Original Article

Clusterin is a biomarker of breast cancer prognosis and correlated with immune microenvironment

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Background: It has been established that clusterin is involved in the invasion of immune cells in the tumor microenvironment, but it remains unknown how it promotes immune invasion in breast cancer.

Methods: We used Tumor Immune Estimation Resource (TIMER) and Gene Expression Profiling Interactive Analysis (GEPIA) databases to assess the relation between expression of clusterin and immunoinfiltration-related marker genes. TIMER database was used to evaluate the expression of clusterin, and its relation to tumor immune invasion was examined. Based on Kaplan-Meier plotter database, we investigated the association between clusterin expression and prognosis in patients with cancer, and the impact of clinicopathological factors and cancer-related outcomes.

Results: Clusterin expression was markedly associated with prognosis of a variety of tumors, specifically breast cancer. Enhanced clusterin expression was markedly associated with molecular typing of breast cancer and expression of multiple markers related to specific immune cell subsets.

Conclusions: These results indicate that clusterin is connected to prognosis of breast cancer patients and tumor immune cell infiltration. This demonstrates that clusterin may be a biomarker of immune cell recruitment into breast tumors and an important biomarker for immune cell infiltration; consequently being a valuable prognostic factor in breast cancer patients.

Keywords: Breast cancer; prognosis; clusterin (CLU); tumor infiltration; biomarker

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Introduction

Breast cancer is one of the most common diseases and main causes of women's mortality worldwide (1). Breast tumors are heterogeneous, with five major subtypes (luminal A and B, HER2, basal, and normal), with different clinical characteristics and prognoses (2). In recent years, immune checkpoint inhibitors (ICIs) have been used in the treatment of malignant tumors. ICIs work by utilizing the host immune system to kill tumor cells (3). Currently, programmed death ligand-1 (PD-L1) is the only convincing predictive biomarker for ICIs in breast cancer, and clinical trials have concentrated on triple-negative breast cancer (4-7). Tumor-infiltrating immune cells are associated with prognosis, particularly tumor-associated macrophages (TAMs) and neutrophils, which are also associated with

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tumor chemotherapy (8). Therefore, clarifying the immune phenotype in the breast cancer microenvironment and how immune cells modulate breast cancer is essential to identify potential new immunotherapeutic targets.

Clusterin (CLU) is an evolutionarily conserved molecular chaperone present in diverse human tissues and fluids, and is considered an important tumor regulator (9-11). CLU regulates several cancer-related cellular events, including cancer cell proliferation, metastasis, stemness, epithelial-mesenchymal transition, survival, therapeutic resistance, and suppression of programmed cell death to support tumor growth and recurrence (12-14). It seems to vary its location and function to preserve cells and ensure their survival, and it is important in neuroprotection and tumors as well as in chemoresistance (15).

In the mouse splenic matrix, CLU mRNA is significantly downregulated after deletion of lymphoid receptors critical for development, maintenance, and function of secondary lymphoid organs (16). This is an early understanding of the function of CLU in the immune system. Semen CLU interaction with dendritic cell (DC)-specific intercellular-adhesion-molecule-captured non-integrins is reported to promote antigen capture by DCs and differentiation of DCs into tolerogens, characterized by an increased ability to promote expansion of $Foxp3^+$ T regulatory (Treg) cells (17). Elevated preoperative secretory CLU expression in breast cancer correlates with cancer-associated fibroblast (CAF) resistance and tumor necrosis factor α (TNF-α)-induced apoptosis in breast cancer cells (18,19). Therefore, we speculated that CLU could be associated with tumor-infiltrating immune cells and affect the treatment of cancer patients.

Here, we performed a comprehensive assessment of the relationship between CLU and patient prognosis using multiple databases (PrognoScan, GEPIA, and Kaplan-Meier plotter), and explored the link between CLU and tumor immune cell infiltration using the Tumor Immunoassay Resource (TIMER). Our findings provide new insights into the functional role of CLU in breast cancer, highlighting a potential mechanistic basis by which CLU affects immune cell–tumor interactions. We present the following article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1882/rc).

Highlight box

Key findings

- Clusterin is connected to prognosis of breast cancer patients and tumor immune cell infiltration.

What is known and what is new?

- Clusterin is involved in the invasion of immune cells in the tumor microenvironment.
- Enhanced clusterin expression was markedly associated with molecular typing of breast cancer and expression of multiple markers related to specific immune cell subsets.

What is the implication, and what should change now?

- Clusterin may be a biomarker of immune cell recruitment into breast tumors and an important biomarker for immune cell infiltration; consequently, being a valuable prognostic factor in breast cancer patients.

Methods

PrognoScan database (http://www.abren.net/PrognoScan/)

The PrognoScan database aims to facilitate a meta-analysis of the prognostic value of genes by comparing the relationship between gene expression and relevant outcomes, including overall survival (OS) in numerous published cancer microarray datasets (20). Accordingly, we used this database to evaluate the relationship between CLU expression and patient prognosis.

GEPIA database (http://gepia.cancer-pku.cn/index.html)

GEPIA is an online database based on web tools that provides customizable and rapid features based on Genotype-Tissue Expression (GTEX) and The Cancer Genome Atlas (TCGA) data. There are several features that make it interactive and customizable, including differential expression analysis, correlation analyses, mapping, similar genetic testing, patient survival analyses, and dimension reduction analysis (21). We utilized GEPIA database to evaluate the link between expression of CLU and patient prognosis and to further evaluate the link between expression of CLU and specific markers associated with tumor immune cell infiltration.

TIMER database (https://cistrome.shinyapps.io/timer/)

TIMER is a database for investigating immune cell infiltration in many cancers. In the database, a variety of statistical methods validated by pathological examination are used to analyze tumor infiltration by neutrophils,

macrophages, DCs, B cells, and CD4/CD8 T cells (22). We first used this database to assess differences in CLU expression levels in specific tumor types and then explored the association between CLU expression and extent of infiltration by specific immune cell subsets. We performed Kaplan-Meier curve analysis to explore the impact of immune cell infiltration or gene expression on patient survival. In addition, we considered whether CLU expression correlated with specific markers of immune infiltrating cell subsets.

Kaplan-Meier plotter (http://kmplot.com/analysis/)

The Kaplan-Meier plotter provides a convenient method for exploring the impact of many different genes on survival of tumor patients with large sample sizes, including breast, ovarian, lung and gastric cancers (23). Based on this database, we investigated the association between CLU expression and prognosis in patients with these cancers, and the impact of clinicopathological factors and cancer-related outcomes. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

As part of the respective analyses, survival plots were generated based on the PrognoScan, TIMER, Kaplan-Meier plotter and GEPIA databases, with data such as hazard ratios (HRs) and P values, or P values derived from the log-rank test. Spearman's correlation was used to measure the degree of correlation between specific variables, and the following R values were used to determine the degree of correlation. The range of 0.00–0.19 represented very weak, 0.20–0.39 weak, 0.40–0.59 moderate, 0.60–0.79 strong, and 1.0 extremely strong. We set a significance level of $P<0.05$.

Results

Assessment of CLU expression differences between tumors and normal tissues

We evaluated differences in expression of CLU in multiple tumor types using the TIMER and TCGA databases. In comparison with normal control subjects, CLU was significantly higher in kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), and thyroid carcinoma (THCA). However, CLU expression was significantly reduced in these tumors compared to normal tissues, including bladder urothelial carcinoma (BLCA), cholangiocarcinoma (CHOL), breast invasive carcinoma (BRCA), esophageal carcinoma (ESCA), colon adenocarcinoma (COAD), kidney chromophobe (KICH), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), rectal adenocarcinoma (READ) and uterine corpus endometrial carcinoma (UCEC). *Figure 1* illustrates a comparison of CLU expression in tumors and adjacent normal tissues from the TCGA dataset.

Relationship between CLU expression and prognosis of cancer patients

We used the PrognoScan database to study the relationship between CLU expression and prognosis of cancer patients (Figures S1-S4). We found that CLU expression was significantly correlated with prognosis of patients, including patients with hematological, breast, colon, lung and prostate cancers (*Figure 2A-2H*). We also used the Kaplan-Meier plotter database to evaluate the relationship between CLU expression and prognosis of these cancer types (*Figure 2I-2P*). The increase in CLU expression was significantly correlated with the poor prognosis of gastric cancer [OS HR =1.69, 95% confidence interval (CI): 1.37–2.09, P=1E-06; disease-free survival (DFS) HR =1.61, 95% CI: 1.26–2.07, P=1.5E-04]. However, the decrease in CLU expression was associated with poor prognosis in breast cancer [OS HR =0.78, 95% CI: 0.64–0.94, P=0.01; DFS HR =0.73, 95% CI: 0.65–0.82, P=5E-08] and lung cancer (OS HR =0.61, 95% CI: 0.53–0.7, P=1.1E-12; progression-free survival HR =0.59, 95% CI: 0.49–0.71, P=5.1E-08). There was no association between CLU expression and OS in ovarian cancer, but decreased expression improved DFS (OS HR =0.9, 95% CI: 0.78–1.03, P=0.12; DFS HR =1.17, 95% CI: 1.03–1.34, P=0.015). Using the GEPIA database, 33 TCGA tumor types were further analyzed to assess the correlation between CLU expression and patient outcomes and found that CLU expression correlated with OS in brain lower grade glioma (LGG), KIRC, pancreatic adenocarcinoma (PAAD), sarcoma (SARC), THCA and LIHC (figure available at https://cdn.amegroups.cn/static/public/tcr-22-1882-1.pdf). These results suggest that CLU expression is associated with different prognosis of multiple tumor types.

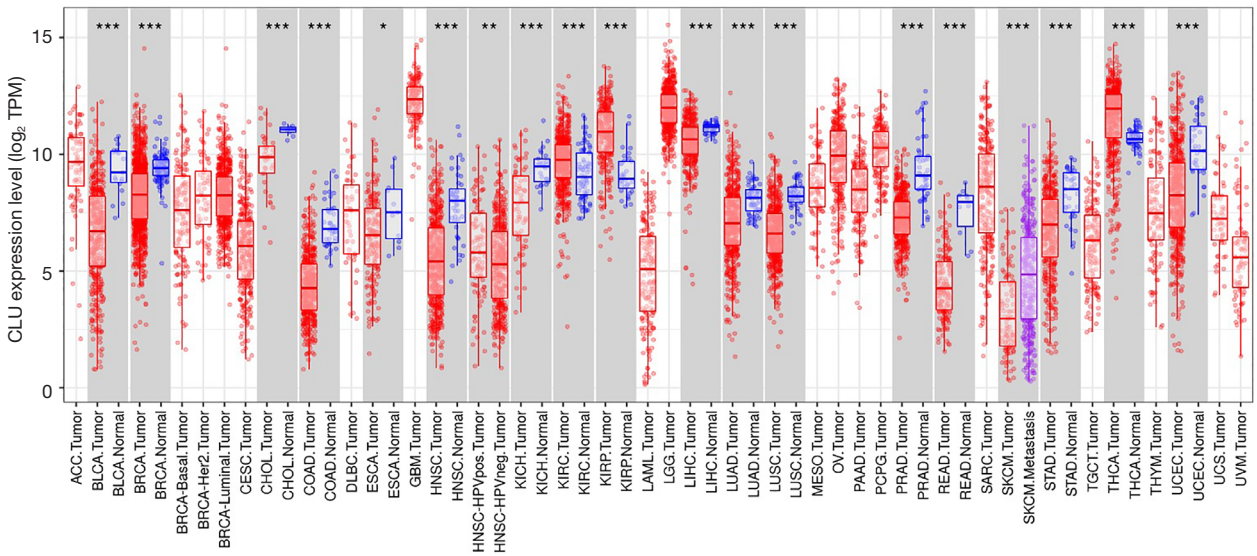

Figure 1 Assessment of clusterin expression in different cancer and normal tissues (TIMER database; *, P<0.05; **, P<0.01; ***, P<0.001). ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; CLU, clusterin; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; TPM, transcripts per million; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; TIMER, Tumor Immune Estimation Resource.

Correlation between CLU expression and prognosis of breast cancer patients with different molecular types

Since we found that CLU expression was associated with good prognosis in breast cancer patients, we examined the relationship between CLU expression and the molecular subtypes among breast cancer patients using Kaplan-Meier plots. CLU expression was significantly correlated with OS, DFS and with patient estrogen receptor (ER) status (positive array), HER2 status (positive array), subtype St Gallen (luminal B), subtype PAM50 (basal), TP53 status (mutated) and lymph node status, but not for grade and Pietenpol subtype (*Table 1*). We found no significant correlation between CLU expression and HER2 positive status (St Gallen) by molecular subtype, suggesting that there was no crosstalk between CLU expression and HER2 pathway.

CLU expression correlates with the infiltration of breast cancer by immune cells

Prior studies have shown that the extent of immune cell infiltration impacts tumor prognosis in a variable way (24,25), especially for breast cancer (26,27). Therefore, we used the TIMER database to examine the relationship between CLU expression and immune cell infiltration across multiple tumor types (figure available at https://cdn.amegroups.cn/static/public/tcr-22-1882-2.pdf). CLU expression was significantly associated with tumor purity in 21 tumor types, and with B cell infiltration in 22 tumor types. CLU was also correlated with the level of $CD8^+$ T cell infiltration in 10 tumor types, $CD4^+$ T cell infiltration in 23 tumor types, macrophage infiltration in 21 tumor types, neutrophil infiltration in 12 tumor types,

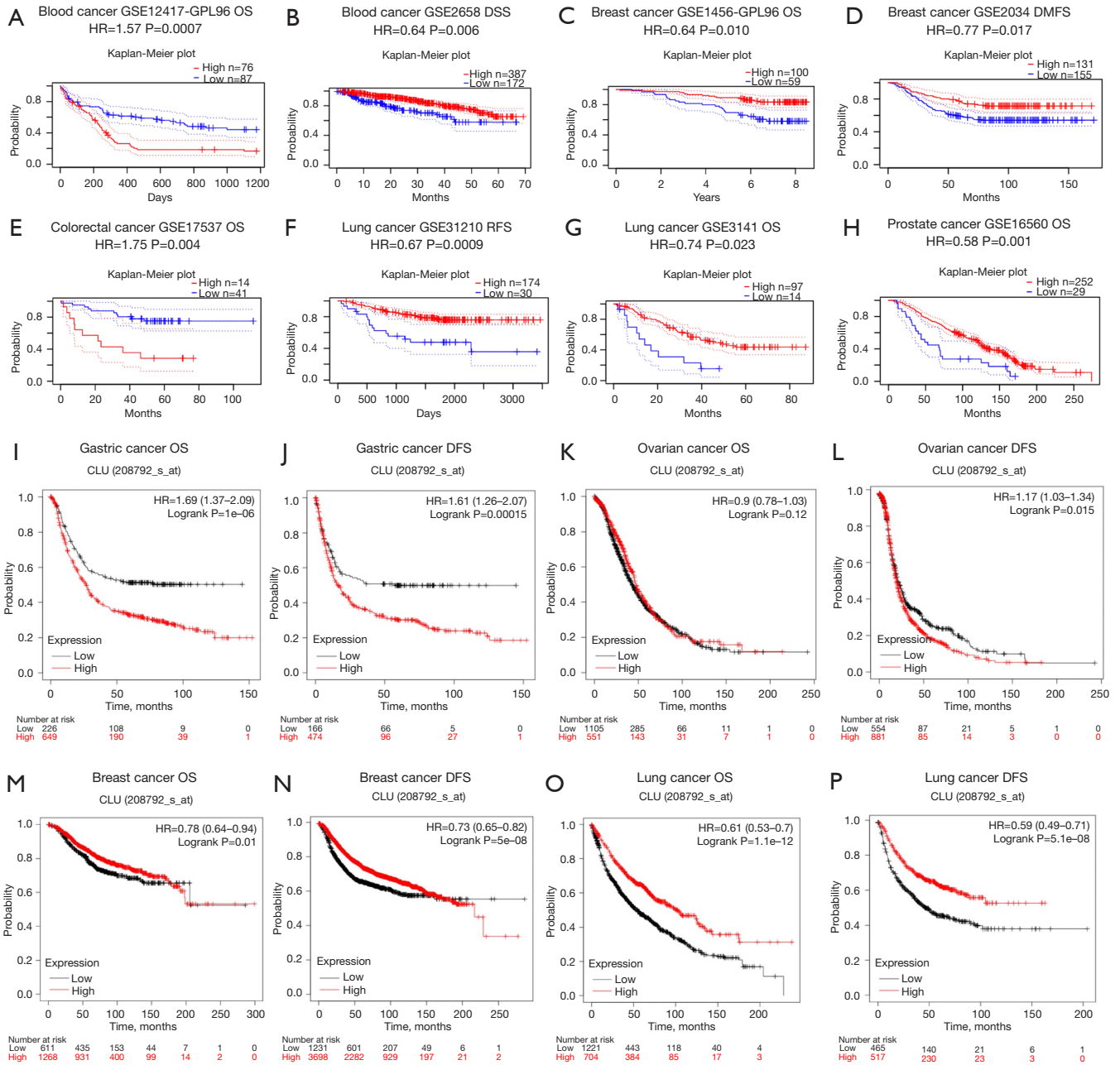

Figure 2 Prognostic correlation between clusterin and different tumors in the PrognoScan (A-H) and Kaplan-Meier plotter (I-P) databases. CLU, clusterin; DFS, disease-free survival; DSS, disease-specific survival; DMFS, distant metastasis free survival; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival; HR, hazard ratio.

Table 1 Kaplan-Meier plotter detects effects of different molecular subtypes of breast cancer on clusterin gene expression and clinical prognosis

Clinicopathological characteristics	Overall survival			Progression-free survival		
	N	HR (95% CI)	P	N	HR (95% CI)	P
ER status—IHC						
Positive	754	0.77 (0.56–1.08)	0.1286	2,633	0.75 (0.64–0.87)	0.0001
Negative	520	1.20 (0.85–1.69)	0.2929	1,190	0.74 (0.60–0.91)	0.0050
ER status—array						
Positive	1,309	0.78 (0.60–1.00)	0.0465	3,768	0.81 (0.72–0.91)	0.0005
Negative	570	0.84 (0.61–1.15)	0.2744	1,161	0.80 (0.66–0.97)	0.0260
PR status—IHC						
Positive	156	2.97 (1.40–6.29)	0.003	926	1.22 (0.91–1.64)	0.1926
Negative	291	0.78 (0.48–1.27)	0.3194	925	0.65 (0.50–0.83)	0.0007
HER2 status—array						
Positive	420	0.67 (0.47–0.96)	0.0296	882	0.79 (0.63–0.98)	0.0311
Negative	1,459	0.81 (0.65–1.02)	0.0759	4,047	0.77 (0.68–0.87)	1.70E-05
Subtype St Gallen						
Basal	404	0.78 (0.53–1.15)	0.2046	846	0.77 (0.61–0.97)	0.0288
Luminal A	794	1.58 (1.13–2.22)	0.0072	2,277	0.91 (0.75–1.10)	0.3202
Luminal B	515	0.66 (0.46–0.95)	0.0239	1,491	0.77 (0.64–0.92)	0.0037
$HER2^+$	166	1.38 (0.78–2.45)	0.2706	315	0.79 (0.55–1.14)	0.2074
Subtype PAM50						
Basal	431	0.52 (0.35–0.77)	0.0009	953	0.73 (0.58–0.91)	0.0055
Luminal A	596	1.56 (1.00–2.43)	0.049	1,809	1.16 (0.92–1.47)	0.2159
Luminal B	439	1.16 (0.81–1.67)	0.4068	1,353	0.83 (0.70–0.99)	0.0358
$HER2^+$	362	1.41 (0.96–2.07)	0.0814	695	0.87 (0.68–1.12)	0.2696
Normal	51	2.08 (0.77–5.61)	0.1384	119	0.72 (0.34–1.50)	0.3777
Lymph node status						
Positive	452	0.70 (0.51–0.98)	0.0348	1,656	0.63 (0.53–0.74)	4.70E-08
Negative	726	1.51 (1.02–2.25)	0.0402	2,368	0.79 (0.67–0.92)	0.0033
Grade						
1	175	1.67 (0.67–4.15)	0.2658	397	1.42 (0.85–2.37)	0.1753
2	443	1.49 (0.98–2.28)	0.0615	1,177	0.67 (0.53–0.84)	0.0007
3	586	1.25 (0.89–1.76)	0.1902	1,300	0.87 (0.71–1.06)	0.1545
TP53 status						
Mutated	130	0.51 (0.26–1.01)	0.0486	188	0.52 (0.31–0.86)	0.0091
Wild type	197	0.73 (0.39–1.38)	0.3298	273	0.78 (0.50–1.23)	0.2861
Pietenpol subtype						
Basal-like 1	103	0.24 (0.07–0.81)	0.0126	251	0.69 (0.44–1.07)	0.0982
Basal-like	58	2.23 (0.77–6.44)	0.1266	101	0.76 (0.40–1.42)	0.3868
Immunomodulatory	149	0.68 (0.31–1.50)	0.3399	300	1.40 (0.88–2.22)	0.1558
Mesenchymal	114	0.72 (0.37–1.43)	0.3517	211	0.78 (0.52–1.17)	0.2296
Mesenchymal Stem-like	39	1.76 (0.60–5.20)	0.2989	81	1.48 (0.68–3.22)	0.3229
Luminal androgen receptor	116	1.22 (0.60–2.24)	0.5164	253	0.84 (0.58–1.23)	0.3743

HR, hazard ratio; CI, confidence interval; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TP53, tumor protein 53; PAM50, prediction analysis of microarray 50.

and DC infiltration in 20 tumor types. Within BRCA, BRCA luminal, and HER2 subtypes, CLU levels were not significantly associated with B cell, $CD4^+$ T cell, $CD8^+$ T cell, macrophage, neutrophil or DC infiltration (*Figure 3A-3C*). However, CLU expression was significantly correlated with the level of tumor purity (R=–0.301, P=5.3E-04), B cells (R=0.308, P=4.99E-04), $CD8^+$ T cells (R=0.26, P=3.71E-03), $CD4^+$ T cells (R=0.221, P=1.46E-02), macrophages (R=0.179, P=4.37E-02), neutrophils (R=0.275, P=3.70E-03) and DCs (R=0.205, P=2.84E-02) in BRCA basal type (*Figure 3D*). In BRCA basal type, CLU expression was significantly correlated with immune cell infiltration, especially B cells. This is consistent with previous results (28,29). We further used the TIMER database to generate Kaplan-Meier plots to investigate the correlation between CLU expression and immune cell infiltration in BRCA and its subtypes. B-cell infiltration and CLU expression were significantly associated with prognosis in BRCA (P=0.046) and HER2 (P=0.017) subtypes but not in BRCA basal and luminal subtypes (*Figure 4*). This suggests that CLU regulates the infiltration of immune cells in breast cancer.

Correlation between CLU expression and immune markers

Using the TIMER and GEPIA databases, we investigated further the connection between CLU expression and immune cell infiltration based on immune marker sets in BRCA. We examined the association between CLU expression and markers of specific cell subsets, including total T cells, $CD8^+$ T cells, M1/M2 macrophages, B cells, natural killer (NK) cells, monocytes, neutrophils, DCs, TAMs, Th1 cells, Th2 cells, Th17 cells, T follicular helper (Tfh) cells, Treg cells and exhausted T cells. After adjusting for tumor purity, CLU expression correlated with TAM markers (CD68 and IL-10), monocyte markers (CD115), DC markers (HLA-DPB1 and HLA-DQB1), M1 macrophage markers [inducible nitric oxide synthase (iNOS) and IRF5], NK cell markers (KIR3DL3), Th1 markers [interferon γ (IFN-γ)], Th2 markers (GATA3, STAT6 and STAT5A), Th17 markers in BRCA (STAT3), Tfh markers (BCL6), Treg cell markers (Foxp3, CCR8 and STAT5b) and T cell exhaustion markers [cytotoxic T-lymphocyte-associated protein 4 (CTLA4), lymphocyte activation gene 3 (LAG3) and GZMB] (*Table 2*). There was a significant correlation between CLU expression and DC markers (HLA-DPB1 and HLA-DQB1), Th2 markers (GATA3, STAT6 and STAT5A), Treg cell markers (FOXP3, CCR8 and STAT5B) and T cell exhaustion markers (CTLA4 and LAG3) in BRCA (P<0.05; *Figure 5*). Therefore, we further evaluated the relationship between CLU expression and these markers in BRCA using the GEPIA database, which revealed similar correlations between CLU and markers of DCs, Th2 cells, Treg cells and T cell exhaustion, and between CLU expression in TIMER and these markers (*Table 3*). This suggested that elevated CLU expression in BRCA increased DC infiltration, and expression of DC markers HLA-DQB1, HLA-DPB1, HLA-DPA1, HLA-DRA, BDCA-4 and BDCA-1 correlated with CLU expression. CLU was closely related to tumor DC penetration. DCs can promote tumor metastasis by enhancing Treg cell responses and suppressing $CD8^+$ T cell cytotoxicity (30). Further work is required to determine whether CLU plays a critical role in regulating DC infiltration and tumor metastasis. We further observed a significant correlation between CLU, T-cell subsets, including Foxp3 CCR8, STAT5b, transforming growth factor β (TGFβ), CTLA4 and LAG3 (*Table 2*), suggesting that CLU may play an immune escape role in the progression of breast cancer, although the mechanism needs to be confirmed.

Discussion

CLU is a secretory glycoprotein and is essentially a heterodimer. It is expressed in a variety of tissues and body fluids. CLU is also considered to be a promising biomarker for cell death, malignancy, cancer progression and drug resistance development (31). CLU plays an important carcinogenic role by promoting various downstream carcinogenic pathways (11,32-34). Protein kinase D3 is a key regulator of CLU and promoted tumor growth in triple-negative breast cancer (35). In HER2-positive breast cancer, trastuzumab treatment upregulates expression of CLU protein, which is positively correlated with the dose. By blocking the CLU expression induced by trastuzumab, OGX-011 treatment might enhance the growth inhibitory effect of monoclonal antibody trastuzumab (36). In this study, we found that in several types of cancer, CLU expression was correlated with the prognosis of patients, and low CLU expression was strongly correlated with poor prognosis of BRCA. BRCA patients with low CLU expression are also more likely to be ER and HER2 negative, suggesting that CLU may be useful as a prognostic indicator. We found that expression of CLU in tumors was associated with many different markers of immune cell subsets, which highlighted the possible role of

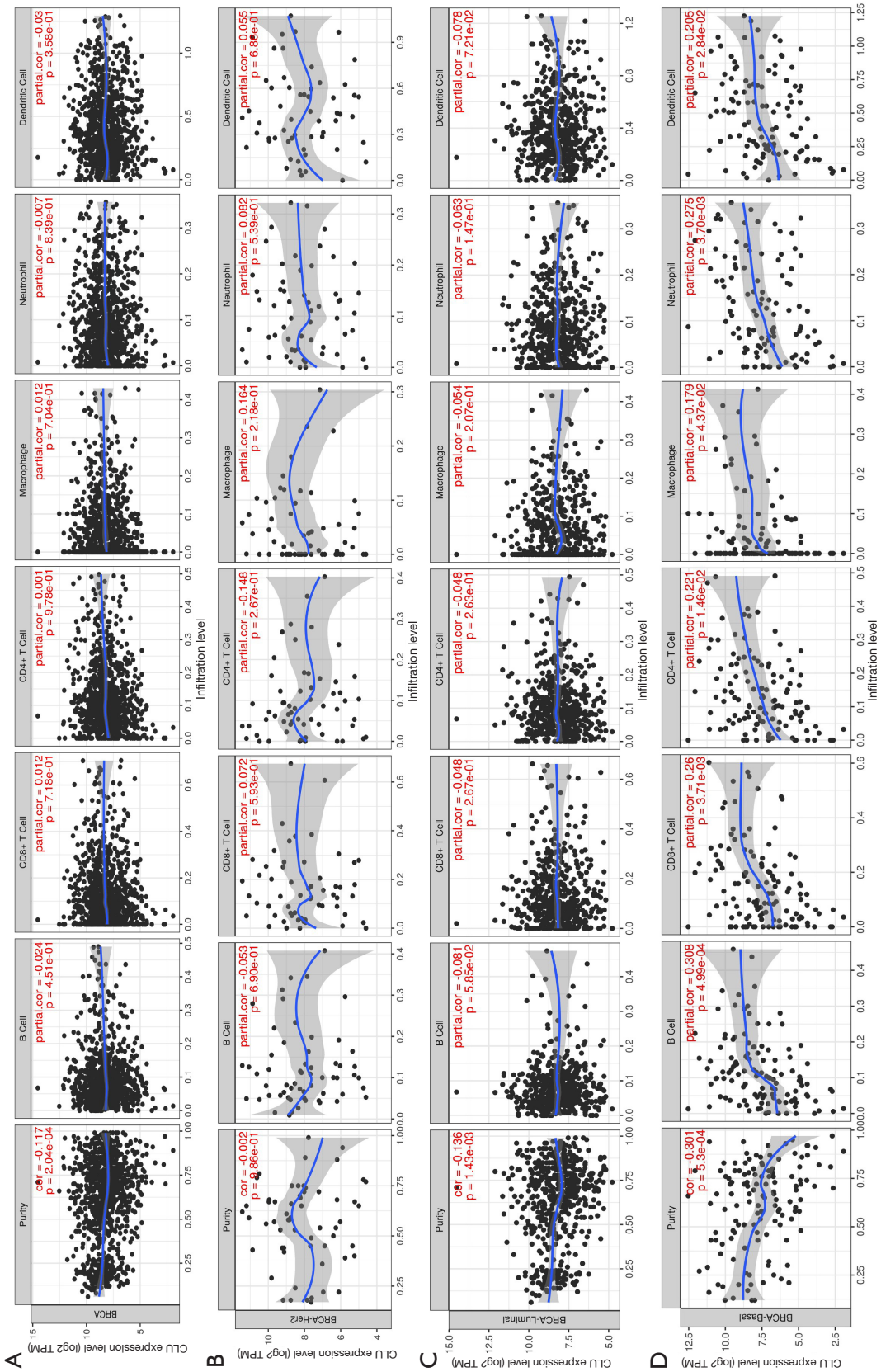

Figure 3 Clusterin expression correlates with the level of immune infiltration in BRCA and its subtypes. (A) Expression of clusterin correlates with the level of immune infiltration in BRCA. (B) Expression of clusterin correlates with the level of immune infiltration in BRCA HER2 subtype. (C) Expression of clusterin correlates with the level of immune infiltration in BRCA luminal subtype. (D) Expression of clusterin correlates with the level of immune infiltration in BRCA basal subtype. BRCA, breast invasive carcinoma; CLU, clusterin; TPM, transcripts per million; CD, cluster of differentiation; partial.cor, partial correlation (a correlation between two variables when the effects of one or more related variables are removed). HER2, human epidermal growth factor receptor 2.

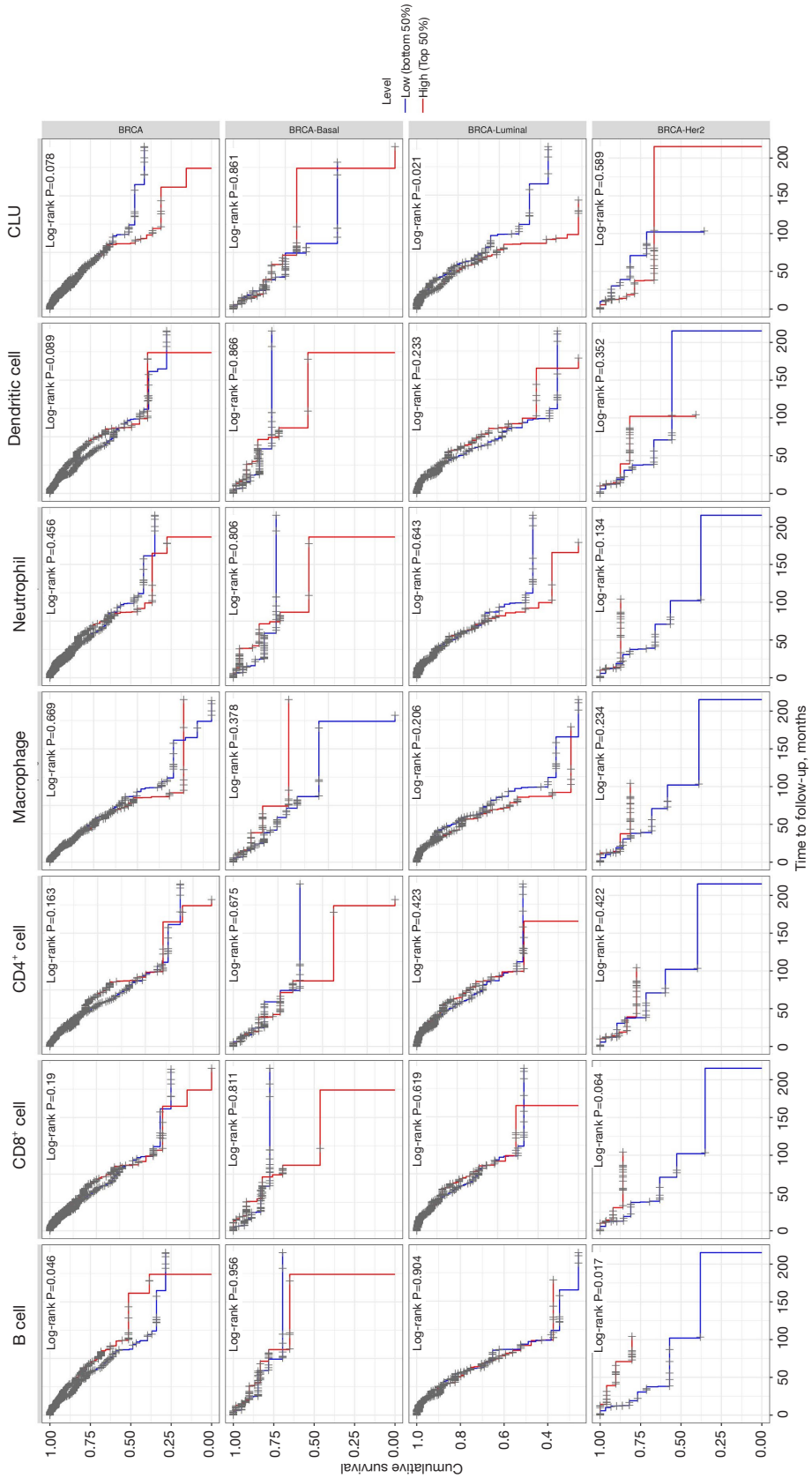

Figure 4 Kaplan-Meier plots showing immune infiltration and clusterin expression levels in BRCA and its subtypes. BRCA, breast invasive carcinoma; CLU, clusterin.

Table 2 Correlation analysis of immune-cell-related genes and markers between clusterin and BRCA

Description	Gene makers	BRCA			
		None		Purity	
		Cor	P	Cor	P
$CD8^+$ T cell	*CD8A*	0.025	0.416	–0.037	0.242
	CD8B	0	0.997	–0.054	0.090
T cell (general)	*CD3D*	0.013	0.655	–0.054	0.089
	CD3E	0.022	0.473	–0.047	0.137
	CD2	0	0.998	–0.068	0.033
B cell	*CD19*	0.024	0.421	–0.033	0.297
	CD79A	0.03	0.327	–0.034	0.289
Monocyte	*CD86*	–0.009	0.765	–0.056	0.080
	CD115 (CSF1R)	0.103	5.87E-04	0.063	0.047
TAM	*CCL2*	0.012	0.68	–0.033	0.298
	CD68	–0.026	0.387	–0.076	0.017
	IL10	–0.035	0.247	–0.081	0.010
M1 macrophage	*INOS (NOS2)*	–0.072	0.0163	–0.078	0.014
	IRF5	0.127	2.38E-05	0.105	9.49E-04
	COX2 (PTGS2)	0.038	0.0202	–0.003	0.932
M2 macrophage	*CD163*	–0.005	0.872	–0.047	0.141
	VSIG4	0.052	0.0864	0.011	0.741
	MS4A4A	0.021	0.494	–0.03	0.340
Neutrophils	*CD66b (CEACAM8)*	0.016	0.586	0.01	0.742
	CD11b (ITGAM)	0.047	0.118	0.018	0.562
	CCR7	0.033	0.286	–0.027	0.391
NK cells	*KIR2DL1*	–0.003	0.927	–0.031	0.322
	KIR2DL3	0.011	0.718	–0.018	0.561
	KIR2DL4	0.009	0.761	–0.022	0.482
	KIR3DL1	0.011	0.717	–0.02	0.525
	KIR3DL2	0.029	0.345	–0.024	0.459
	KIR3DL3	–0.035	0.24	–0.073	0.022
	KIR2DS4	0.008	0.797	–0.021	0.499
DC	*HLA-DPB1*	0.131	1.22E-05	0.091	3.95E-03
	HLA-DQB1	0.092	2.19E-03	0.065	4.02E-02
	HLA-DRA	0.06	4.54E-02	0.011	0.740
	HLA-DPA1	0.102	6.96E-04	0.061	5.48E-02
	BCDA-1 (CD1C)	0.096	1.41E-03	0.039	0.221
	BDCA-4 (NRP1)	0.079	8.52E-03	0.031	0.334
	CD11c (ITGAX)	0.009	0.759	–0.039	0.217

Table 2 (*continued*)

Table 2 (*continued*)

Description	Gene makers	BRCA			
		None		Purity	
		Cor	P	Cor	P
Th1 cell	*T-bet (TBX21)*	0.022	0.476	–0.042	0.181
	STAT4	0.045	0.134	–0.009	0.774
	STAT1	–0.035	0.246	–0.054	9.07E-02
	IFN-γ (IFNG)	–0.038	0.21	–0.091	3.97E-03
	TNF-α (TNF)	–0.026	0.38	–0.029	0.368
Th2 cell	*GATA3*	0.126	2.87E-05	0.153	1.21E-06
	STAT6	0.194	9.09E-11	0.181	8.58E-09
	STAT5A	0.217	3.33E-13	0.202	1.38E-10
	IL13	–0.028	0.355	–0.042	0.186
Tfh cell	*BCL6*	0.151	5.14E-07	0.147	3.11E-06
	IL21	–0.025	0.41	–0.049	0.119
Th17 cell	*STAT3*	0.12	6.81E-05	0.117	2.10E-04
	IL17A	0.048	0.109	0.019	0.557
Treg cell	*FOXP3*	–0.063	3.62E-02	–0.118	2.04E-04
	CCR8	–0.078	1.01E-02	–0.118	2.03E-04
	STAT5B	0.185	6.38E-10	0.168	1.04E-07
	TGFβ (TGFB1)	0.084	5.36E-03	0.041	0.202
T cell exhaustion	*PD-1 (PDCD1)*	0.023	0.447	–0.032	0.307
	CTLA4	–0.063	3.68E-02	–0.12	1.42E-04
	LAG3	–0.061	4.18E-02	–0.096	2.37E-03
	TIM-3 (HAVCR2)	0.003	0.92	–0.038	0.230
	GZMB	–0.032	0.287	–0.087	5.91E-03

Cor, R value of Spearman's correlation; None, correlation without adjustment; Purity, correlation adjusted by purity; BRCA, breast invasive carcinoma; CD, cluster of differentiation; TAM, tumor associated macrophage; CCL2, chemokine ligand 2; IL, interleukin; INOS, inducible nitric oxide synthase; IRF, interferon regulatory factor; COX2, cyclooxygenase 2; VSIG4, V-set and immunoglobulin domain-containing protein 4; CCR, chemokine receptor; KIR2DL1, killer cell immunoglobulin-like receptor 2DL1; NK cell, natural killer cell; DC, dendritic cell; HLA, human leukocyte antigen; STAT4, signal transducer and activator of transcription 4; IFN, interferon; FOXP3, forkhead box p3; TGF-β, transforming growth factor beta; CTLA4, cytotoxic T-lymphocyte-associated protein 4; LAG3, lymphocyte activation gene-3; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3.

CLU in the immune interaction between BRCA and such tumors, making CLU a promising biomarker for further investigation. We evaluated the expression of CLU using an independent GEPIA database because it is related to the prognosis of many different types of cancer. In these cancers, the expression of CLU in tumor tissues was significantly different from that in normal tissues. TCGA data set analysis indicated that there was elevated CLU expression in KIRC, KIRP and THCA, whereas expression was decreased in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, LUAD, KICH, LIHC, LUSC, READ, PRAD, STAD and UCEC relative to adjacent control tissues. In a series of different cancers, depending on what method was used in the study, or what mechanism was involved, CLU expression may have changed. In these databases, we consistently observed that decreased CLU expression was

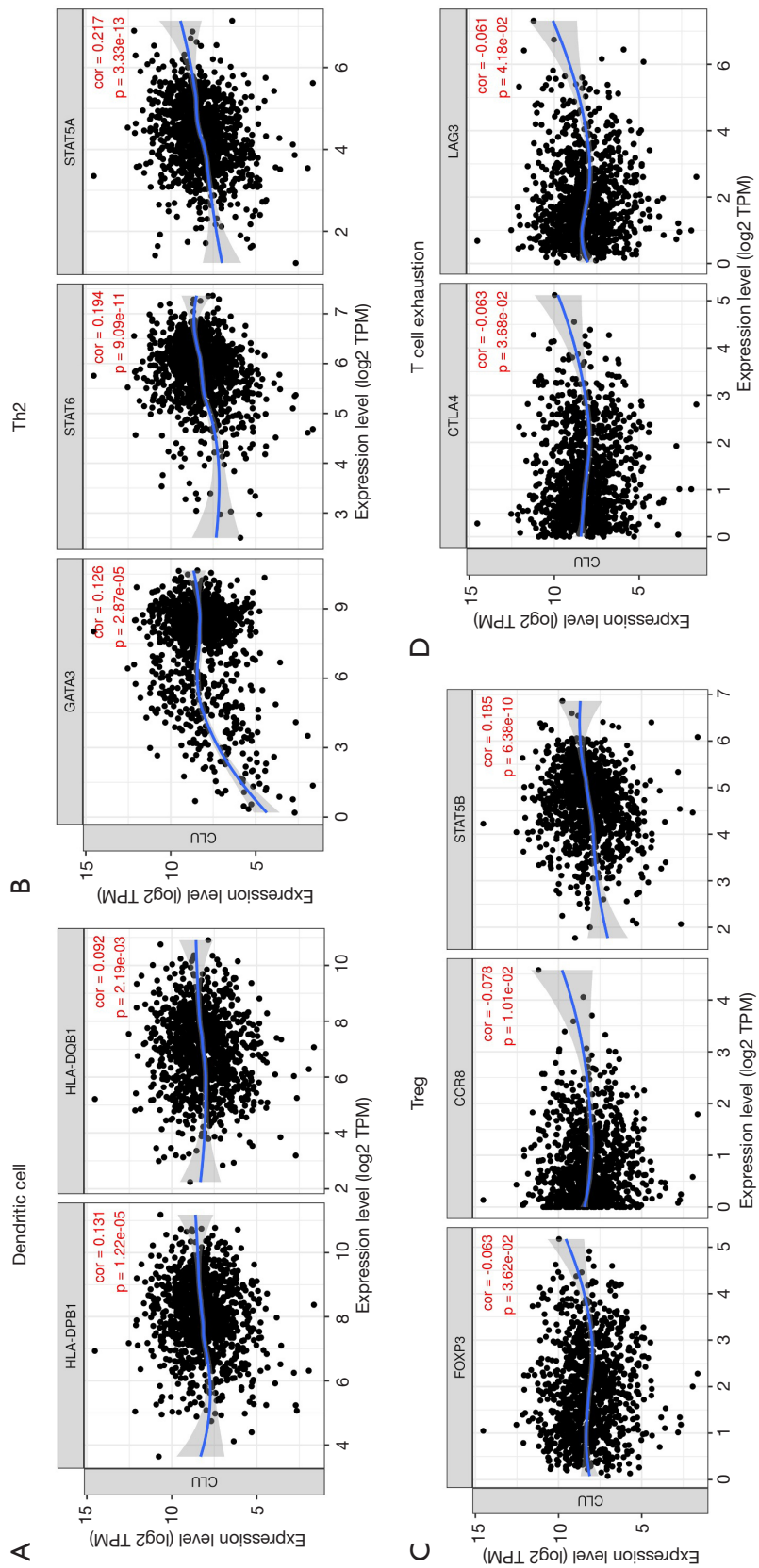

Figure 5 Correlation analysis between clusterin expression and immunological markers in BRCA. (A-D) Scatterplots of correlations between clusterin expression and gene markers of DCs (A), Th2 cells (B), Treg cells (C), and T cell exhaustion (D) in BRCA. BRCA, breast invasive carcinoma; CLU, clusterin; HLA, human leukocyte antigen; TPM, transcripts per million; GATA3, GATA binding protein 3; STAT, signal transducer and activator of transcription; FOXP3, forkhead box p3; CCR, chemokine receptor; CTLA4, cytotoxic T-lymphocyte-associated protein 4; LAG3, lymphocyte activation gene 3; Treg, T regulatory cells; Cor, R value of Spearman's correlation; DCs, dendritic cells.

Table 3 Correlation analysis of CLU with genes and markers related to DCs, Th2 cells, Treg cells and T cell failure in GEPIA

Description	Gene makers	BRCA			
		Tumor		Normal	
		R	P	R	P
DCs	*HLA-DPB1*	0.130	1.20E-05	0.460	4.10E-07
	HLA-DQB1	0.060	0.049	0.290	2.20E-03
	HLA-DRA	0.064	0.036	0.270	3.80E-03
	HLA-DPA1	0.110	3.70E-04	0.220	0.021
	BCDA-1 (CD1C)	0.086	4.50E-03	0.220	0.019
	BDCA-4 (NRP1)	0.130	1.40E-05	0.120	0.200
	CD11c (ITGAX)	0.025	0.410	0.310	0.001
Th2 cells	*GATA3*	0.150	3.20E-07	0.062	0.520
	STAT6	0.210	3.70E-12	0.170	0.070
	STAT5A	0.230	5.20E-15	–0.074	0.440
	IL13	0.018	0.560	9.10E-03	0.920
Treg cells	*FOXP3*	–0.069	0.024	0.220	0.020
	CCR8	–0.047	0.120	0.120	0.220
	STAT5B	0.220	2.00E-13	–0.058	0.540
	TGFβ (TGFB1)	0.095	1.70E-03	0.460	3.30E-07
T cell exhaustion	*PD-1 (PDCD1)*	0.011	0.710	0.290	1.80E-03
	CTLA4	–0.059	0.054	–0.065	0.490
	LAG3	–0.082	7.10E-03	0.150	0.130
	TIM-3 (HAVCR2)	0.028	0.360	0.350	1.40E-04
	GZMB	–0.048	0.120	0.340	2.30E-04

CLU, clusterin; GEPIA, Gene Expression Profiling Interactive Analysis; BRCA, breast cancer; IL, interleukin; CCR, chemokine receptor; DCs, dendritic cells; HLA, human leukocyte antigen; CD, cluster of differentiation; STAT, signal transducer and activator of transcription; FOXP3, forkhead box p3; CTLA4, cytotoxic T-lymphocyte-associated protein 4; LAG3, lymphocyte activation gene-3; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3.

associated with poor prognosis of BRCA. In the TCGA database, elevated CLU levels were associated with poor prognosis in LGG patients, while LIHC results were the opposite. Similarly, the Kaplan-Meier database found that the decrease in CLU was associated with poor prognosis of breast and lung cancers. Decreased expression of CLU was associated with poorer prognosis, as well as ER status (array), HER2 status (array), subtype, and lymph node status. These results suggest that CLU may be a valuable biomarker for the prognosis of BRCA.

The expression of CLU is also correlated with immune infiltration in many cancers, including BRCA. We found that expression of CLU was weakly positively correlated with infiltration of B cells, $CD8^+$ T cells, $CD4^+$ T cells, DCs and neutrophils in BRCA. We further found that B cell infiltration was significantly correlated with the prognosis of BRCA. The correlation between CLU and expression of some immune marker genes strongly suggests that CLU can control the infiltration and interaction of immune cells in the tumor microenvironment in BRCA. We observed a weak correlation between CLU and Th2 marker STAT5A. This suggests that CLU regulates humoral immunity. We further found that CLU levels in BRCA were associated with markers of Treg cells and T cell failure (CTLA4 and LAG3). It is suggested that CLU can promote Treg cell response and inhibit T-cell immunity. We found that

expression of CLU is linked to expression of multiple T cell markers (Th2, Tfh and Th17) in BRCA. This may reflect that CLU is involved in regulating T cell response in BRCA, and suggests that CLU plays a role in regulating the recruitment and activation of immune cells in BRCA.

In conclusion, CLU may play an important regulatory role in tumor immune cell infiltration, and is also a valuable prognostic biomarker for patients with breast cancer.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1882/rc

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1882/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1882/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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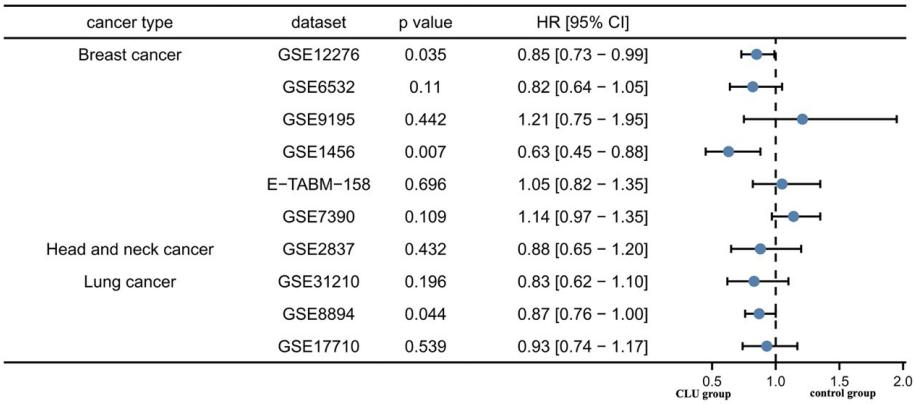

Figure S1 Relationship between CLU expression and prognosis (relapse-free survival) in patients with different tumors in PrognoScan database. HR, hazard ratio; CI, confidence interval; CLU, clusterin.

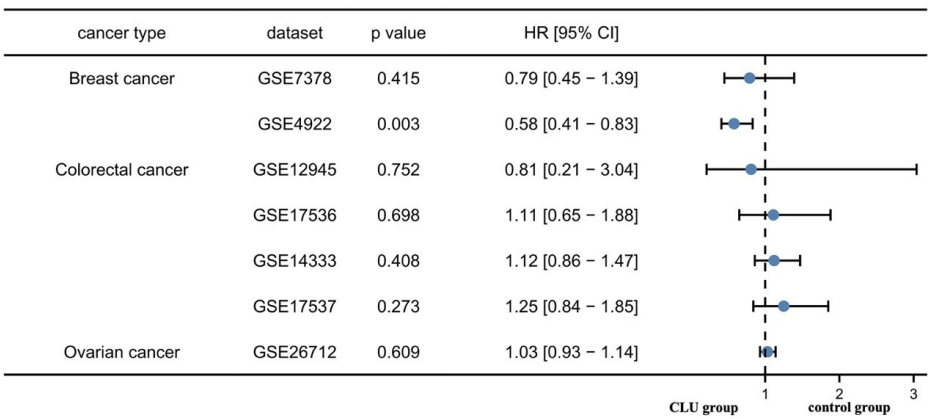

Figure S2 Relationship between CLU expression and prognosis (disease-specific survival) in patients with different tumors in PrognoScan database. HR, hazard ratio; CI, confidence interval; CLU, clusterin.

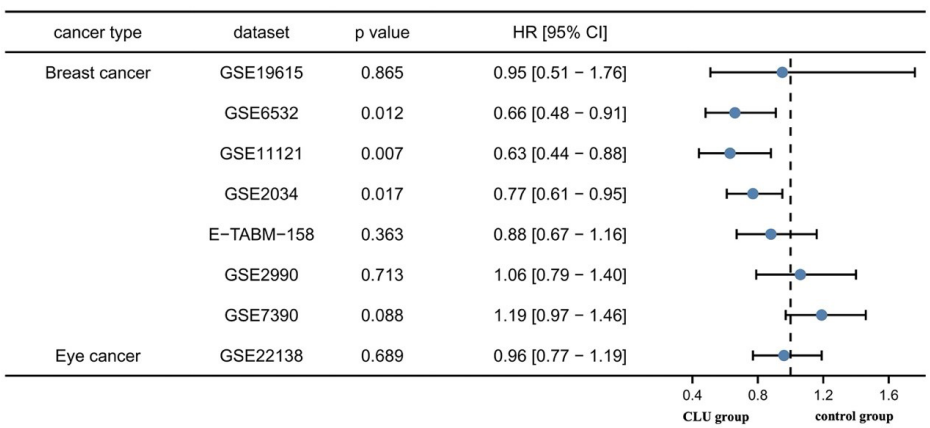

Figure S3 Relationship between CLU expression and prognosis (distant metastasis free survival) in patients with different tumors in PrognoScan database. HR, hazard ratio; CI, confidence interval; CLU, clusterin.

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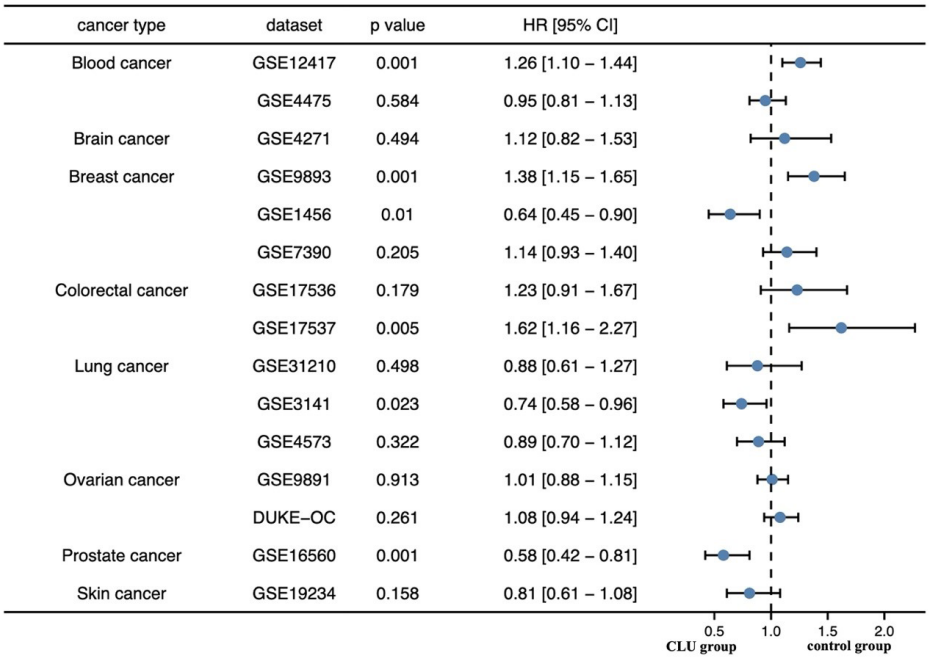

Figure S4 Relationship between CLU expression and prognosis (overall survival) in patients with different tumors in PrognoScan database. HR, hazard ratio; CI, confidence interval; CLU, clusterin.

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