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CORRESPONDENCE

Aberrant DNA polymerase beta expression is associated with dysregulated tumor immune microenvironment and its prognostic value in gastric cancer

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Abstract

Background Gastric cancer is caused by different exogenous risk factors. Polymerase beta (POLB) is critical to repair oxidative and alkylating-induced DNA damage in genome maintenance. It is unknown whether overexpression of POLB genes in GC modulates tumor immunogenicity and plays a role in its prognostic value.

Methods RNA-Seq of GC data retrieved from TCGA and GEO database and patient survival were compared using Kaplan-Meier statistical test. The TIMER algorithm was used to calculate the abundance of tumor-infiltrating immune cells. Furthermore, ROC analysis was applied to evaluate the prognostic value of POLB overexpression.

Results Our data analysis of TCGA and GEO gastric cancer genomics datasets reveals that POLB overexpression is significantly associated with intestinal subtypes of stomach cancer. In addition, POLB overexpression is associated with low expression of innate immune signaling genes. In contrast, POLB-overexpressed tumor harbors high mutation frequency and MSI score. Furthermore, POLB-overexpressed tumor with high immune score exhibits a better prognosis. Interestingly, our ROC analysis results suggested that POLB overexpression has a potential for prognostic markers for stomach cancer.

Conclusions Our analysis suggests that aberrant POLB overexpression in stomach cancer impacts the diverse aspects of tumor immune microenvironment. In addition, POLB might be a potential prognosis marker and/or an attractive target for immune-based therapy in GC. However, our observation still requires further experimental-based scientific validation studies.

Introduction

Stomach cancer remains highly prevalent and accounts for a notable proportion of global cancer mortality [1]. A variety of risk factors, including sodium chloride intake, Helicobacter pylori infection and smoking, induce inflammation of gastric tissue and promotes gastric carcinogenesis [2]. Intrinsic or exogenous agents exacerbate reactive oxygen species-related oxidized DNA base lesions and single-stranded DNA breaks (SSBs) that are primarily repaired by the base excision repair (BER) pathway [3]. BER is critical for the removal and error-free repair of up to 20,000 endogenous DNA lesions per cell per day [4–6]. Our work and other studies have shown that BER is critical to prevent spontaneous or exogenous risk factor-induced gastric cancer [7]. Moreover, there are a significant number of genetic germline variant and somatic mutations (30%) in BER genes that are significantly modulate the risk of cancer and treatment response [8–10]. BER expression is deregulated in several types of cancer, which leads to genomic instability, impacting treatment outcomes [11–14]. Moreover, abnormal expression of BER genes in gastric cancer has been reported [15, 16]. Mutations in BER genes can lead to deficient or aberrant repair that subsequently causes cancer-driving chromosomal instability and/or mutations (genomic instability). POLB belongs to the X-family of a DNA polymerase that participates in BER [17, 18]. POLB not only functions as a DNA polymerase but also catalyzes the excision of the deoxyribose phosphate (5'-dRP) [19]. Several somatic mutations in human cancers have been identified in the *PolB* gene and characterized their biological significance [10, 20].

With the establishment of public bioinformatics databases and the advancement of bioinformatics analysis techniques used to find biomarkers at the transcriptional level [21, 22], in this study, by exploring the data from TCGA and GEO

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public database, we analyzed the relationships between the expression of POLB genes and the tumor stage, pathological classification, and patient overall survival (OS). Furthermore, there is a significant need to explore whether POLB overexpression is associated with tumor immune microenvironment and its potential prognostic predictors in GC. Meanwhile, the tumor immune assessment resources (TIMER) and CIBERSORT were applied to study the potential biological functions of POLB gene. Interestingly, we used ROC analysis to get the optimal critical value of mRNA of POLB expression for prognostic value of the risk of stomach cancer patients. Overall, in this study, the expression of POLB in GC tumors and the relationship with gastric tumor immunogenicity and tumor immune infiltration was investigated.

Methods

Data acquisition

RNA sequencing (RNA-Seq) and clinical information (histological grade, survival status, and tumor stages, tumor subtypes, lymph node metastasis status) of GC ($n=415$) was retrieved from the Cancer Genome Atlas (TCGA pan cancer datasets: cBioProtal (http://cbioportal.org). Additional dataset of tumor samples ($n=71$) and normal adjacent tissues ($n=19$) database were also obtained from GEO (GSE13861) to characterize POLB in tumor versus normal tissues. Further patients' gene chip database of samples from non-cancerous tissues ($n=294$) and tumor ($n=375$) were retrieved from TNAM platform. The patients are categorized as POLB high and low to analyze the tumor data including the tumor stage, lymph node metastasis status, histological grade characteristics.

Exclusion and inclusion criteria

POLB is the primary source of interest for this analysis, and only individuals with valid RNA-Seq V2 RSEM data for POLB were included. Individuals with a z score of ≤ -0.5 were placed into the low-expressing group while individuals with a z score of ≥ 0.5 were placed into the high-expressing group. Individuals falling between -0.5 and 0.5 were excluded from analyses.

Immune cell infiltration in GC

The TIMER algorithm was used to calculate the tumor abundance of six infiltrating immune cells ($CD4^+$ T cells, $CD8^+$ T cells, B cells, neutrophils, macrophages, and dendritic cells) based on RNA-Seq expression profiles data. The correlation between the POLB and immune cells was calculated by Spearman correlation analysis using TIMER 2.0.

Estimation of stromal and immune cells in tumor tissues

The ESTIMATE algorithm-generated matrix, immune scores and stromal scores were used to estimate the level of infiltrating matrix and immune cells in GC tissue and tumor purity through expression profiles. Results for the tumor-infiltrating immune component were yielded with data extracted from the TCGA database, which was analyzed by the CIBERSORT algorithm.

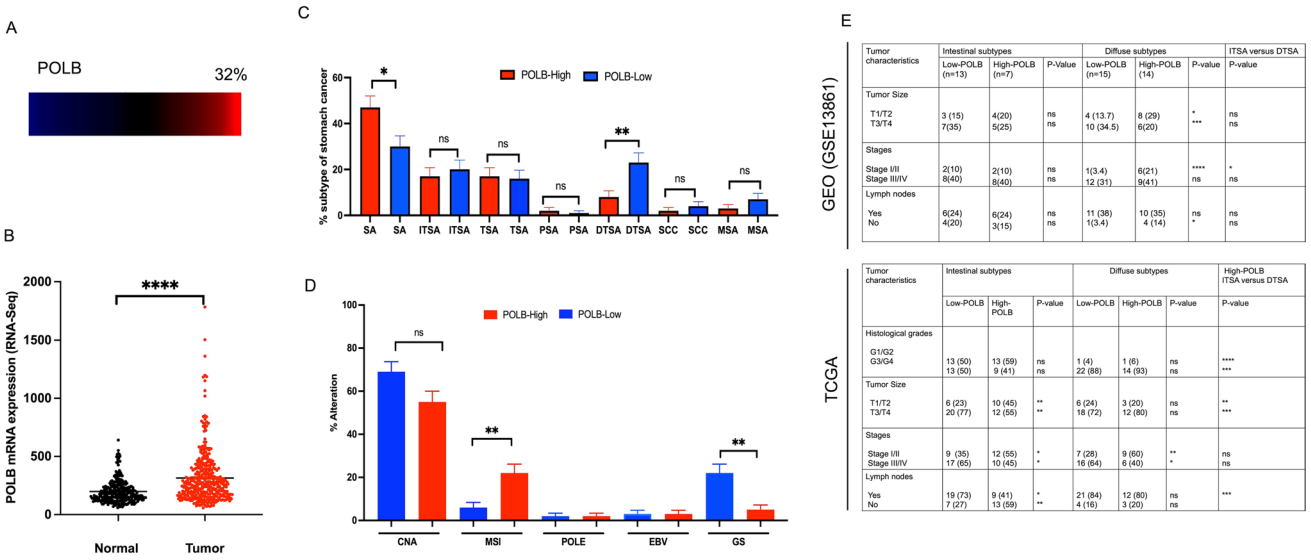
Statistical analysis

Group comparisons for continuous data were conducted using t tests, and quantitative variables were analyzed with the Wilcoxon signed-rank test or the Spearman rank correlation test. Kaplan–Meier analysis was used to assess overall survival. Uni-variable associations between POLB overexpression and clinicopathologic variables were tested using nonparametric tests. We applied ROC analysis of POLB mRNA expression tumor versus normal to obtain a numerical representation of the classifier performance the "area under the curve" (AUC) value. An AUC of 0.5 corresponds to no classification power at all, and an AUC value of 1 denotes a perfect biomarker. Z score > 0.5 applied as cutoff value for POLB overexpression to categorize the sample and calculate the sensitivity and specificity graph. Statistical significance was set at $P<0.05$.

Results

DNA polymerase *beta* overexpression is associated with subtypes of stomach *cancer*

Gastric cancer is a highly heterogeneous disease. According to Laurén's classification, gastric cancer is categorized as intestinal type and diffuse type [23]. In this part of study, we have examined the mRNA expression of POLB in TCGA dataset ($n=415$; Pan cancer). We have found that POLB is overexpressed in 32% (132/415) of gastric tumor (Fig. 1A) and significantly high in tumor versus normal tissues (Fig. 1B). Further 44% of those gastric tumors overexpressed POLB belong to a subtypes of stomach adenocarcinoma, 17% (23/132) are intestinal subtype stomach adenocarcinoma and 8% diffuse subtype stomach adenocarcinoma. We have found that patients with intestinal subtypes of stomach adenocarcinoma harbor significant high level of POLB overexpression (Fig. 1C, $P^*<0.05$). In contrast, patients with diffuse subtype of stomach adenocarcinoma are significantly



Tumor characteristics	Intestinal subtypes			Diffuse subtypes			ITSA versus DTSA
	Low-POLB (n=13)	High-POLB (n=7)	P-Value	Low-POLB (n=15)	High-POLB (14)	P-value	P-value
Tumor Size							
T1/T2	3 (15)	4(20)	ns	4 (13.7)	8 (29)	*	ns
T3/T4	7(35)	5(25)	ns	10 (34.5)	6(20)	***	ns
Stages							
Stage I/II	2(10)	2(10)	ns	1(3.4)	6(21)	****	*
Stage III/IV	8(40)	8(40)	ns	12 (31)	9(41)	ns	ns
Lymph nodes							
Yes	6(24)	6(24)	ns	11 (38)	10 (35)	ns	ns
No	4(20)	3(15)	ns	1(3.4)	4 (14)	*	ns

Tumor characteristics	Intestinal subtypes			Diffuse subtypes			High-POLB ITSA versus DTSA
	Low-POLB	High-POLB	P-value	Low-POLB	High-POLB	P-value	P-value
Histological grades							
G1/G2	13 (50)	13 (59)	ns	1 (4)	1 (6)	ns	****
G3/G4	13 (50)	9 (41)	ns	22 (88)	14 (93)	ns	***
Tumor Size							
T1/T2	6 (23)	10 (45)	**	6 (24)	3 (20)	ns	**
T3/T4	20 (77)	12 (55)	**	18 (72)	12 (80)	ns	***
Stages							
Stage I/II	9 (35)	12 (55)	*	7 (28)	9 (60)	**	ns
Stage III/IV	17 (65)	10 (45)	*	16 (64)	6 (40)	*	ns
Lymph nodes							
Yes	19 (73)	9 (41)	*	21 (84)	12 (80)	ns	***
No	7 (27)	13 (59)	**	4 (16)	3 (20)	ns	

Fig. 1 POLB is overexpressed in gastric tumor and modulates in subtypes of stomach cancer. **A** Percent of POLB overexpression in stomach cancer (32%); **B** expression of POLB is high in tumor (T) versus normal non-cancerous tissues; **C** Stomach subtypes including intestinal, diffuse, tubular, singlet, mucin stomach adenocarcinoma; **D** Molecular subtypes of stomach cancer in POLB high- versus low-expressed tumor with chromosomal instability (CIN), microsatellite instability (MSI), Epstein-Barr virus infected (EBV), DNA polymerase E (POLE), genetically stable (GS); **E** Clinical characteristics of the intestinal and diffuse subtypes of stomach cancer from TCGA (lower panel) and GSE13861(upper panel). The Mann-Whitney U test analysis was performed using graph pad prism. * represent *$P<0.05$; ** $P<0.01$; ***$P<0.001$; ****$P<0.0001$ and ns represent no statistical significance difference

high in POLB low-expressed tumor versus POLB high-expressed tumor (Fig. 1C, $P^{**}<0.01$). Our study also examines whether aberrant expression of POLB is associated with different molecular subtypes of stomach cancer (Fig. 1D). Results show that majority of POLB-overexpressed tumor significantly increases in MSI molecular subtypes of gastric cancer (Fig. 1D, **$P<0.01$). In addition, chromosomal instability is high in POLB-overexpressed tumor as compared to tumor with low POLB expression. In contrast, tumor with low expression of POLB has more genetically stable (GS) as compared to high POLB-expressed tumor (Fig. 1E**,** **$P<0.01$).

Our TCGA data show that high POLB expression in diffuse subtypes of cancer is associated with histological high-grade tumor (TCGA, Fig. 1E). POLB-overexpressed intestinal subtype of stomach tumor harbors high percentage of patients with T1/T2 tumor sizes, high number early tumor stage I/ II and more lymph node metastasis versus low POLB-expressed intestinal subtype of stomach tumor (TCGA, Fig. 1E, $P^{*}<0.01$). On the other hand, POLB overexpression in diffuse subtype of gastric cancer is associated with more T1/T2 and stage I/II tumors as compared with low POLB-overexpressed diffused subtypes of tumor groups (GEO dataset; Fig. 1E**,** *$P<0.05$ & $P^{****}<0.0001$). Furthermore, high POLB-overexpressed diffuse subtypes of stomach cancer exhibit high percentage of patients with high histological tumor grades (G3/G4; 93% versus 41%), high tumor size (T3/T4; 80% versus 55%) and more lymph node metastasis (80% versus 41%) as compared with high POLB-overexpressed intestinal subtypes of stomach cancer (TCGA database; Fig. 1E, ***$P<0.001$).

Aberrant expression of POLB is associated with change of immune score and correlation with immune cell infiltration

Increasing evidence suggests that cancer progression is strongly influenced by host immune response, which is represented by immune cell infiltrates in the tumor microenvironment. We compared changes in immune and stromal scores between high expression versus low expression of POLB gene in tumor samples, and we found that POLB-overexpressed tumors are associated with significantly low immune score, stromal score and ESTAMATE score (Fig. 2A-C; ****$P<0.001$ & ***$P<0.01$). We also determine whether immune score is different in the stomach cancer subtypes, and interestingly, high immune score was exhibited in DTSA tumor with low POLB expression versus high (Fig. 2 D, $P^{*}<0.05$). However no significant difference in immune score found in intestinal types of stomach cancer in high POLB versus low-expressed tumor (Fig. 2E). However, ESTMATE score is significantly high in DTSA subtypes of cancer than ITSA (Fig. 2F, $P^{*}<0.05$). Intriguingly, we performed a comprehensive investigation on the correlations between the expression levels of POLB and immune cells infiltration on tumor using TIMER database.

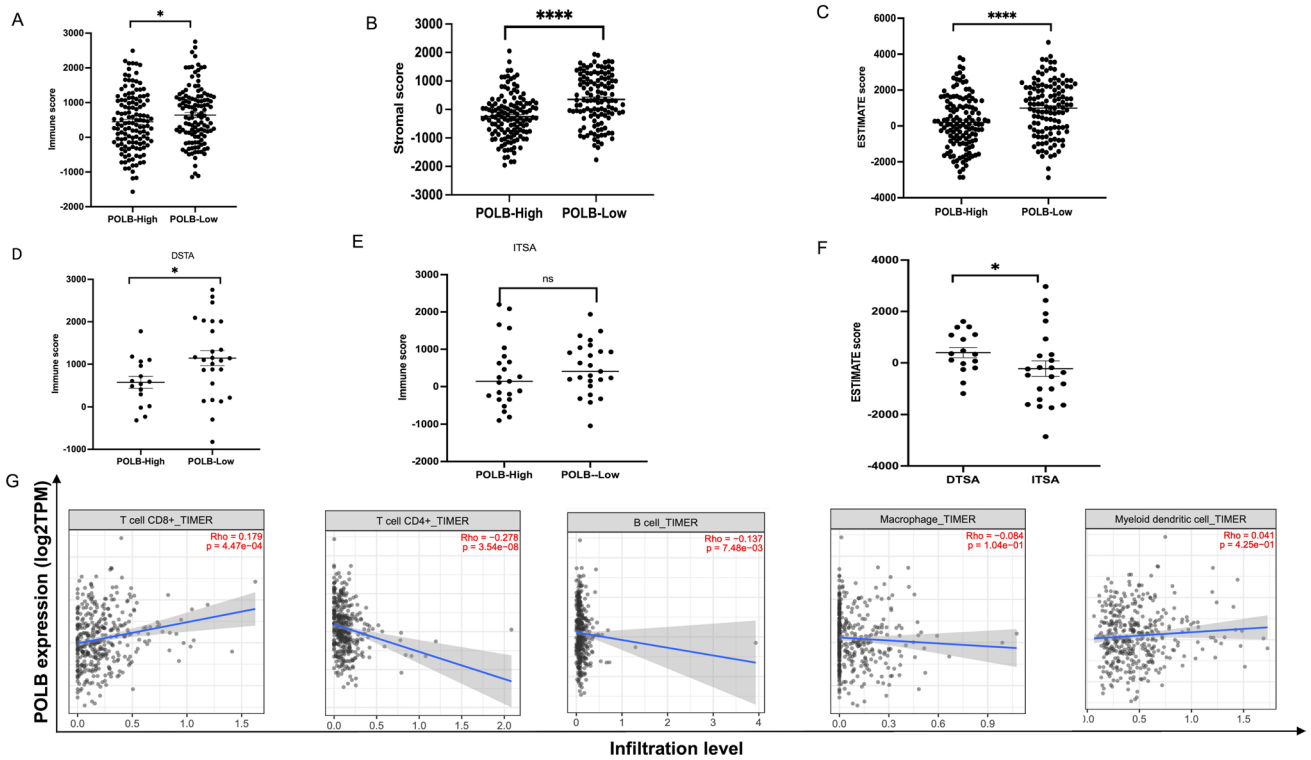


Fig. 2 The landscape of the tumor immune microenvironment and its impact on gastric cancer. (**A**) Immune, stromal (**B**) and ESTIMATE score (**C**) of the tumor with high versus low POLB mRNA expression; (**D**) immune score of DTSA tumor with POLB high versus low; (**E**) immune score of ITSA with POLB high versus low POLB expression; (**F**) estimate score of DTSA versus ITSA; (**G**) overexpression of POLB is associated with low level of infiltration of immune cell to the tumor microenvironment. Correlation of immune infiltration versus the POLB expression in tumor. The correlation of POLB expression versus immune cell (CD8 + T, CD4 + T, B cells macrophage and DC cells); the Mann-Whitney U test was used to compare the expression of POLB versus immune score as well as stromal score. Spearman analysis was performed the correlation coefficient of POLB and immune infiltration. Rho represent correlation coefficient; * represent $*P<0.05$; $****P<0.0001$ and ns represent no statistical significance difference

Interestingly, we found that high POLB expression was associated with reduced innate and adaptive immune cell abundance, including CD4 + T cell (correlation = − 0.3; $P = 3.4e-08$), B cells (correlation = − 0.2; $P = 5.53e$), macrophage (correlation = − 0.084; $P = 1.04e-04$) and DC (correlation = 0.04; $P = 4.3e-01$) (Fig. 2G). However, moderate increase CD8 + T of infiltration was observed in POLB-overexpressed tumors (Fig. 2G; correlation GC = 0.2, $P = 4.5e-04$). These results showed that overexpression of POLB is significantly associated with reduced the infiltration of the dominate immune cells in tumor microenvironment.

Association of POLB with immune checkpoint genes and innate immune signaling landscape in GC

We examined whether altered level of POLB expression is associated with innate immune signaling genes expression and immune checkpoints mediator genes. We perform spearman correlation analysis on tumor that overexpressed versus low expressed of subtypes of stomach cancer using publicly available cancer genomic database including TCGA (Pan cancer) and GEO (GSE13861). We found that diffuse stomach cancer with high POLB expression has very weak positive correlation with innate immune signaling genes (IRF1, IRF3, IRF7, ISG15, ISG20, STING1, CXCL10) except CCL5 (Fig. 3A upper panel). In contrast, intestinal subtypes of stomach cancer with high POLB expression are moderately correlated with innate immune signaling genes expression (Fig. 3A; lower panel). Furthermore, the diffuse subtypes of stomach cancer with high POLB expression have weak positive correlation with innate immune signaling genes. However, STING1 and IRF3 have a strong positive correlation with high POLB-expressed diffuse subtypes of gastric cancer (Fig. 3B; upper panel). In addition, intestinal subtypes of stomach cancer with high POLB expression have positive correlation with IRF3 (Fig. 3B lower panel). Furthermore, our study examined whether POLB expression in tumor is associated with altered expression of immune checkpoint genes (CTLA4, PD-1, PDL-1 and PDL-2). Diffuse subtypes cancer with high POLB expression are more pronounced positive correlation with CTLA4, PDL-1 and PDL-2 gene expression (TCGA database: Fig. 3C; upper

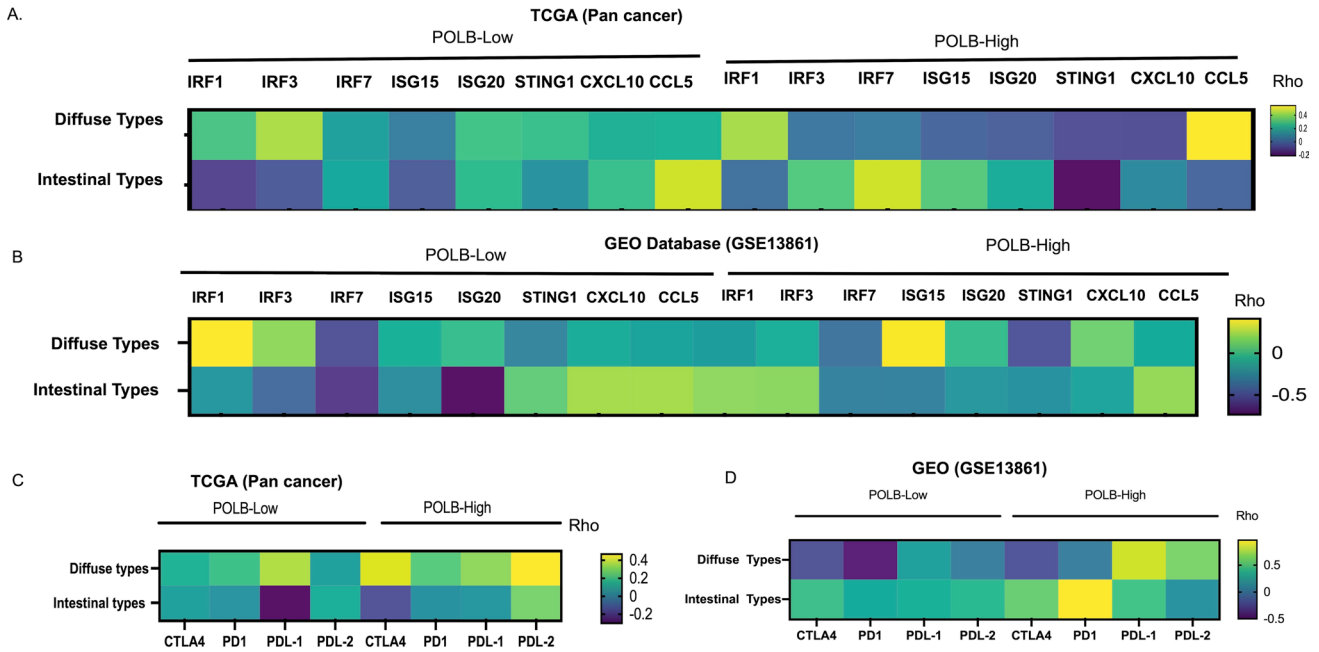


Fig. 3 Heatmap of innate immune signaling gene expression versus POLB mRNA expression in stomach cancer. **A** Heatmap of innate immune signaling genes (IFNB, ISG15, ISG20, CCL5, CXCL10, IRF1, IRF3, IRF7, STING) expression versus POLB mRNA expression in stomach cancer from TCGA database; **B** Heatmap of POLB mRNA expression versus innate immune signaling genes from GSE13861 database; **C** The relationship between the POLB expression versus immune check point genes (CTLA4, CD274 PDCD1, PDCD1GL2) from TCGA database; **D** POLB expression versus immune check point genes (CTLA4, CD274 PDCD1, PDCD1GL2) from GSE13861database. The Spearman's correlation was performed to analyze using GraphPad. Rho represent the correlation coefficient

panel). In contrast, POLB high expression in intestinal cancer has a negative correlation, and in the CTLA4 expression, it has moderate positive correlation with PDL-2 expression (TCGA: Fig. 3C lower panel). In addition, the GEO database analysis of diffuse subtypes of stomach cancer with high POLB expression has strong positive correlation with PDL-1 and PDL-2 mRNA expression than intestinal stomach cancer subtypes (Fig. 3D). However, expression of CTLA 4 and PD-1 in intestinal subtypes of cancer is strongly correlated with high POLB-expressed tumor (Fig. 3D).

High expression of POLB with high immune score is associated with better overall survival in GC patients

To determine the co-occurrence of high immune score with aberrant POLB expression in tumor impact overall survival of GC patients, Kaplan–Meier survival curves were generated based upon the designation of individuals with high immune score and with high POLB expression versus those with low POLB expression in tumor. POLB-overexpressed tumor with high immune score increases the overall survival outcome versus low immune score groups (Fig. 4A). In particular, the diffuse and intestinal subtypes of gastric cancer with high immune score co-occurrence with high POLB expression have better overall survival than low POLB-expressing groups (Fig. 4B and C). Furthermore, the results showed that high expression of genes involved in antitumor immune response such as PD-1 and CTLA4 in POLB-overexpressed tumor is associated with the better overall survival outcomes (Fig. 4D and E).

The prognostic value of dysregulated POLB expression

To further understand the role of POLB gene expression in the prognosis of gastric cancer, we used the ROC curve to study the optimal critical values of POLB expression using TCGA and GSE13861 database. Combined with sensitivity and specificity of POLB expression in high versus low expression, normal versus tumor, we have found that a strong significant AUC is observed in POLB overexpression as compared to control (Fig. 5A; AUC 0.87, 95% CI 0.85–0.9, $P < 0.0001$). The results showed in Fig. 5A using TCGA database with the cutoff value z score > 0.5 demonstrated 92% of sensitivity (95% CI; 86.01% to 95.63%) and 80% specificity (95% CI; 64.11% to 89.96). In addition, the GEO genomic database of GSE13861 analysis provides a significant AUC value (Fig. 5B; 0.75, 95% CI 0.67–0.85; $P < 0.0001$). In addition, our analysis of evaluating POLB

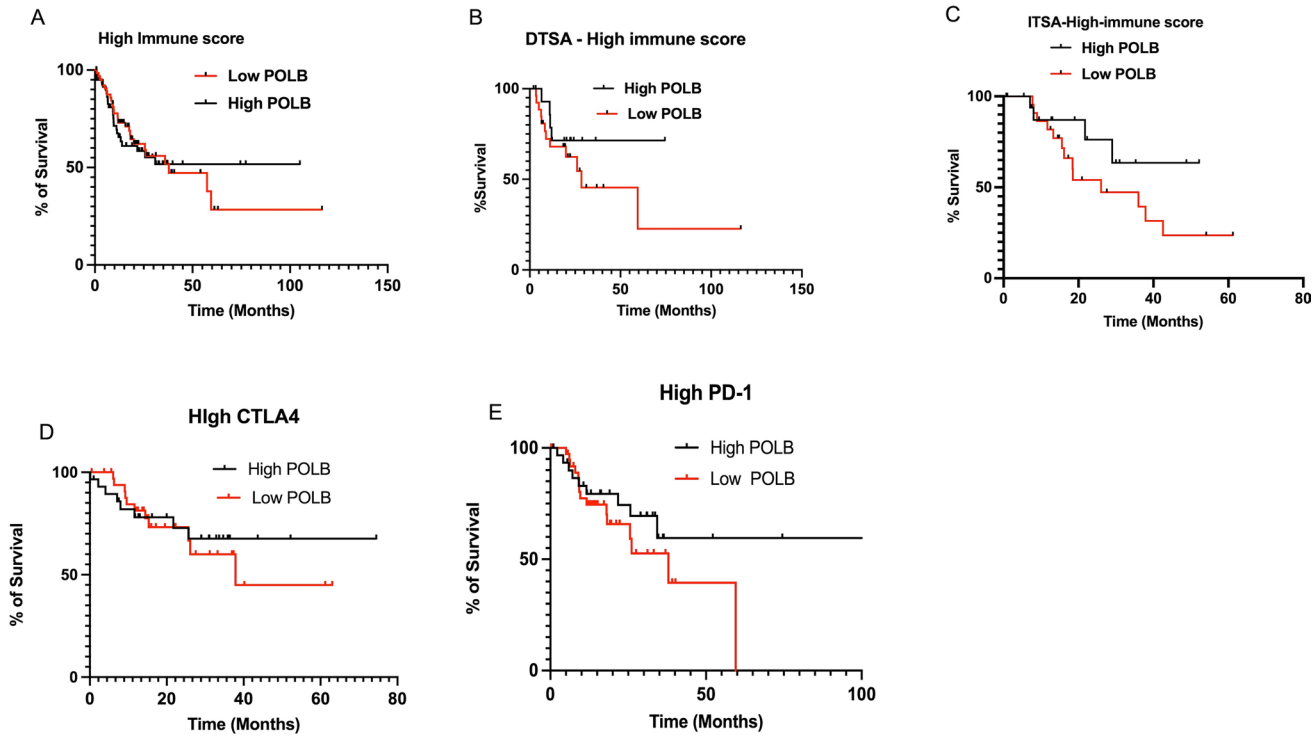
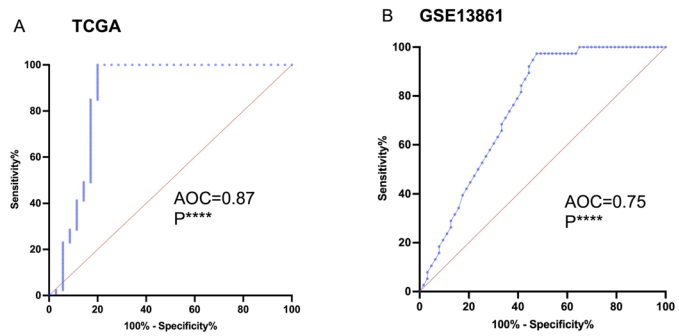


Fig. 4 The co-occurrence of POLB expression with high immune score is associated with better GC patient overall survival. **A** Kaplan–Meier survival analysis of POLB expression (low versus high) groups with high immune score; **B** The overall survival of diffuse stomach cancer subtypes in patients with tumor harbor high immune score with high POLB high expression versus low; **C** The overall survival of intestinal stomach cancer subtypes in patients with tumor harbor high immune score with POLB high expression versus low; **D** The overall survival of patients with high CTLA4 and high POLB expression versus low groups; **E** The overall survival of patients with high PD-1 with high POLB expression versus low groups. For Kaplan–Meier curves, *P*-values, were generated by log-rank tests. $p < 0.05$ was considered as statistically significant



C

Study Types	AOC	95% CI	P-value
TCGA (control/high)	0.87	0.847-0.99	****
ITSA	0.999	0.999-1	****
DTSA	1	1-1	****
GSE13861	0.75	0.66-0.85	****
ITSA	0.85	0.71-0.99	***
DTSA	0.88	0.78-0.99	****

Fig. 5 Diagnostic ROC curves of DNA POLB gene expression. **A** Diagnostic ROC curves of POLB expression from TCGA database; **B** Diagnostic ROC curves of POLB gene expression from GSE13861 database; **C** Diagnostic ROC values of TCGA and GSE13861 database with subtypes of stomach cancer

overexpression using GSE13861 gene chip database with the cutoff value 8.9 showed 84% sensitivity (95% CI, 69.58% to 92.56%) and 59% of specificity (95% CI; 46.42% to 70.04%). From both databases, the ROC value is significantly high and has a great prognostic value for stomach cancer (Fig. 5B). However, the ROC value in intestinal and diffuse subtypes of stomach cancer from TCGA and GSE13861 database analysis shows that the AUC value (0.99 and 1) is significantly high but not able to differentiate the diffuse and intestinal subtypes of gastric cancer, respectively (Fig. 5C).

Discussion

In this study, we present a comprehensive evaluation of aberrant POLB expression which is associated with innate immune signaling and tumor immunogenicity in gastric cancer. We further showed that POLB overexpression is associated with the Laurén's and molecular subtypes of stomach cancer and molecular subtypes. It is well established that POLB has critical role in repairing endogenous and exogenous oxidative DNA damage [24, 25]. However, the aberrant expression of POLB impact on gastric cancer subtypes and its association with tumor immune microenvironment is unknown. Our analysis shows 32% of gastric cancer harbor POLB overexpression. Our data agree with previous reviews reporting that 30% of human tumors have elevated expression of POLB [11]. Furthermore, most of the tumor with POLB overexpression co-occurred with MSI molecular subtypes. Our in silico data agree with previous report on POLB overexpression inducing MSI in vitro [26]. We also compared our results to the previously published studies that investigated the transcriptomic differences between two histopathology subtypes of gastric adenocarcinomas, intestinal (well-differentiated) and diffuse (undifferentiated), with a distinct morphologic appearance, pathogenesis, and genetic profiles diffused versus intestinal stomach cancer subtypes of cancer [23, 27–29]. It is interesting to notice that POLB-overexpressed tumor has significantly occurred in intestinal subtypes of stomach cancer. In contrast, a significant number of tumors with low POLB expression are observed in the diffuse subtypes of gastric cancer. It is known that diffuse subtypes of gastric cancer are aggressive and have a higher rate of peritoneal involvement compared to the intestinal type [30, 31]. Our in silico analysis shows that overexpression of POLB is associated with stomach adenocarcinoma (Fig. 1). Interestingly, diffuse subtypes of stomach cancer with high POLB expression have a significant tumor size, high histologically grade and harbor more lymph node metastasis versus intestinal subtypes of stomach cancer (Fig. 1E) suggesting that dysregulated POLB expression may contribute for the clinicopathological difference between subtypes of stomach cancer and may provide important information for future to develop type specific therapeutic approaches.

Notably, previous studies have shown that tumor immune microenvironment has implication in gastric cancer progression and susceptibility for immunotherapy [32, 33]. In addition, cells with DNA damage repair defects tend to be sensitive to immunotherapy as a result of enhanced neoantigen generation, upregulation of programmed death ligand 1 (PD-L1), and induction of innate immune signaling [34]. Previous studies have shown that tumor infiltration by immune cells is linked with prognosis of gastric cancer [35]. The tumor microenvironment composition including CD8 + T, CD4 + T, macrophages and tumor-associated fibroblasts relates with clinical outcomes in various cancers including gastric cancer [36]. Other studies have shown that DNA repair deficiency has been shown to be associated with immunogenicity in other types of cancer [37] but the association of POLB overexpression and tumor innate immune signaling and immunogenicity is unknown. Therefore, we analyzed immune response dynamics in relation with POLB high and low expression using TCGA-STAD and GSE13861 database, including infiltrating lymphocytes, immune subtypes, innate immune signaling genes expression. Most of the innate immune signaling genes expression is suppressed in POLB-overexpressed diffuse subtypes of stomach cancer relatively compared with intestinal subtypes (Fig. 3). Our in silico studies may suggest that a better antitumor immunity exists in intestinal subtypes of tumor microenvironment (Fig. 3). Further, our results showed that tumor harboring high POLB expression was negatively corelated with CD4 + T, B cells, macrophage, DC infiltration. Interestingly, we observed an infiltration of CD8 + T cells in POLB-overexpressed tumors. Programmed death 1 (PD-1) and its ligands programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) mediate cancer cell immune escape. Several reports have shown that PDL-2 is highly expressed in human cancers including HNSCC, cervical cancer and gastric cancer where it plays immunosuppressive role [38, 39]. In our study, we found a positive correlation between POLB overexpression and PDL-2 in GC. Our result suggest that PDL-2 may serve asa potential target to enhance immunotherapy response. Furthermore, our analysis shows that POLB-overexpressed tumor co-occurred with low immune score suggested that high POLB expression may contribute to suppress antitumor immunity. Our findings are in compliance with previous research that indicated that overexpression of DNA damage response abrogates immune cell killing of cancer cells [40]. Furthermore, low expression of IFN signaling genes in POLB-overexpressed tumor suggests low tumor immunogenicity. A recent study has shown that IFN signaling pathways are essential for antigen presentation and T cell recognition and killing, as well as immunotherapy effect [41]. POLB-overexpressed stomach tumor with low immunogenicity may require additional stimulant to enhance immunogenicity and provide a better future immunotherapy response [41]. We observed that POLB expression is inversely related to the innate signaling genes (IFNB1, IRF3, IRF7, STING1). However, an analysis of the relationship between POLB and chemokine CXL10 and CCL5 suggests positive correlation between them in different subtypes of GC tumor. Given the involvement of CXL10 in promoting migration and invasiveness in gastric cancer, POLB might

promote tumor metastasis in gastric cancer by stimulating CXL10 [42]. In contrast to less innate immune signaling gene expression, our analysis within the high immune score co-occurrence with high POLB expression results in better survival outcomes as compared with low POLB-expressed groups (Fig. 4). The diffuse and intestinal subtypes of cancer with high POLB expression and high immune score show better survival outcomes. This observation suggested that POLB-overexpressed tumor exhibited with high mutation frequency associated with high microsatellite score may contribute for tumor neoantigen-based immunogenicity in the tumor microenvironment and better survival outcomes (Fig. 4 and Supplement Figure 1). Considering that gastric cancer often goes undetected until advanced stages and has a poor prognosis, pinpointing the molecular markers and mechanisms driving its progression is crucial. Therefore, we investigated the prognostic significance of POLB expression in GC patients. We found that higher expression of mRNA of POLB shows a statistically significant difference compared to those with control with high AUC value of with high sensitivity and specificity. Our study indicated positive correlation between POLB expression and progression-free analysis. This finding suggests that POLB expression might not significantly extend the overall lifespan of GC patients, but it could enhance the progression-free survival.

Conclusion

In summary, this study provides key evidence that POLB overexpression influences tumor immune infiltrates and genes involved in innate immune signaling. The altered POLB-mediated BER pathway in tumors may directly affect the efficacy of immune checkpoint inhibitor by affecting immunogenicity, immune cell infiltration, and the related regulating molecules. Finally, observations based on TCGA-STAD data and GSE13861 represent correlations, and the causality between POLB expression and tumor immune response requires mechanistic investigations. POLB-overexpressed tumors harbor relatively low innate immune signaling genes expression that may contribute in suppressing antitumor immunity of human tumors, raising the possibility that targeting POLB genes may represent a novel therapeutic strategy for future immune-based therapy. Even though our data show high sensitivity and specificity (Fig. 4), there is a potential to improve the prognostic value with joint score using other DNA repair genes in stomach cancer in the future work. Furthermore, it indicates that the AUC diagnostic value of tumor markers to differentiate intestinal and diffuse subtype in the prognosis of gastric cancer needs to be further improved. Overall, additional in vitro and in vivo research is needed to analyze the relationship between POLB overexpression and gastric cancer to uncover the molecular mechanism of tumor immunogenicity. A better understanding of the relationship between dysregulated POLB expression and tumor microenvironment would facilitate efforts in the future to select patients based on the POLB expression for prognostic purpose and optimize the efficacy of immune-based therapy. Furthermore, our data suggest that a systemic understanding of the DNA repair landscape in a tumor may help assess the tumor's vulnerability to immune-based therapy. We recognized that this study has some limitations that excluded patients with a z score ranging between −0.5 and 0.5 which may impact the interpretation of the result. In addition, we acknowledge the curation of different genomic dataset from different source leads to several diverse outcomes including patient populations, tumor heterogeneity and experimental conditions that inherent to each dataset may have played a role that resulted in variations in correlation strength between POLB expression and innate immune signaling genes as well as immune checkpoint genes. However, our understanding from analysis of these datasets suggests that POLB expression is corelated with innate immune signaling in different subtypes of stomach cancer, with varying degrees of correlation across the TCGA and GEO datasets. Furthermore, future in vitro and in vivo study required to provide experimental evidence to further explore the molecular mechanism of how dysregulated POLB regulates gastric tumor immunogenicity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10238-024-01498-7.

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Author contributions A. S. participated in writing MS and data analysis; D.K. performed data analysis, interpretation, and manuscript writing. All authors read and approved the manuscript.

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Data availability All extracted data from cBioPortal (www.cBioPortal.org), The Cancer Genome Atlas datasets and GEO database used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval Not applicable for this study.

Consent to participate Not applicable for this study.

Consent for publication Not applicable for this study.

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