Progesterone receptor gene serves as a prognostic biomarker associated with immune infiltration in gastric cancer: a bioinformatics analysis

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Background: Gastric cancer (GC) is one of the most prevalent cancers globally with a poor prognosis. The progesterone receptor gene (PGR), which encodes the progesterone receptor (PR), can modulate the immune response in many cancers, but its correlation with prognosis and immune infiltration in GC remains unclear. We aimed to investigate the relationship between PGR expression and prognosis in GC patients.

Methods: The expression profile was obtained and the relationship between PGR expression and cancer prognosis was analyzed by Oncomine, Tumor Immune Estimation Resource (TIMER), Kaplan-Meier plotter, the PrognScan database and Gene Expression Profiling Interactive Analysis (GEPIA) database using the minimum P value approach and log-rank method for prognosis analysis. Next, the correlation between the expression level of PGR and immune cell infiltration by the Spearman method using TIMER and GEPIA.

Results: A correlation of elevated PGR expression with poorer prognosis in GC was observed, with overall survival (OS) hazard ratio (HR) was 1.74 [95% confidence interval (CI): 1.4–2.17, P value =6.2e-7] and progression-free survival (PFS) HR was 2.09 (95% CI: 1.58–2.78, P value =1.6e-7). Also, high expression of PGR was related to worse OS and PFS in patients of each N stage in GC, with the highest OS and PFS HR values in stage N1. PGR expression in stomach adenocarcinoma (STAD) was significantly correlated with levels of B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils and dendritic cells. It was also observed that PGR expression was related to a variety of immune cell markers.

Conclusions: PGR expression correlated with prognosis and immune cell infiltration in GC, indicating PGR is a potential prognostic biomarker in GC patients.

Keywords: Bioinformatics; gastric cancer (GC); immune infiltration; progesterone receptor gene; prognosis

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Introduction

Gastric cancer (GC) remains one of the most common malignancies worldwide with a poor prognosis, posing a

risk to human health (1). The pathogenesis and mechanism of GC are complex and immunological mechanisms play a role in regulating GC development (2,3). By targeting

the host immune system, immunotherapy has shown great promise in several solid cancers and has evolved into a future therapeutic option in GC (4). Immunotherapies, including several immune checkpoints such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death-1 (PD-1) and programmed death ligand-1 (PD-L1), have proven efficacious in several solid cancers (4,5). However, unlike other solid tumors, clinical responses to immunotherapy in GC still needs to be evaluated because immunotherapy has shown poor clinical efficacy in some clinical trials (4,6,7). The tumor microenvironment has significant influences on the therapeutic response, clinical outcome and immune cells within the microenvironment (8). Several studies have shown that tumor-associated macrophages (TAMs), regulatory T-lymphocytes (Tregs) and other tumor-infiltrating lymphocytes are associated with the development and outcome of cancers (9-11). To date, however, there has been little discussion about the regulation of immune cells and immunotherapy targets of GC, which still needs to be clarified.

Biomarkers contribute greatly to the diagnosis and management of cancer and related follow-up programs. The conventional biomarkers of GC are widely used in clinical practice, and mainly include CEA, CA19-9, CA72-4, CA125, etc. (12). However, because of their insufficient specificity and sensitivity, more novel biomarkers need to be investigated. So far, PI3K/Akt/mTOR (13), long noncoding RNAs (14), PD-L1 (15) and other biomarkers are related to the prognosis in GC patients.

The progesterone receptor gene (PGR), which is located at 11q22-q23, is a member of the steroid receptor superfamily of nuclear receptors (16). It encodes the human progesterone receptor (PR), which is involved in establishing and maintaining a pregnancy (17). Two main PR isoforms, PR-A and PR-B, have different expressions and functions (18). Progesterone, which can promote the downregulation of proinflammatory cytokines and chemokines, must bind to the PR to exert its effect (19). Mutations and polymorphisms of PGR are related to tumorigenesis and risks for cancers, including ovarian, breast and endometrial cancers (16,20,21). However, the relationship between PGR expression and the GC prognosis remains unclear. So far, PGR regulation in GC patients remains controversial, according to one study, PR may play a role in the progression of GC (22) but others have reported that PR was not significantly related to GC prognosis (23). PR can modulate the immune response by

altering Th1 and Treg activity (24). Also, studies have found that the PR is related to infiltration of a subpopulation of Tregs, which indicated a poorer prognosis for breast cancer (25,26). A higher percentage of CD25+/FOXP3+/CD127 T cells within CD3+/CD4+ T cells has been observed in PR (-) breast cancer patients (19), and Treg cell infiltration was more prominent in breast cancer patients with estrogen receptor (ER) (+) and PR (-) tumors (26). Until now, few studies have examined the association between PGR and immune cell infiltration in GC.

The purpose of this study was to analyze the relationship between PGR expression and prognosis of cancer patients using databases, including Oncomine, PrognScan, and Kaplan-Meier plotter. Also, the correlation of PGR and immune cell infiltration of tumors was investigated by the Tumor Immune Estimation Resource (TIMER). The findings of this study bring new insights into the potential prognostic role of PGR in GC and the possible mechanisms of PGR and immune cell interaction, which may help improve the early diagnosis and prognosis of GC in clinical practice.

We present the following article in accordance with the MDAR checklist (available at <https://dx.doi.org/10.21037/tcr-21-218>).

Methods

Data collection from The Cancer Genome Atlas (TCGA)

The gene expression profiles of GC patients, including clinical data including sex, tumor stage, TNM classification, and survival status, were downloaded from the TCGA portal (v22.0, <https://portal.gdc.cancer.gov/>) with project ID: TCGA-GC.

PGR differential expression

Oncomine (<http://www.oncomine.org>) is an online database that contains 715 datasets and 86,733 samples to support various analyses for cancer research (27). To evaluate the relationship between PGR expression levels and cancer patient prognosis in different types of cancers, we used the Oncomine database, with the threshold of P value of 0.001, fold change of 1.5, and gene ranking of all.

Survival analyses of PGR

PrognScan (<http://www.prognoscan.org/>) is a large

public dataset of gene expression and clinical information, providing a novel platform for evaluating potential tumor markers and therapeutic targets (28). The relationship between PGR expression and patient survival in various types of cancers was analyzed through a minimum P value approach. Kaplan-Meier plotter (<http://kmplot.com/analysis/>) is an online platform that can be used to evaluate the effect of 54,675 genes on survival based on 10,461 cancer samples, including 5,143 breast, 1,816 ovarian, 2,437 lung, and 1,065 gastric cancers (29). In this study, the associations between survival and PGR expression in patients with breast, ovarian, lung and gastric cancers were analyzed using the Kaplan-Meier plotter database by the log-rank method. To investigate whether PGR is an independent prognostic biomarker in GC, Cox analysis was performed. All clinical variables were obtained for a multivariate Cox analysis, aiming to evaluate whether each of the variables had an independent prognostic value.

Correlation between PGR and infiltrating immune cells

TIMER (<http://cistrome.org/TIMER/>) is an analytical web tool that includes 10,897 samples of 32 cancer types from TCGA, supporting the comprehensive analysis of tumor-infiltrating immune cells (30). We used it to analyze the relationship between PGR expression levels with abundant immune infiltrates, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells (DCs). Correlations between PGR expression and gene markers of tumor-infiltrating immune cells reported in previous studies (31,32) were investigated using this database. The Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>), which incorporates 9,736 tumor samples and 8,587 normal controls from TCGA and the Genotype Tissue Expression (GTEx), is an online tool that is capable of integrated analysis of cancer expression profiling data (33). GEPIA was used to assess the relationship between PGR expression and patient survival and immune cell infiltration of tumors by the Spearman method in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

Survival curves were calculated by PrognScan and KaplanMeier plots, containing results with a hazard ratio (HR) and P or Cox P values from a log-rank test. P values,

fold changes, and ranks are shown in the results from the Oncomine database. We used Spearman correlations to examine correlations between variables, with the strength of a correlation determined by the following r values: 0.00–0.19 “very weak”, 0.20–0.39 “weak”, 0.40–0.59 “moderate”, 0.60–0.79 “strong” and 0.80–1.0 “very strong”. Two-sided $P < 0.05$ was considered significant.

Results

Expression levels of PGR in different cancers

To investigate the differences PGR expression in tumor and normal tissues in multiple cancers, we first used the Oncomine database for analysis. Compared with normal tissues, the expression levels of PGR showed difference in bladder, breast, cervical, colorectal, gastric, head and neck, lung, ovarian and prostate cancers (Figure S1A). Table S1 shows the detailed information on the PGR expression in various types of cancers. We performed analysis using the TIMER database further to evaluate the different PGR expressions in different tumor tissues. The expression levels of PGR in tumor tissues of BLCA (urothelial bladder carcinoma), BRCA (invasive breast carcinoma), COAD (colon adenocarcinoma), HNSC (head and neck cancer), KIRC (kidney renal clear cell carcinoma), KIRP (kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), PRAD (prostate adenocarcinoma), READ (rectum adenocarcinoma), STAD (stomach adenocarcinoma), UCEC (uterine corpus endometrial carcinoma), KICH (kidney chromophobe) and THCA (thyroid carcinoma) showed significant difference compared with adjacent normal tissues (Figure S1B).

PGR expression and prognosis of multiple cancers

Because PGR expression was significantly different between cancer and normal tissues in multiple cancers, we then evaluated whether the PGR expression levels can predict the prognosis of cancer patients. We analyzed their correlations using the PrognScan database (Table S2). The results demonstrated a significant statistical correlation between expression of PGR and the prognosis of various types of cancer (Figure S2). We then used the GEPIA database further to analyze the prognostic potential of PGR in different types of cancer. The results showed that high PGR expression correlated with poorer prognosis of ACC

(adrenocortical carcinoma), MESO (mesothelioma) and STAD, but was linked to better prognosis of BRCA, KIRC, LGG (brain lower grade glioma), LIHC, SARC (sarcoma) and UCEC (*Figure 1*). We then performed a survival analysis using the Kaplan-Meier plotter database to evaluate further the association between expression of PGR and patients' prognosis in different cancers (*Figure 2*). Higher PGR expression's correlation with poorer prognosis in GC was observed, with overall survival (OS) HR of 1.74 [95% confidence interval (CI), 1.4–2.17, P value =6.2e-7] and progression-free survival (PFS) HR of 2.09 (95% CI, 1.58–2.78, P value =1.6e-7). However, PGR expression was found to be related with better prognosis in lung cancer (OS HR =0.56, 95% CI, 0.47–0.66, P=1.3e-11; PFS HR =0.53, 95% CI, 0.34–0.82, P=0.0036), breast cancer (OS HR =0.6, 95% CI, 0.44–0.83, P=0.0015; relapse-free survival HR =0.53, 95% CI, 0.45–0.62, P=1.6e-15) and ovarian cancer (OS HR =0.75, 95% CI, 0.61–0.91, P=0.0046; PFS HR =0.67, 95% CI, 0.56–0.81, P=3.4e-5). These results suggested that PGR expression was correlated with prognosis in multiple cancers.

Relationship of elevated PGR expression levels and GC patients' prognosis of different clinicopathological factors

To further explain the relationship between PGR expression and GC, we analyzed PGR expression levels and different clinicopathological factors in GC by the Kaplan-Meier plotter database. In both female and male patients, elevated PGR expression levels had a statistically significant relationship with worse OS and PFS (P<0.05) (*Table 1*). Also, except for stage 1, stage 2, stage M1 and poorly differentiated cancer, high expression of PGR was associated with worse OS and PFS in patients with different clinicopathological factors, such as stage, stage N, stage M, Lauren classification and differentiation (*Table 1*). Notably, PGR expression was related to each N stage in GC and had the highest OS and PFS HR values in stage N1 (OS HR =9.66, 95% CI, 1.29–72.12, P=0.0068; PFS HR =9.89, 95% CI, 1.33–73.78, P=0.006). Because stage N represents the different lymph node metastasis status, which is a strong risk factor for GC (34,35), these results indicated that PGR expression might be involved in GC patients with lymph node metastasis.

To investigate whether PGR is an independent prognostic factor of GC, a Cox proportional-hazards model was constructed. The Kaplan-Meier plotter database was used to perform the multivariate Cox analyses, which can

confirm the significant value of PGR in the prognosis of GC (*Table S3*). The results showed the critical value of stage T, stage N, Lauren classification and PGR as independent prognosis factors in OS (*Table S3*). The Cox analysis based on PFS showed that stage N, Lauren classification and PGR were risk factors. Taking together, PGR is a potential independent risk factor in GC.

Correlation of the expression of PGR in gastric cancer and immune infiltration

Tumor-infiltrating lymphocytes are an attempt by the host to eradicate malignancies, and be an independent predictor of survival in multiple cancer (36,37). Hence, we used the TIMER database to investigate the correlation between expression of PGR and immune cell infiltration in 39 types of cancer. We found that PGR expression had a significant relationship with tumor purity in 26 types of cancer, with B cells in 20 cancers, CD8+ T cells in 24 cancers, CD4+ T cells in 26 cancers, macrophages in 30 cancers, neutrophils in 23 cancers, and DCs in 23 cancers (*Figure S3*). Compared with PGR expression in DLBC (diffuse large B-cell lymphoma) which had no significant relationship with B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils or DCs infiltration, PGR expression in STAD showed significant correlations with levels of B cells (r=0.225, P=1.26e-5), CD8+T cells (r=0.248, P=1.41e-6), CD4+T cells (r=0.524, P=3.27e-27), macrophages (r=0.652, P=3.30e-36), neutrophils (r=0.25, P=1.13e-6) and dendritic cells (r=0.421, P=2.19e-17) (*Figure 3A,B*). Also, Kaplan-Meier plots using the TIMER database were constructed to investigate further whether PGR expression affected immune infiltration in STAD and DLBC. It was observed that macrophage infiltration (P=0.004) and PGR expression (P=0.045) had significantly correlations with STAD prognosis, but no such significant relations were found in DLBC (P>0.05) (*Figure 3C,D*). Thus, these findings indicated that PGR expression was strongly related to immune infiltration in GC, especially in terms of macrophages.

Correlation between PGR expression and immune markers

To investigate the relationship between the expression of PGR and multiple immune markers in STAD, their correlations were analyzed by the TIMER and GEPIA databases. Immune markers of different immune cells, including CD8+ T cells, T cells (general), B cells,

Figure 1 Correlation of PGR expression with prognosis in multiple types of cancer. Comparison of overall survival and disease-free survival with expression of PGR in ACC (A), BRCA (B), KIRC (C), LGG (D), LHC (E), MESO (F), SARC (G), STAD (H) and UCEC (I). PGR, progesterone receptor gene; ACC, adrenocortical carcinoma; BRCA, breast invasive carcinoma; KIRC, kidney renal clear cell carcinoma; LGG, brain lower grade glioma; LHC, liver hepatocellular carcinoma; MESO, mesothelioma; SARC, sarcoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

Figure 2 Survival curves of PGR expression in different types of cancer in the Kaplan-Meier survival curves of gastric cancer. (C,D) OS and RFS survival curves of breast cancer. (E,F) OS and PFS survival curves of lung cancer. (G,H) OS and PFS survival curves of ovarian cancer. PGR, progesterone receptor gene; OS, overall survival; RFS, relapse-free survival; PFS, progression-free survival.

Table 1 Relationship between the expression of PGR gene with clinical prognosis of different clinicopathological characteristics by Kaplan-Meier plotter

Clinicopathological characteristics	Overall survival (n=881)			Progression-free survival (n=645)		
	N	Hazard ratio	P value	N	Hazard ratio	P value
Gender						
Female	236	2.47 (1.53–4)	0.0001	201	3.02 (1.56–5.8)	0.0006
Male	544	1.79 (1.31–2.45)	0.0002	437	1.8 (1.26–2.55)	0.0009
Stage						
1	67	1.87 (0.56–6.2)	0.301	60	1.72 (0.52–5.74)	0.3703
2	140	2.3 (0.9–5.89)	0.0747	131	1.6 (0.79–3.26)	0.1914
3	305	1.71 (1.11–2.64)	0.0147	186	1.79 (1.14–2.79)	0.0098
4	148	2.02 (1.31–3.13)	0.0013	141	1.74 (1.07–2.83)	0.0247
Stage T						
2	241	1.79 (1.17–2.74)	0.0062	239	1.64 (1.02–2.63)	0.0392
3	204	1.58 (1.12–2.23)	0.0085	204	1.51 (1.08–2.11)	0.0144
4	38	2.76 (1.15–6.67)	0.0189	39	2.19 (0.98–4.89)	0.0517
Stage N						
0	74	9.66 (1.29–72.12)	0.0068	72	9.89 (1.33–73.78)	0.006
1	225	2.16 (1.43–3.27)	0.0002	222	2.55 (1.45–4.49)	0.0007
2	121	1.57 (0.97–2.52)	0.0622	125	1.49 (0.94–2.35)	0.0463
3	76	2.26 (1.22–4.18)	0.0079	76	2.05 (1.11–3.8)	0.0194
1+2+3	422	1.77 (1.36–2.31)	1.7e-5	423	1.59 (1.23–2.05)	0.0003
Stage M						
0	444	1.97 (1.4–2.78)	8.3e-5	443	1.89 (1.36–2.64)	0.0001
1	56	1.87 (0.93–3.73)	0.073	56	1.64 (0.79–3.38)	0.1799
Lauren classification						
Intestinal	320	2.08 (1.38–3.14)	0.0003	263	1.99 (1.33–2.97)	0.0006
Diffuse	241	2 (1.41–2.85)	8.3e-5	231	2.05 (1.44–2.92)	4.5e-5
Mixed	32	2.98 (0.96–9.2)	0.0468	28	1.89 (0.69–5.16)	0.2076
Differentiation						
Poorly differentiated	165	1.25 (0.77–2.03)	0.3627	121	1.28 (0.81–2.03)	0.288
Moderately differentiated	67	2.32 (0.9–5.97)	0.0737	67	2.67 (1.04–6.82)	0.0332

PGR, progesterone receptor gene.

monocytes, TAMs, M1 and M2 macrophages, neutrophils, natural killer (NK) cells, DCs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, Tregs and exhausted T cells, were chosen to be analyzed in STAD, with DLBC as the control. Adjusted by tumor purity, the results showed that PGR

expression had a significant relationship with most of the markers of CD8+ T cells, T cells (general), B cells, monocytes, TAMs, M1 macrophages, M2 macrophages, neutrophils, NK cells, DCs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, Tregs and exhausted T cells in STAD (*Table 2*).

Figure 3 PGR expression's correlation with immune cell infiltration in STAD and DLBC. (A) PGR expression was significantly correlated with levels of B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils and dendritic cells in STAD. (B) PGR expression had no significant correlations with immune cells infiltration in DLBC. (C) Kaplan-Meier plots of expression levels of PGR and immune cells in STAD. (D) Kaplan-Meier plots of expression levels of PGR and immune cells in DLBC. PGR, progesterone receptor gene; STAD, stomach adenocarcinoma; DLBC, diffuse large B-cell lymphoma

Table 2 Correlation analysis between PGR and relate genes and markers of immune cells in TIMER

Description	Gene markers	STAD				DLBC			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
CD8+ T cell	CD8A	0.308	***	0.287	***	0.206	0.16	0.126	0.431
	CD8B	0.172	***	0.153	***	0.137	0.3533	0.099	0.539
T cell (general)	CD3D	0.245	***	0.217	***	0.029	0.845	−0.049	0.762
	CD3E	0.295	***	0.272	***	0.136	0.356	0.036	0.821
	CD2	0.32	***	0.301	***	0.102	0.491	0.02	0.9
B cell	CD19	0.377	***	0.36	***	−0.05	0.733	0.076	0.637
	CD79A	0.386	***	0.365	***	0.065	0.661	0.045	0.78
Monocyte	CD86	0.337	***	0.324	***	0.169	0.252	0.278	0.078
	CD115 (CSF1R)	0.443	***	0.475	***	0.431	*	0.308	0.05
TAM	CCL2	0.438	***	0.428	***	0.273	0.06	0.282	0.0741
	CD68	0.195	***	0.189	**	0.194	0.187	0.083	0.605
	IL10	0.385	***	0.376	***	0.343	0.017	0.223	0.161
M1 Macrophage	INOS (NOS2)	−0.037	0.456	−0.042	0.415	0.238	0.104	0.114	0.477
	IRF5	0.254	***	0.244	***	0.394	*	0.331	*
	COX2 (PTGS2)	0.238	***	0.231	***	0.377	*	0.268	0.089
M2 Macrophage	CD163	0.388	***	0.382	***	0.121	0.413	0.152	0.343
	VSIG4	0.378	***	0.384	***	0.05	0.738	0.079	0.625
	MS4A4A	0.468	***	0.466	***	0.255	0.0789	0.295	0.0611
Neutrophils	CD66b (CEACAM8)	0.124	0.0112	0.127	0.013	0.105	0.476	0.07	0.663
	CD11b (ITGAM)	0.438	***	0.433	***	0.179	0.222	0.045	0.778
	CCR7	0.456	***	0.439	***	0.211	0.151	0.105	0.514
Natural killer cell	KIR2DL1	0.174	**	0.153	**	0.181	0.217	0.034	0.832
	KIR2DL3	0.118	0.0157	0.078	0.13	0.352	0.0142	0.343	0.0279
	KIR2DL4	−0.042	0.398	−0.07	0.171	0.403	*	0.292	0.0637
	KIR3DL1	0.137	*	0.109	0.0338	0.173	0.238	0.167	0.298
	KIR3DL2	0.17	**	0.138	*	0.227	0.12	0.108	0.501
	KIR3DL3	−0.02	0.692	−0.025	0.621	0.064	0.664	0.037	0.819
	KIR2DS4	0.043	0.38	0.006	0.9	0.23	0.116	0.1	0.532
Dendritic cell	HLA-DPB1	0.298	***	0.278	***	0.188	0.201	−0.139	0.387
	HLA-DQB1	0.138	**	0.11	0.0324	0.168	0.254	−0.079	0.622
	HLA-DRA	0.205	***	0.185	**	0.119	0.421	0.075	0.640
	HLA-DPA1	0.242	***	0.222	***	0.094	0.526	0.089	0.581
	BDCA-1 (CD1C)	0.555	***	0.563	***	0.202	0.168	0.175	0.273
	BDCA-4 (NRP1)	0.599	***	0.592	***	0.515	**	−0.222	0.157
	CD11c (ITGAX)	0.388	***	0.368	***	0.278	0.055	0.102	0.524

Table 2 (continued)

Table 2 (continued)

Description	Gene markers	STAD				DLBC			
		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P
Th1	T-bet (TBX21)	0.287	***	0.261	***	0.279	0.0545	0.268	0.0869
	STAT4	0.396	***	0.382	***	0.353	0.014	0.32	0.0415
	STAT1	0.055	0.26	0.047	0.361	0.19	0.196	0.142	0.377
	IFN- γ (IFNG)	-0.043	0.38	-0.066	0.198	0.175	0.235	0.133	0.406
	TNF- α (TNF)	0.074	0.133	0.042	0.42	0.311	0.0315	0.179	0.262
Th2	GATA3	0.403	***	0.394	***	0.202	0.169	0.158	0.324
	STAT6	0.251	***	0.261	***	0.593	***	0.554	**
	STAT5A	0.423	***	0.414	***	0.016	0.915	-0.18	0.26
	IL13	0.15	*	0.151	*	0.104	0.438	0.262	0.0978
Tfh	BCL6	0.537	***	0.528	***	0.106	0.472	0.258	0.104
	IL21	0.102	0.0369	0.079	0.126	0.094	0.527	0.088	0.586
Th17	STAT3	0.429	***	0.421	***	0.477	**	0.352	0.0239
	IL17A	-0.197	***	-0.212	***	0.028	0.85	-0.048	0.767
Treg	FOXP3	0.243	***	0.214	***	0.194	0.187	0.125	0.435
	CCR8	0.398	***	0.389	***	0.225	0.124	0.22	0.166
	STAT5B	0.604	***	0.589	***	0.341	0.176	0.224	0.159
	TGF β (TGFB1)	0.496	***	0.482	***	0.418	*	0.286	0.0694
T cell exhaustion	PD-1 (PDCD1)	0.149	*	0.116	0.0235	0.149	0.312	0.097	0.546
	CTLA4	0.12	0.0147	0.091	0.0769	0.136	0.357	0.132	0.412
	LAG3	0.094	0.0558	0.061	0.236	0.102	0.491	0.098	0.542
	TIM-3 (HAVCR2)	0.32	***	0.31	***	0.232	0.112	0.234	0.141
	GZMB	0.041	0.401	-0.086	0.0935	0.031	0.835	0.024	0.881

*P<0.01; **P<0.001; ***P<0.0001. PGR, progesterone receptor gene; STAD, stomach adenocarcinoma; DLBC, diffuse large B-cell lymphoma; TAM, tumor-associated macrophage; Th, T helper cell; Tfh, follicular helper T cell; Treg, regulatory T cell; Cor, r value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity.

However, PGR expression was only found to be related to three markers of M1 macrophages, NK cells and Th17 cells in DLBC.

Among these markers, PGR expression was strongly related to most of the markers of TAMs, M1 macrophages, and M2 macrophages in STAD, especially CD86, CD115, CCL2, CD68, IL10, IRF5, COX2, CD163, VSIG4 and MS4A4A (Figure S4A,B,C,D,E,F,G,H). We further analyzed PGR expression with these markers in STAD via the GEPIA database and similar results were found (Table 3). Also, elevated PGR expression was related to most markers of DCs and Tregs in STAD, including HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, BDCA-1, BDCA-4,

CD11c, FOXP3, CCR8, STAT5B, and TGF β (Tables 2,3). Therefore, our findings suggested that PGR expression may affect the immune mechanism and microenvironment of GC.

Discussion

PGR has been well recognized as the gene that encodes the PR to mediate the actions of the progesterone in preparation and completion of pregnancy (38). Emerging evidences has shown the distinct roles of progesterone and PR in the pathogenesis of multiple types of cancers (39). In this study, we found that PGR expression levels were

Table 3 Correlation analysis between PGR and relate genes and markers of monocyte, TAM, macrophage, DC and Treg in GEPIA

Description	Gene markers	STAD			
		Tumor		Normal	
		R	P	R	P
Monocyte	CD86	0.31	***	−0.08	0.64
	CD115 (CSF1R)	0.45	***	0.31	0.065
TAM	CCL2	0.41	***	0.61	***
	CD68	0.18	**	−0.41	0.013
	IL10	0.36	***	0.17	0.32
M1 Macrophage	INOS (NOS2)	0.02	0.69	0.26	0.12
	IRF5	0.23	***	−0.42	0.011
	COX2 (PTGS2)	0.27	***	0.88	***
M2 Macrophage	CD163	0.32	***	0.71	***
	VSIG4	0.36	***	0.55	**
	MS4A4A	0.44	***	0.64	***
Dendritic cell	HLA-DPB1	0.25	***	−0.11	0.53
	HLA-DQB1	0.11	0.03	−0.29	0.089
	HLA-DRA	0.19	**	−0.21	0.22
	HLA-DPA1	0.24	***	−0.13	0.46
	BDCA-1 (CD1C)	0.49	***	−0.014	0.94
	BDCA-4 (NRP1)	0.54	***	0.87	***
	CD11c (ITGAX)	0.35	***	0.19	0.26
Treg	FOXP3	0.21	***	−0.45	*
	CCR8	0.38	***	−0.27	0.11
	STAT5B	0.57	***	0.9	***
	TGFβ (TGFB1)	0.47	***	0.28	0.092

*P<0.01; **P<0.001; ***P<0.0001. PGR, progesterone receptor gene; STAD, stomach adenocarcinoma; DLBC, diffuse large B-cell lymphoma; TAM, tumor-associated macrophage; Treg, regulatory T cell; Cor, r value of Spearman's correlation.

related to the prognosis of different cancers and elevated PGR expression had a strong relationship with a poorer prognosis of GC. Also, PGR expression affected lymph node metastasis of GC patients, and it was observed that immune cell infiltration, as well as immune markers, were related to PGR expression. These results suggested that PGR is a potential prognostic biomarker in relation to immune cell infiltration in GC.

In this study, we investigated the PGR expression and its association with clinical prognosis in different cancers. Progesterone, mediated by the PR, has diverse effects on multiple tissues and organs and therefore influence

different types of cancer such as breast and endometrial cancer (40,41). In this study, we found that PGR expression levels were significantly different in various cancer tissues in the Oncomine and TCGA databases when compared with normal tissues, indicating that PGR may affect the pathogenesis of multiple types of cancer (Figure S1). Several researchers have found that the PR can be used as a favorable prognostic predictor in ovarian, prostate and breast cancers (42–44). However, until now, the prognostic role of PGR in GC has been controversial (45–49). In this study, analysis of the prognostic relationships between PGR and cancer patients' survival indicated that PGR

expression was correlated with poorer prognosis of multiple cancers including ACC, MESO and STAD, and with better prognosis of BRCA, KIRC, LGG, LIHC, SARC and UCEC (*Figure 1*). Following this result, the Kaplan-Meier plotter database analysis showed that elevated PGR expression correlated with poor OS and PFS in GC (*Figure 2*). As shown in *Table 1*, high expression PGR was significantly related to different clinicopathological factors of GC, including stages 2–4, stage T2–T4, stage N0–N3, and stage M0, and the highest OS and PFS HR values were observed in stage N1. Taken together, these results indicated that PGR might be a prognostic predictor of GC.

Tumor-infiltrating immune cells, including macrophages, DCs, and Tregs, actively participate in tumor progression and metastatic processes (50). Previous reports have shown that progesterone and the PR elicit various functional effects on immune cell types, including DCs, monocytes, lymphocytes, and macrophages (51). However, the mechanism of PGR in immune cell infiltration regulation of cancer is still largely uncharacterized. Our current study found that PGR expression correlated with immune cell infiltration in different cancers, especially in GC. PGR expression in STAD was significantly correlated with levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and DCs, suggesting PGR might influence immune cell infiltration and the tumor microenvironment in STAD (*Figure 3*).

Further, the correlation between the expression of PGR and various immune cell markers was analyzed. Macrophages play an important role in carcinogenesis, and TAMs are active in promoting solid-tumor metastasis (52). TAM infiltration into GC tissue is positively correlated with cancer invasion, nodal status, and clinical stage (53). The PR was reported to modulate the differentiation of macrophages into M1 or M2 phenotypes (51). A strong and positive correlation between TAMs, M1 and M2 macrophage markers, including CD86, CD115, CCL2, CD68, IL10, IRF5, COX2, CD163, VSIG4 and MS4A4A, and PGR expression in STAD was found in this study (*Table 2*, *Figure S4*). This suggested that PGR may influence TAM polarization in cancer, resulting in the promotion of tumor proliferation.

Treg cells are closely associated with the progress of a variety of cancers (54). FOXP3+Tregs are reported to be closely related to the incidence and development of GC, and increase FOXP3+Tregs infiltration in GC tissues can predict poor prognosis (55). FOXP3, a key protein in Treg differentiation and function, may influence tumor

differentiation and immune escape in GC patients (56). In this study, we found that PGR expression was positively related to markers of Tregs, such as FOXP3, CCR8, STAT5B, and TGFB1 (*Tables 2,3*). This result indicated that PGR could activate the Treg response in GC, promoting tumor pathogenesis. These findings suggested that PGR has the potential to play a role in regulating immune cell infiltration in STAD, and the relationship between immune cell infiltration and immune markers with PGR expression may partly explain the correlation of PGR expression with poor prognosis in STAD.

Conclusions

We found that elevated PGR expression was related to poor prognosis and immune cell infiltration in GC, indicating that PGR is a potential prognostic biomarker in GC patients.

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Footnote

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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 PGR expression levels in different cancer and normal tissues. (A) Oncomine database was used to compare PGR expression levels in different cancers with normal tissues. (B) The comparison of PGR expression levels in multiple cancer types and normal tissues by the TIMER database (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Table S1 PGR expression in cancers *vs.* normal tissue in Oncomine database

Cancer	Cancer type	P value	Fold change	Rank (%)	Sample	Reference (PMID)
Brain and CNS	Desmoplastic Medulloblastoma	1.96E-4	2.381	1%	62	AL137566
	Medulloblastoma	3.94E-5	5.526	2%	85	2328727
	Mixed Glioma	1.05E-5	1.511	2%	38	8889549
Breast	Breast Cancer	2.11E-6	4.305	1%	288	8889549
	Breast Cancer	1.56E-4	3.385	1%	76	8889549
	Mucinous Breast Carcinoma	1.41E-4	5.542	1%	336	AL137566
	Breast Cancer	8.18E-19	3.287	1%	341	31598691
	Mixed Lobular and Ductal Breast Carcinoma	7.52E-4	5.431	2%	593	31598691
	Invasive Ductal and Lobular Carcinoma	3.09E-4	7.096	5%	593	31598691
	Breast Cancer	2.35E-31	3.455	3%	1911	AL137566
	Tubular Breast Carcinoma	6.86E-7	1.617	5%	2136	31598691
	Breast Cancer	3.33E-4	2.442	10%	917	AL137566
Colorectal	Rectal Adenocarcinoma	8.19E-4	1.595	2%	154	AL137566
	Rectosigmoid Adenocarcinoma	1.49E-4	2.480	2%	237	31598691
Gastric	Diffuse Gastric Adenocarcinoma	4.98E-6	3.453	3%	43	AL137566
	Diffuse Gastric Adenocarcinoma	5.33E-4	1.871	4%	200	AL137566
Ovarian	Ovarian Endometrioid Adenocarcinoma	1.09E-5	3.984	1%	113	2328727
	Ovarian Endometrioid Adenocarcinoma	1.80E-4	2.946	1%	241	31598691
	Borderline Ovarian Surface Epithelial-Stromal Tumor	3.87E-13	8.160	1%	90	AL137566
	Borderline Ovarian Surface Epithelial-Stromal Tumor	9.61E-5	1.527	3%	39	31598691
	Borderline Ovarian Surface Epithelial-Stromal Tumor	5.73E-11	8.273	2%	295	AL137566
Prostate	Prostate Cancer	1.80E-10	1.889	8%	1911	AL137566

PGR, progesterone receptor gene; CNS, central nervous system.

Table S2 Correlation between PGR expression and prognosis of different cancers in Prognoscan database

Cancer type	Dataset	Endpoint	P-value	Hazard ratio (95% CI)
Bladder cancer	GSE5287 (n=30)	OS	0.866906	1.18 [0.17–7.98]
	GSE13507 (n=165)	OS	0.610353	1.53 [0.30–7.82]
Blood cancer	GSE12417-GPL96 (n=163)	OS	0.829778	0.89 [0.30–2.63]
	GSE8970 (n=34)	OS	0.081255	1.37 [0.96–1.96]
	GSE2658 (n=559)	DSS	0.377941	0.90 [0.72–1.13]
Brain cancer	GSE4271-GPL96 (n=77)	OS	0.680079	0.93 [0.65–1.33]
	GSE7696	OS	0.826305	1.40 [0.07–29.12]
	GSE16581 (n=67)	OS	0.900187	1.08 [0.34–3.40]
Breast cancer	GSE12276 (n=204)	RFS	0.000004	0.84 [0.78–0.91]
	GSE6532-GPL570 (n=87)	RFS	0.020421	0.84 [0.73–0.97]
	GSE1379 (n=60)	RFS	0.018115	0.83 [0.72–0.97]
	GSE1456-GPL97 (n=159)	RFS	0.017101	0.74 [0.57–0.95]
	GSE6532-GPL570 (n=87)	DMFS	0.015345	0.71 [0.53–0.94]
	GSE9195 (n=77)	DMFS	0.033456	0.76 [0.59–0.98]
	GSE11121(n=200)	DMFS	0.005517	0.79 [0.67–0.93]
	GSE2034 (n=280)	DMFS	0.002136	0.83 [0.73–0.93]
	E-TABM-158 (n=117)	DMFS	0.296147	1.22 [0.84–1.79]
	GSE1456-GPL96 (n=159)	OS	0.042492	0.80 [0.64–0.99]
	GSE7390 (n=198)	OS	0.001387	0.84 [0.76–0.94]
	GSE12945 (n=62)	OS	0.252414	7.96 [0.23–277.96]
Colorectal cancer	GSE17536 (n=177)	DSS	0.664883	0.61 [0.06–5.81]
	GSE17537 (n=55)	OS	0.559584	0.52 [0.06–4.67]
	GSE22138 (n=63)	DMFS	0.000533	5.29 [2.06–13.57]
Head & neck cancer	GSE2837 (n=28)	RFS	0.768132	0.45 [0.00–86.58]
Lung cancer	GSE31210 (n=204)	RFS	0.003040	0.59 [0.41–0.84]
	GSE3141 (n=111)	OS	0.009047	0.65 [0.47–0.90]
	GSE17710 (n=56)	RFS	0.364172	1.15 [0.85–1.56]
Ovarian cancer	GSE9891 (n=278)	OS	0.002163	0.58 [0.41–0.82]
	GSE8841 (n=81)	OS	0.005308	0.37 [0.18–0.74]
	GSE17260 (n=110)	OS	0.132846	0.87 [0.73–1.04]
Skin cancer	GSE19234 (n=38)	OS	0.459531	0.69 [0.26–1.84]

PGR, progesterone receptor gene; OS, overall survival; RFS, relapse-free survival; DSS, disease free survival; DMFS, distant metastasis-free survival.

Figure S2 Correlation between PGR expression and prognosis in different cancers in the PrognoScan database. (A-H) Kaplan-Meier survival curves of breast, eye, lung and ovarian cancers. OS, overall survival; RFS, relapse-free survival; DMFS, distant metastasis-free survival.

Table S3 Multivariate analysis of the correlation of PGR expression and clinical characteristics with prognosis in GC patients

Parameter	HR	95% CI	P-value
Overall survival			
Gender	1.72	1.32-2.24	0.059
Stage	1.28	0.96-1.72	0.089
Stage T	1.78	1.09-2.91	0.020
Stage N	1.85	1.32-2.59	0.00027
Stage M	1.53	0.86-2.72	0.14
Lauren classification	2.60	1.67-4.06	1.2e-05
Differentiation	0.73	0.46-1.15	0.170
PGR	1.63	1.32-2.02	4.5e-06
Progression-free survival			
Gender	1.72	1.28-2.31	0.280
Stage	0.75	0.51-1.12	0.160
Stage T	0.77	0.55-1.08	0.130
Stage N	1.73	1.06-2.81	0.027
Stage M	1.41	0.79-2.52	0.250
Lauren classification	2.26	1.44-3.55	0.00029
Differentiation	0.61	0.37-1.02	0.056
PGR	1.80	1.39-2.32	5.7e-06

PGR, progesterone receptor gene; GC, gastric cancer

Figure S3 Correlation of PGR expression with immune infiltration levels in different cancers. Adrenocortical carcinoma (ACC) (A), bladder urothelial carcinoma (BLCA) (B), breast invasive carcinoma-basal (BRCA-Basal) (C), breast invasive carcinoma-Her2 (BRCA-Her2) (D), breast invasive carcinoma-Luminal (BRCA-Luminal) (E), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (F), cholangiocarcinoma (CHOL) (G), colon adenocarcinoma (COAD) (H), esophageal carcinoma (ESCA) (I), glioblastoma multiforme (GBM) (J), head and neck squamous cell carcinoma (HNSC) (K), head and neck squamous cell carcinoma-HPVpos (L), head and neck squamous cell carcinoma-HPVneg (HNSC-HPVneg) (M), kidney chromophobe (KICH) (N), kidney renal clear cell carcinoma (KIRC) (O), kidney renal papillary cell carcinoma (KIRP) (P), brain lower grade glioma (LGG) (Q), liver hepatocellular carcinoma (LIHC) (R), lung adenocarcinoma (LUAD) (S), lung squamous cell carcinoma (LUSC) (T), mesothelioma (MESO) (U), ovarian serous cystadenocarcinoma (OV) (V), pancreatic adenocarcinoma (PAAD) (W), pheochromocytoma and paraganglioma (PCPG) (X), prostate adenocarcinoma (PRAD) (Y), rectum adenocarcinoma (READ) (Z), sarcoma (SARC) (AA), skin cutaneous melanoma (SKCM) (AB), skin cutaneous melanoma-metastasis (SKCM-Metastasis) (AC), skin cutaneous melanoma-primary (SKCM-Primary) (AD), testicular germ cell tumors (TGCT) (AE), thyroid carcinoma (THCA) (AF), thymoma (THYM) (AG), uterine corpus endometrial carcinoma (UCEC) (AH), uterine carcinosarcoma (UCS) (AI), uveal melanoma (UVM) (AJ).

Figure S4 Analysis of the correlation between PGR and immune markers of monocyte, TAM, M1 and M2 in STAD and DLBC. (A-D) Correlations between PGR expression and gene markers of monocytes (A), TAMs (B), and M1 (C) and M2 macrophages (D) in STAD. (E-H) Correlations between PGR expression and gene markers of monocytes (E), TAMs (F), and M1 (G) and M2 macrophages (H) in DLBC. STAD, stomach adenocarcinoma; DLBC, diffuse large B-cell lymphoma; M1, M1 macrophage; M2, M2 macrophage; TAM, tumor-associated macrophage.