaining T cells in tumors, infections, and autoimmune responses [25]. CD74 is an important molecule involved in antigen presentation. In the present study, it had a strong positive correlation with other antigen presentation genes such as HLA-A, HLA-DRA and HLA-DOB, but has a weak correlation with HLA-G, a molecule which is involved in immune escape. In addition, we analyzed the correlation between CD74 and CD274, the gene encoding PD-L1. The result showed that the mRNA expression level of CD74 was significantly positively correlated with that of CD274 in 37 tumor types (Figure S2).

Interestingly, CD74 also showed a high correlation with some co-inhibitory receptors, such as immune cell exhaustion markers Hepatitis A virus cellular receptor 2 (HAVCR2) and Lymphocyte activation gene-3 (Lag-3) [26]. In addition, CD74 had a high correlation with CCL5 and CCR5. The CCL5-CCR5 axis is a major regulator of immunosuppressive myeloid cells (IMC) of monocyte and granulocytic lineages, and it has the function of promoting tumor proliferation and migration [27].

The aforementioned results suggest that CD74 expression is positively correlated with the expression of antitumor immune cells and molecules, potentially indicating its involvement in regulating the tumoricidal process or directly contributing to tumoricidal activity.

CD74 expression was correlated with the response to ICB therapy in bladder cancer

The expression of CD74 demonstrated a moderate to strong correlation with cytotoxic T cell infiltration in tumor tissues from patients with melanoma, bladder cancer, glioma, and clear cell renal cell carcinoma who received immunotherapy, and the high expression of CD74 was associated with better prognosis of patients. (Table 1 and Figure S1).

Patients in the IMvigor 210 cohort were divided into high and low expression groups using optimal expression cutoff values of CD74. A total of 99 patients had CD74 high expression tumors and 249 patients had CD74 low expression tumors. A higher proportion of patients had

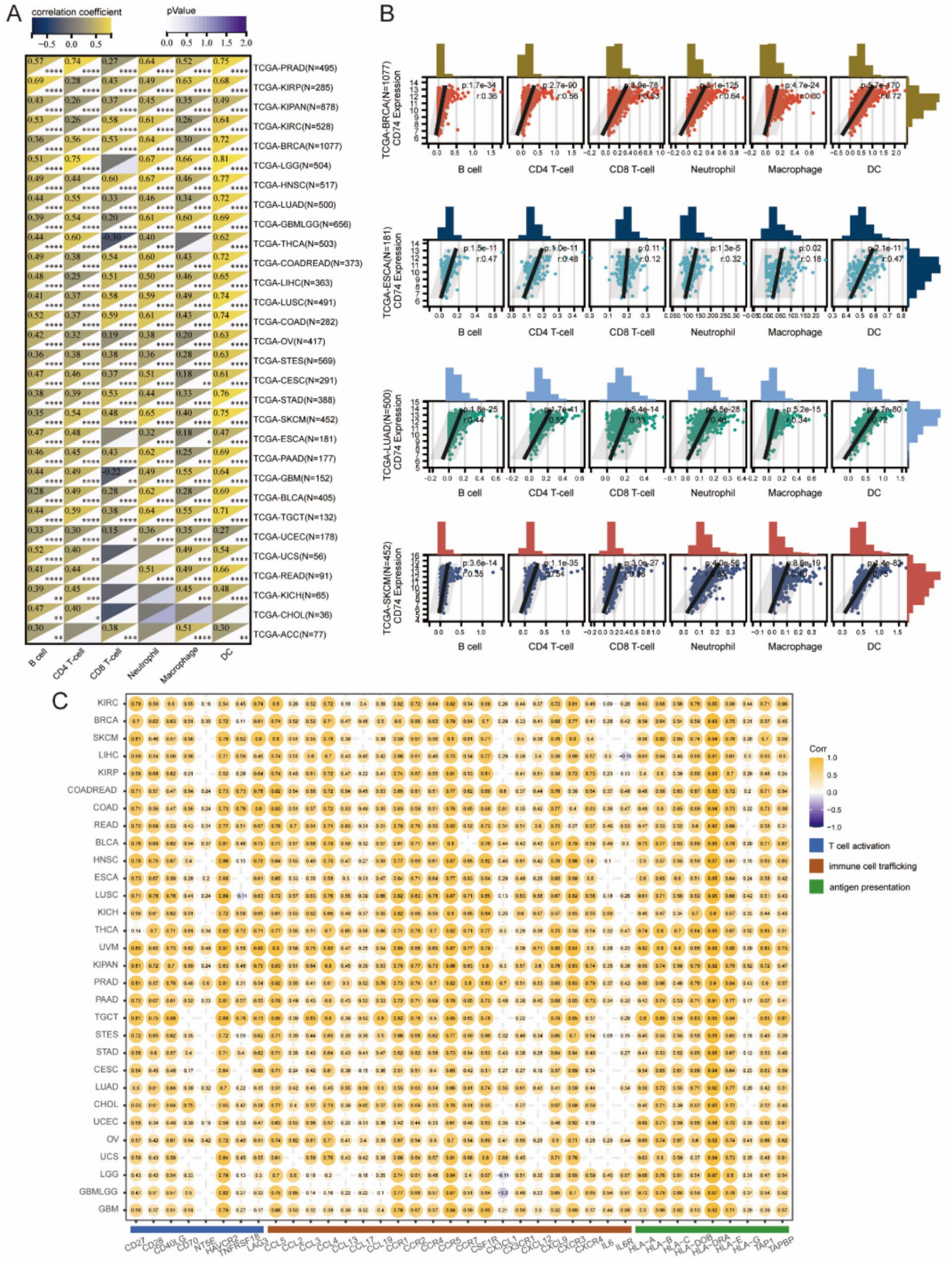

Fig. 3 Correlation between CD74 expression and immune cell infiltration and immune-related genes in the pan-caner setting. (**A**) Correlation between CD74 expression and immune cell infiltration using data from the TIMER database. (**B**) Correlation between CD74 expression and immune cell infiltration in BRCA, ESCA, LUAD and SKCM using data from UCSC database. (**C**) Correlation between CD74 expression and 38 genes related to three types of immune pathways. BRCA, breast invasive carcinoma; ESCA, esophageal carcinoma; LUAD, lung Adenocarcinoma; SKCM, skin cutaneous melanoma.

Table 1 The predictive value of CD74 expression in ICB therapy in different cancer types

Study	Cancer	Treatment	CTL Correlation	Survival end point type	Survival risk score	Sample count
Nathanson_2017	Melanoma	CTLA4_post	0.925	OS	-1.988	15
		CTLA4_pre	0.837	OS	1.257	9
Gide_2019	Melanoma	CTLA4 + PD1	0.873	OS	-1.391	32
Riaz_2017	Melanoma	PD1_Prog	0.833	PFS	-2.355	26
Gide_2019	Melanoma	PD1	0.826	PFS	-2.294	41
Mariathasan_2018	Bladder	PD-L1	0.731	OS	-0.295	348
Zhao_2019	Glioblastoma	PD1_pre	0.712	OS	0.923	15
		PD1_Post	0.15	OS	-1.855	9
Liu_2019	Melanoma	PD1_Prog	0.691	PFS	-3.021	47
Miao_2018	ccRCC	PD1 or PD-L1 ± CTLA4	0.533	OS	-0.753	33

CTL, cytotoxic T cell infiltration; ccRCC, clear cell renal cell carcinoma; OS, overall survival; PFS, progression-free survival

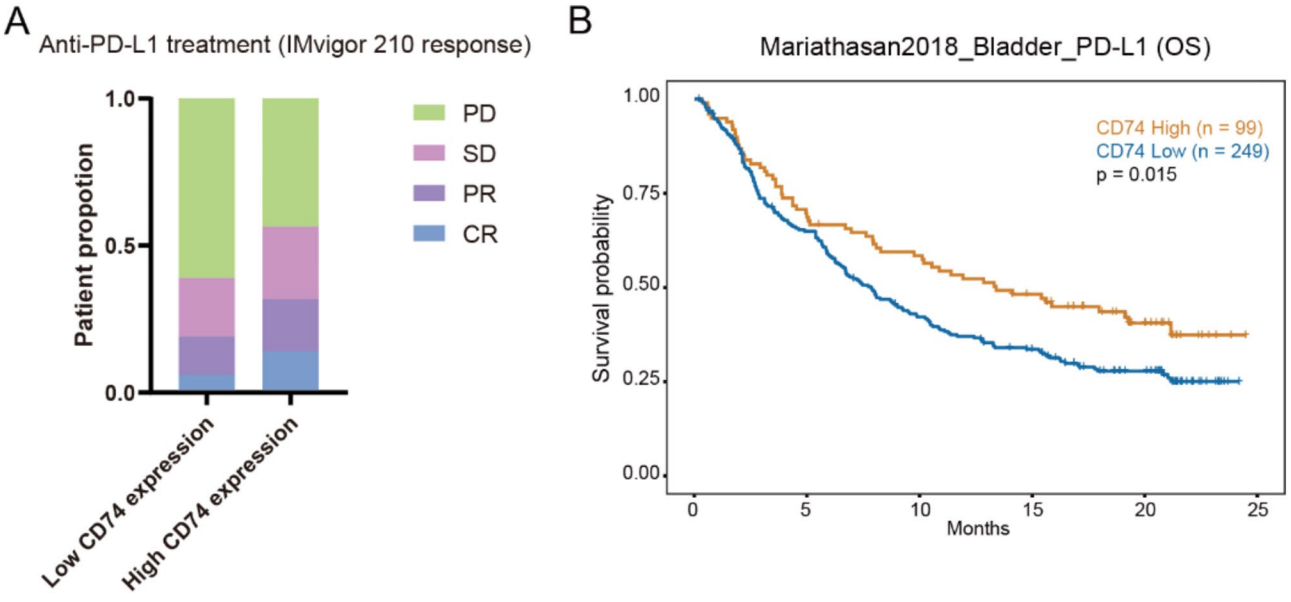

Fig. 4 Correlation between CD74 expression and the response to ICB therapy using publicly available data. (**A**) The treatment response to ICB therapy in patients with high tumor CD74 expression and patients with low tumor CD74 expression. (**B**) Kaplan-Meier curve demonstrates that the OS of patients with high tumor CD74 expression is significantly better than those with low tumor CD74 expression using data from the IMvigor 210 cohort. ICB, immune checkpoint blockade; PD, progression disease.

tumors with complete response (CR), partial response (PR) and stable disease (SD) in the CD74 high expression group than those in the CD74 low expression group (Fig. 4A). Furthermore, the expression of CD74 was prognostic in this patient cohort. Patients with CD74 high expression tumors had prolonged OS than those with CD74 low expression tumors (Fig. 4B).

Validation of CD74 as a potential biomarker for the response to ICB in esophageal cancer

mIHC staining of FFPE specimens from gastric cancer patients revealed co-localization between CD74 and CD80, suggesting that CD74 is expressed in professional antigen-presenting cells (APCs) in the TME (Fig. 5A). Next, IHC staining was performed on samples from 38 patients with esophageal cancer who received neoadjuvant immunotherapy combined with chemotherapy. Of these, 15 had matching biopsy specimens before treatment and postoperative surgical resected specimens, and 23 had only postoperative surgical resected specimens. Clinicopathological parameters are summarized in Table 2. CD74 expression in the responders was significantly higher than that in the non-responders (Fig. 5B, D, E). Interestingly, the expression of CD74 increased after treatment in the responders, while there was no significant change after treatment in the non-responders (Fig. 5C). Further, we plotted the ROC (receiver operating characteristic) curve and the area under the curve (AUC) of CD74 expression for discriminating the responders from non-responders was 0.75 (95%CI, 0.594 to 0.904) (Fig. 5F). Using the maximum value of Youden index as the cut-off value, the patients were divided into high CD74 expression group and low CD74 expression group. The results showed that the response rate was 70.4% in

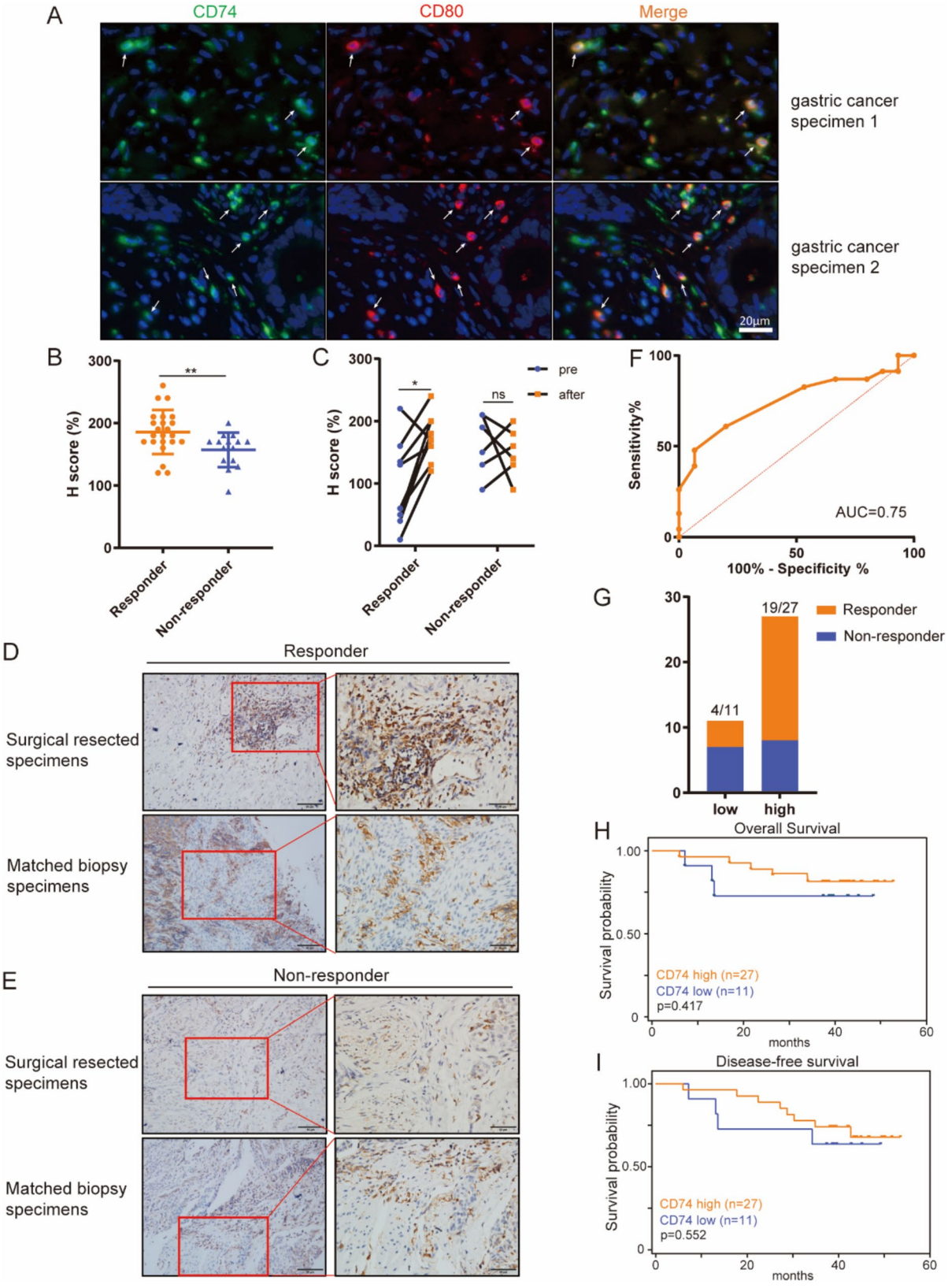

Fig. 5 (See legend on next page.)

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Fig. 5 Validation of the predictive value of CD74 expression in ICB treatment response in esophageal cancer. (**A**) mIHC staining of FFPE specimens from gastric cancer patients demonstrates that the expression of CD74 is co-localized with CD80, a marker for macrophage. White arrows indicate colocalized cells. (**B**) The tumor expression of CD74 in responders is significantly higher than the non-responders who were treated with ICB. (**C**) The expression of CD74 before and after ICB treatment in responders and non-responders. In the responders, the CD74 expression is significantly elevated after treatment, which is not observed in the non-responders. (**D**, **E**) Representative figures of CD74 expression in specimens before and after treatment in responders (**D**) and non-responders (**E**). (**F**) ROC curve analysis demonstrates that CD74 expression has good performance in discriminating the responders from the non-responders. (**G**) Response rates to ICB therapy in the CD74 high and low expression groups. (**H**-**I**) Kaplan–Meier curve analysis of the correlation between CD74 protein expression and overall survival (**H**) and disease-free survival (**I**) in esophageal cancer patients with ICB treatment. FFPE, formalin-fixed and paraffin-embedded; mIHC, multiplex immunohistochemistry; ROC, receiver operating characteristic.

the CD74 high expression group, and only 36.4% in the CD74 low expression group (Fig. 5G). Moreover, the correlation between CD74 expression and overall survival (OS) and disease-free survival (DFS) in these 38 patients were analyzed. Patients with higher CD74 expression showed a trend for improved OS and DFS compared to those with CD74 low expression, although the differences were not statistically significant, which may due to the low patient number in each group (Fig. 5H-I). Our results demonstrated that CD74 expression was significantly elevated in the response group compared to the non-response group, suggesting that CD74 may serve as a biomarker for predicting the response to ICB therapy in patients with esophageal cancer.

Discussion

In this study, we analyzed the expression of CD74 in pan-cancer and the correlation between CD74 expression and different immune cells. CD74 was differentially expressed in multiple cancer types and was significantly associated with patient survival. Furthermore, the expression of CD74 was positively correlated with cytotoxic T cell infiltration and the expression of molecules associated with T cell activation, immune cell trafficking, and antigen presentation. Among bladder cancer and esophageal cancer patients who had received immunotherapy, CD74 expression was higher in the responders, and it was significantly elevated after treatment. Moreover, patients with high CD74 expression demonstrated a trend for improved DFS and OS compared to those with low CD74 expression. This suggested that CD74 may have a predictive value for the efficacy of ICB therapy.

Although the mechanism of ICB therapy has not been fully revealed, it is clear that a successful antitumoral response requires the activation of cytotoxic T cells, which is dependent on the activation and presentation of antigens by APCs. CD74 plays an important role in antigen presentation, which may be the reason why we observed a positive correlation between CD74 expression and cytotoxic T cells infiltration in a majority of cancer types in this study. We also found that CD74 expression was significantly positively correlated with that of CD274, which encodes the protein PD-L1. This suggests that they are co-expressed in the tumor microenvironment, which may explain why patients with high CD74 expression are more responsive to immune checkpoint inhibitors. Several interesting molecules were found in the correlation analysis in this study. For example, in GBM and THCA, the correlation between CD74 expression and CD8 T-cells showed a negative correlation. Since GBM exhibits a higher degree of CD8 T-cell exhaustion due to its unique immune microenvironment [28], We speculate that the negative correlation between CD74 and CD8 T-cells is due to the lower expression level of cytotoxic CD8 T-cell signature genes, as its expression negatively correlated with that of exhaustic CD8 T-cell. For THCA, to the best of our knowledge, limited research has reported the degree of infiltration and the exhaustion state of CD8 T-cells in its TME. However, some studies have indicated that the prognostic value of CD8 T-cell infiltration in THCA remains controversial. A higher degree of CD8 T-cell infiltration may be associated with a worse prognosis [29]. Therefore, there is no clear explanation for the negative correlation between CD74 and CD8 T-cell in THCA. The expression of CD74 was highly correlated with the expression of HAVCR2 in various tumors. HAVCR2 (also known as Tim-3), a co-inhibitory receptor, has been shown to negatively regulate the type I immunity in previous studies [26]. Recent studies have found that the binding of HAVCR2 to its ligand phosphatidyl serine (PtdSer) affects the phagocytosis of DC and antigen cross-presentation, but not the function of T cells which are not phagocytic [26]. Whether HAVCR2 plays a role in antigen presentation remains to be further explored. CCL5-CCR5 axis is involved in immunosuppression, promoting tumor growth and malignant progression. In this study, we found that the expression of CCL5 and CCR5 were positively correlated with CD74 expression. These results seem counterintuitive. In tumors, high expression of the CCL5-CCR5 axis is typically associated with poor prognosis. However, studies have shown that as an inflammatory chemokine, CCL5 expression is positively correlated with the infiltration of CD8 T-cells. CCL5 promotes T cell chemotaxis through interaction with CCR5 [30]. Furthermore, both CCL5 and CCR5 levels were elevated in the serum of patients with metastatic renal cell carcinoma treated with bevacizumab or bevacizumab in combination with atezolizumab as shown in a recent study [31]. Since immune regulation is a chain reaction, elevated levels of a single

Table 2 Patient characteristics

	No. (%)	% exclude unknown
Age at diagnosis		
Median (IQR) years	64 (60–68)	
Gender		
Men	28 (73.7)	(73.7)
Women	10 (26.3)	(26.3)
Tumor location		
Upper	1 (2.6)	(2.6)
Upper-middle	4 (10.5)	(10.5)
Middle	18 (47.4)	(47.4)
Middle-lower	8 (21.1)	(21.1)
Lower	7 (18.4)	(18.4)
Histological type		
Squamous cell carcinoma	35 (92.1)	(94.6)
Adenocarcinoma	2 (5.3)	(5.4)
Unknown	1 (2.6)	
Histological grade		
G1	3 (7.9)	(7.9)
G2	31 (81.6)	(81.6)
G3	4 (10.5)	(10.5)
Treatment course		
Mean (SD)	2.6 (0.8)	
2	20 (52.6)	(52.6)
3	14 (36.8)	(36.8)
4	2 (5.3)	(5.3)
5	2 (5.3)	(5.3)
Mandard score		
1	12 (31.6)	(31.6)
2	11 (28.9)	(28.9)
3	7 (18.4)	(18.4)
4	3 (7.9)	(7.9)
5	5 (13.2)	(13.2)

IQR, interquartile range; SD, standard deviation.

or a few molecules may arise from feedback regulation in the loop. Further studies are needed to elucidate the regulation network of CD74, as well as their roles in the process of antigen presentation.

Research on the relationship between CD74 expression and the efficacy of immunotherapy is very limited. CD74 may be a key factor that shows different effects in different tumors or may even in different subtypes of the same tumor. The roles of CD74 in different cancers are contradictory from literature. A recent study of glioma showed that in the tumor immune microenvironment, CD74 was highly expressed in monocyte myeloid-derived suppressor cells (M-MDSCs). Targeting M-MDSCs with Ibudilast, an inhibitor of MIF-CD74 interaction, reduced CD74 expression, inhibited the function of MDSC and enhanced the activity of CD8+T cells [32]. MIF-CD74 in microglia inhibits interferon-γ secretion and thereby M1 polarization through ERK1/2 phosphorylation [33]. In another study, CD74 expression in microglia was associated with anti-tumor M1 polarization, which was positively correlated with patient prognosis [34]. In cutaneous melanoma, CD74 was a hub gene in fatty acid metabolism, which was associated with improved overall survival, progression-free survival, and disease-specific survival [35]. In general, the tumor-promoting effect of CD74 is mostly related to MIF, and how it affects tumor progression through antigen presentation still needs further study.

There are some limitations in this study. Although we revealed the expression profile of CD74 in esophageal cancer patient tissues and its predictive value of the efficacy of immunotherapy, the role of CD74 has not been explored in other cancer types. On the other hand, CD74 expression was increased in responders after treatment, and a causal relationship between the two has not been established. The underlying mechanism may be that the elevated expression of CD74 affects immune molecules in the microenvironment, or the microenvironment after treatment is more favorable for CD74 expression. Future studies are needed to explore the role of CD74 in pan-cancer and the regulation mechanism of its expression in TME.

Conclusions

The expression of CD74 was positively correlated with the degree of cytotoxic T cell infiltration. CD74 may be a potential predictive biomarker of the treatment efficacy of ICB.

Abbreviations

ACC	Adrenocortical carcinoma
APCs	Antigen-presenting cells
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
COADREAD	Colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma
CR	Complete response
CTL	Cytotoxic T cell infiltration
DC	Dendritic cell
ESCA	Esophageal carcinoma
FFPE	Formalin-fixed and paraffin-embedded
GBM	Glioblastoma multiforme
GBMLGG	Glioma
GO	Gene oncology
HAVCR2	Hepatitis A virus cellular receptor 2
ICB	immune checkpoint blockade
IMC	Immunosuppressive myeloid cells
KEGG	Kyoto encyclopedia of genes and genomes
KIPAN	Pan-kidney cohort
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
Lag-3	Lymphocyte activation gene-3
LGG	Brain lower grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung Adenocarcinoma
LUSC	Lung squamous cell carcinoma
MHC II	Class II major histocompatibility complex
mIHC	Multiplex immunohistochemistry
M-MDSCs	Monocyte myeloid-derived suppressor cells

mUC	Metastatic urothelial bladder cancer
NK	Natural killer
OS	Overall survival
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PD-L1	Programmed death ligand 1
PPI	Protein-protein interaction
PR	Partial response
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SD	Stable disease
SKCM	Skin cutaneous melanoma
STAD	Stomach adenocarcinoma
STES	Stomach and esophageal carcinoma
TCGA	The Cancer Genome Atlas
TGCT	Testicular germ cell tumors
THCA	Thyroid carcinoma
TIDE	Tumor Immune Dysfunction and Exclusion
TIMER	The tumor immune estimation resource database
TMB	Tumor mutational burden
TME	Tumor microenvironment
UCEC	Uterine corpus endometrial carcinoma
UCSC	The university of California Santa Cruz

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

W-QS, D-XC, and C-PL performed the experiments and data analysis. W-QS and D-XC drafted the manuscript. Z-SD, T-TZ, FP, H-LC and W-NL contributed to the clinical data acquisition and analysis. C-PL and S-HW performed the pathological assessments. S-QQ, J-HF and Z-YW designed the study and revised the draft. All authors have read and approved the article.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shantou Central Hospital.

Competing interests

The authors declare no competing interests.

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