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Positive Correlation between PMS2 Deficiency and PD-L1 Expression in Pancreatic Cancer

Cailing Jiang[1], Chunyan Dang[1], Ruilong He[1], Limin Cheng[2], Yiying Bai[1], Xinyu Bai[1], Xin Wang[1], Qianhui Chen[1], Hongbin Yang[3], Zhengxin Zhang[4], Xiaotong Zhang[3], Yan Chen[4], Qian Xu[4], Lei Liu[3*], Li Zhang[1*]

[1]Department of Oncology, The Affiliated Hospital of Chengde Medical University, Chengde, China
[2]Department of Hepatobiliary Surgery, The Affiliated Hospital of Chengde Medical University, Chengde, China
[3]Department of Immunology, Chengde Medical University, Chengde, China
[4]Department of Pathology, Chengde Medical University, Chengde, China
Email: 1103470411@qq.com, 489384479@qq.com, chinahrl@126.com, chenglimin1972@163.com, 1085924089@qq.com, 2961373360@qq.com, 1144935430@qq.com, 1430275373@qq.com, 1747723679@qq.com, z13069456262@163.com, 754600854@qq.com, 543636594@qq.com, 349412667@qq.com, *homingreceptor@hotmail.com, *cd_zhangl@126.com

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Abstract

Background: Pancreatic cancer is one of the most lethal types of cancer, and immunotherapy has become a promising remedy with advancements in tumor immunology. However, predicting the clinical response to immunotherapy in pancreatic cancer remains a dilemma for clinicians. **Methods:** GEPIA database was used to analyze the differential expression of MMR and PD-L1 genes in 33 common cancer types including pancreatic cancer. The expression levels of MMR and PD-L1 genes were downloaded from the GEPIA and GEO databases to analyze the correlation between MMR genes and PD-L1, and the clinicopathological and survival information were downloaded from the TCGA databases to analyze the relationship between the expression of MMR, PD-L1 and clinicopathological characteristics, prognosis. Meanwhile, the tumor tissue samples of 41 patients with pancreatic cancer were collected, and the protein expression levels of MMR and PD-L1 were detected by immunohistochemical assay. Furthermore, we analyzed the correlation between MMR and PD-L1, and the correlation between the expression of MMR, PD-L1 and clinicopathological characteristics, prognosis of pancreatic cancer patients. **Results:** Bioinformatics analysis showed that MLH1, MLH3, MSH2, MSH3, and PMS2 were highly expressed in most cancer types including pancreatic cancer ($P < 0.05$). TCGA data revealed that MLH1 expression was related to gender ($P = 0.012$), clinical stage (I vs II: $P = 0.016$), MSH2 expression was related to clinical stage ($P < 0.05$), T stage (T3 vs T4: $P = 0.039$), and MSH3 expression was related to T stage ($P < 0.05$). Besides, both MSH2 expression ($P < 0.001$) and MSH6 ($P = 0.044$) were significantly associated with

prognosis. GEPIA data also showed that MSH2 expression was related to prognosis ($P = 0.008$). The correlation analysis revealed that the expressions MSH2, MLH1, PMS2 had strong correlations with PD-L1 both in GEPIA and GEO databases. Real-world data indicated that of the 41 pancreatic cancer patients, 5 cases had MLH1 deletion, 5 cases had MSH2 deletion, 4 cases had PMS2 deletion, and 12 cases had PD-L1 positive expression. Notably, PMS2 deletion was associated with PD-L1 positive expression ($P = 0.035$). In addition, MLH1 was related to clinical stage ($P = 0.033$), age ($P = 0.048$), and MSH2 was related to clinical stage ($P = 0.033$). However, MLH1 ($P = 0.697$), MSH2 ($P = 0.956$), PMS2 ($P = 0.341$), and PD-L1 ($P = 0.734$) appeared to have no impact on overall survival among patients with pancreatic cancer. **Conclusion:** Both bioinformatics and real-world data showed that there were correlation between PMS2 deletion and PD-L1 expression, and correlation between MLH1, MSH2 and clinical stage.

Keywords

Pancreatic Cancer, PD-L1, PMS2, Mismatch Repair Protein, Correlation

1. Introduction

Pancreatic cancer (PC) is one of the most lethal digestive system malignant tumors in the world. The latest epidemiological data show that PC has the sixth highest rate of mortality in China [1], and will have the second highest mortality rate in the United States in 2030 [2]. Surgical resection is the preferred treatment for early-stage PC, however, most patients are diagnosed at an advanced stage, leading to a 5-year survival rate of 5% [3]. For patients with advanced or inoperable PC, chemotherapy is one of the main first-line treatments, but resistance occurs commonly. Therefore, effective treatment for PC is urgently needed [4].

Immunotherapy, an innovative therapeutic method that treats cancer by evoking anti-tumor immunity, is a promising strategy for the treatment of solid tumors and hematologic malignancies [5]. By expressing programmed death-1 (PD-1) ligands, PD-L1 and PD-L2, tumor cells and antigen-presenting cells suppress T cell immune responses via PD-1/PD-L1 interaction, leading to immune escape and tumorigenesis [6]. To our knowledge, PD-L1 expression has been detected in approximately 30% of PC cases, which means that at least 30% of patients should theoretically have a favorable response to PD-1/PD-L1 inhibitors [7]. However, clinical trial evidence shows that the expected efficacy has not been achieved in immunotherapy for PC, and anti-PD-1/PD-L1 monotherapy is almost ineffective [8].

Studies have demonstrated that mismatch repair (MMR) deficiency serves as a potential indicator of response to PD-1/PD-L1 inhibitors and a prognostic molecular marker in patients with cancer [9]. MMR protein is present within cells with proliferative capability, playing a role in DNA mismatch repair during

DNA replication. In the human MMR gene family, MSH2, MSH6, MLH1, and PMS2 are key components of mismatch repair, while MSH3, PMS1, and MLH3 play complementary roles [10]. MMR-deficient cells and tumors display high microsatellite instability (MSI-H) which induces the production of more gene mutations and tumor neoantigens. Due to the abnormal structure of the neoantigens that may be recognized by cells of the innate and adaptive immune system, which in turn generate the anti-tumor immune response. On the other hand, the immunosuppressive factors activate the PD-1/PD-L1 pathway in the tumor microenvironment, which reduces the anti-tumor activity of T cells. The blockade of PD-1 or PD-L1 will relieve T cell immunosuppression in the tumor microenvironment and further inhibit tumor growth. In the past, several studies have demonstrated that MMR status may be a predictive factor for the response to PD-1/PD-L1 inhibition therapy based on bioinformatics and real-world data [11]. However, far less MMR status in PC has been reported. In the present study, based on bioinformatics and real-world data, we analyzed the correlation between MMR and PD-L1, and the correlation between MMR, PD-L1 and clinicopathological characteristics, prognosis in PC, thus providing a theoretical basis for the clinical application of immune checkpoint inhibitors in the treatment of PC patients.

2. Material & Methods

2.1. Data Sources and Online Analysis

Gene Expression Profiling Interactive Analysis (GEPIA) is an interactive web server for analyzing RNA sequencing expression data of tumors and normal samples from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects. The website was used to analyze the differential expression, the correlation between MMR and PD-L1, and the prognosis of MMR and PD-L1. The screening conditions are $|\log_2 FC| > 1$ and P-value < 0.05. The expression of MMR, PD-L1 (from RNA-seq data), and the clinical data of patients with PC were extracted at TCGA-Pancreatic Adenocarcinoma (TCGA-PAAD) cohort (https://cancergenome.nih.gov/). The GSE62452 dataset is available in the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The relationship between MMR and PD-L1, the relationships between MMR, PD-L1 and clinicopathological characteristics, prognosis was analyzed by using the ggpubr package, survival package, and surviviner package in R language.

2.2. Patients and Clinical Data

From 2018 to 2022, the tissue samples from 41 patients with PC at the Affiliated Hospital of Chengde Medical University, Chengde, China were collected for this retrospective study. Participants consisted of 19 males and 22 females between the ages of 46 and 79 (mean age 63). Tumor locations were as follows: the head of the pancreas (23 patients) and the body and tail of pancreas (18 patients). The clinical stage of patients was 29 in stage I + II and 12 in stage III + IV. According to the tumor, lymph node, and metastasis (TNM) staging, 34 cases were stage T1

or T2, and 7 cases were T3 or T4. N staging described 12 patients with lymph node metastasis and 29 patients without lymph node metastasis. Metastases were present in 11 cases and absent in 30. In all 41 samples, 16 cases were obtained from surgically resected tumors and 25 cases were obtained from puncture pathological examination. Follow-up was conducted by telephone interviews until September 30, 2022 or death. The study had been approved by the Medical Ethics Committee of the Affiliated Hospital of Chengde Medical University and written informed consent had been obtained from every patient.

2.3. Immunohistochemical Assay

Tumors were fixed in 10% formalin, dehydrated using a graded ethanol series, embedded in paraffin blocks, and sectioned into 4 μm thick slices. Sections were de-waxed, dehydrated, and treated in 5% hydrogen peroxide in alcohol to block endogenous peroxidase activity. Antigen retrieval was performed according to the particular antibody to be used. Next, the sections were incubated with the antibodies for PD-L1 (1:100, Abcam), MSH2 (1:100, Abcam), PMS2 (1:100, Abcam), and MLH1 (1:100, Abcam). The slides were then incubated with peroxidase-conjugated secondary antibody (ZSGB-BIO; Beijing, China), and 3,3'-diaminobenzidine (DAB) reagent was used as the chromogen. For PD-L1 staining, cell surface membrane staining > 1% was considered positive [12]. Samples showing complete loss of nuclear staining for each MMR protein were defined as deficient for MMR proteins. Adjacent stromal cells and inflammatory cells with intact nuclear staining served as positive controls.

2.4. Statistical Analysis

SPSS22.0 software was used for statistical analysis. All count data were expressed as ratios and compared using the chi-square test or Fisher exact probability method. Correlations were assessed by a Spearman test. Survival curves were calculated by the Kaplan-Meier method. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Expression of MMR and PD-L1 in the GEPIA Database

The expression profiles of MMR genes (MLH1, MSH2, MSH6, PMS2, MSH3, PMS1, MLH3) and PD-L1 were identified in 33 common cancer types including PC by GEPIA, and the results showed that MLH1, MLH3, MSH2, MSH3, and PMS2 were highly expressed in a variety of tumors, including PC ($P < 0.05$). While there was no significant difference between PC and normal tissue for MSH6, PMS1, and PD-L1 genes ($P > 0.05$, **Figure 1**).

3.2. Correlation between MMR, PD-L1 and Clinicopathological Characteristics of PC in the TCGA

The expression of MLH1 had relationships with sex ($P = 0.012$), and clinical

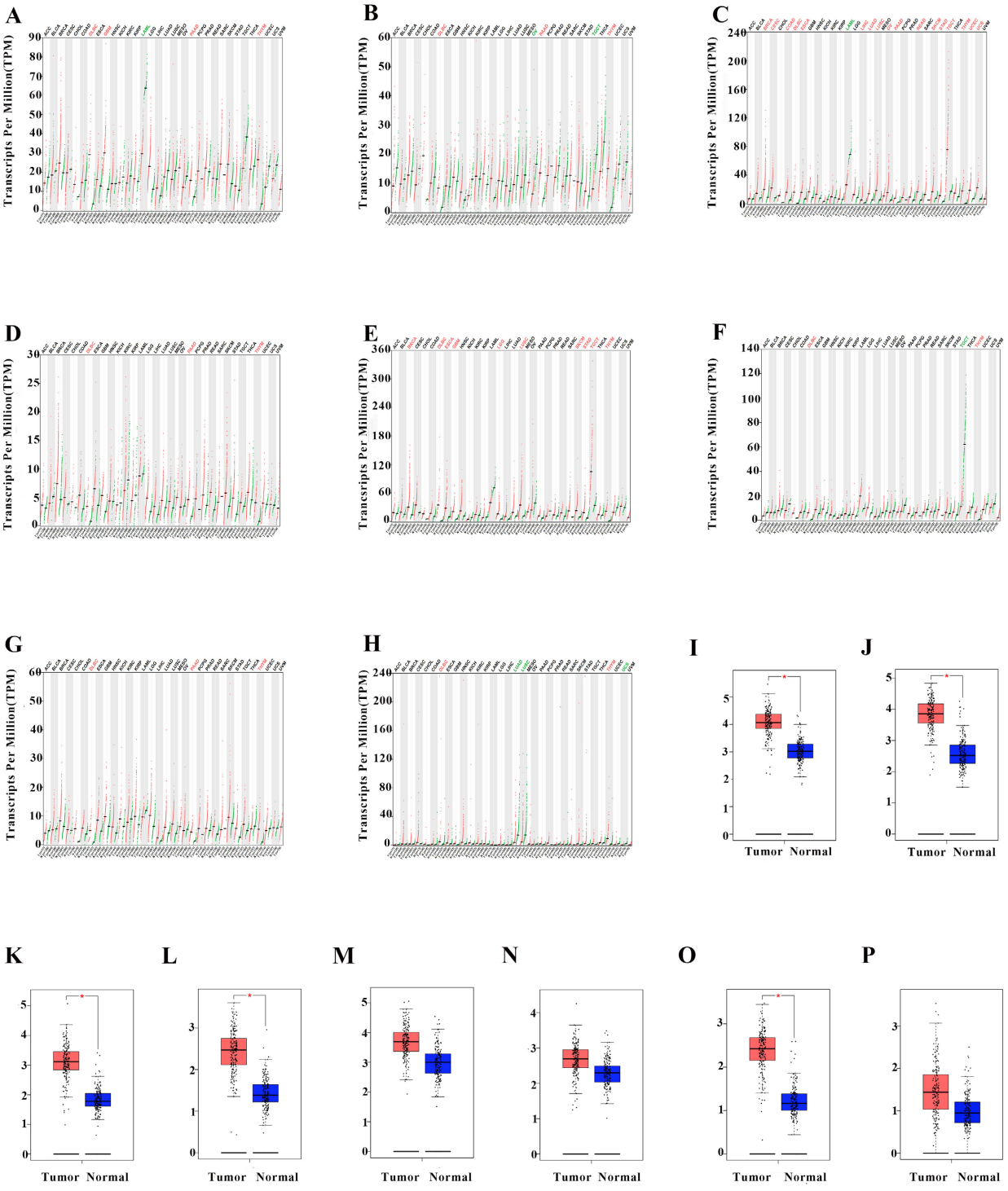

Figure 1. Expression of MMR and PD-L1 in the GEPIA database. (A)-(H). The differential expression analysis of MLH1 (A), MLH3 (B), MSH2 (C), MSH3 (D), MSH6 (E), PMS1 (F), PMS2 (G), and PD-L1 (H) in 33 common cancer types. (I)-(P). The differential expression of MLH1 (I), MLH3 (J), MSH2 (K), MSH3 (L), MSH6 (M), PMS1 (N), PMS2 (O), and PD-L1 (P) between PC and normal tissues. *$P < 0.05$, compared to normal.

stage (stage I vs stage II: $P = 0.016$), while had no relationship with age ($P = 0.730$), T stage ($P > 0.05$), lymph node metastasis ($P > 0.05$), or distant metasta-

sis (P = 0.760). MSH2 was related to the clinical stage (stage I vs stage III: P = 0.023; stage II vs stage III: P = 0.041), and T stage (T3 vs T4: P = 0.039), while was not related to gender (P = 0.950), age (P = 0.660), lymph node metastasis (P = 0.940), and distant metastasis (P = 0.510). MSH3 was related to the T stage (T1 vs T2: P = 0.048; T1 vs T3: P = 0.027; T1 vs T4: P = 0.033), while was not related to sex (P = 0.330), age (P = 0.900), clinical stage (P > 0.05), lymph node metastasis (P = 0.720), and distant metastasis (P = 0.580). The correlations were not observed between MLH3, MSH6, PMS1, PMS2, PD-L1 and clinicopathological characteristics (P > 0.05, Figure 2).

3.3. Correlation between MMR, PD-L1 and Prognosis of PC in the TCGA and the GEPIA

The survival analysis results showed that the PC patients with high level MSH2 had a shorter overall survival time based on both the TCGA database (P < 0.001) and the GEPIA database (P = 0.008). Besides, the survival-associated data from the TCGA-PAAD dataset also suggested that high MSH6 expression had poorer overall survival (P = 0.044, Figure 3). However, the other five MMR genes (MLH1, MS2, MSH3, PMS1 MLH3) and PD-L1 had no relationship with the prognosis of patients with PC (P > 0.05).

3.4. Correlation between MMR and PD-L1 in GEPIA and GEO Databases

As shown in Figure 4, based on the GEPIA database, there were correlations between all seven MMR genes and PD-L1 (P < 0.05). Since correlation coefficient greater than 0.5 is defined as a significant correlation, MSH2, MLH1, and PMS2 strongly correlated with PD-L1. The same results were obtained from dataset GSE62452 in the GEO database. Therefore, the three MMR genes (MLH1, MSH2, and PMS2) and PD-L1 were used for subsequent real-world studies.

3.5. Expression of MLH1, MSH2, PMS2 and PD-L1 in the Real-World Study

The expression levels of the MLH1, MSH2, PMS2, and PD-L1 proteins in tissue samples from 41 patients with PC were detected by immunohistochemistry, and the results showed that in 41 cases, MLH1 (Figures 5(A)-(C)), MSH2 (Figures 5(D)-(F)), and PMS2 (Figures 5(G)-(I)) were absent in 5 (12.195%), 5 (12.195%), and 4 (9.756%) cases, respectively, and present in 36 (87.805%), 36 (87.805%), and 37 (90.244%), respectively. Twelve of these patients were PD-L1-positive (defined as > 1% PD-L1$^+$ tumor cells; Figures 5(J)-(L)).

3.6. Correlation between MLH1, MSH2, PMS2 and PD-L1 in the Real-World Study

As shown in Table 1, the PD-L1-positive rate was 75% (3/4) in the PMS2-deficient group, higher than that in the PMS2-proficient group (24.3%, 9/37), suggesting that PD-L1 positivity was significantly associated with PMS2 deficiency

(P = 0.035). While there were no correlations between PD-L1 and MLH1 (P = 0.112), MSH2 (P = 0.585), based on the data from the 41 PC.

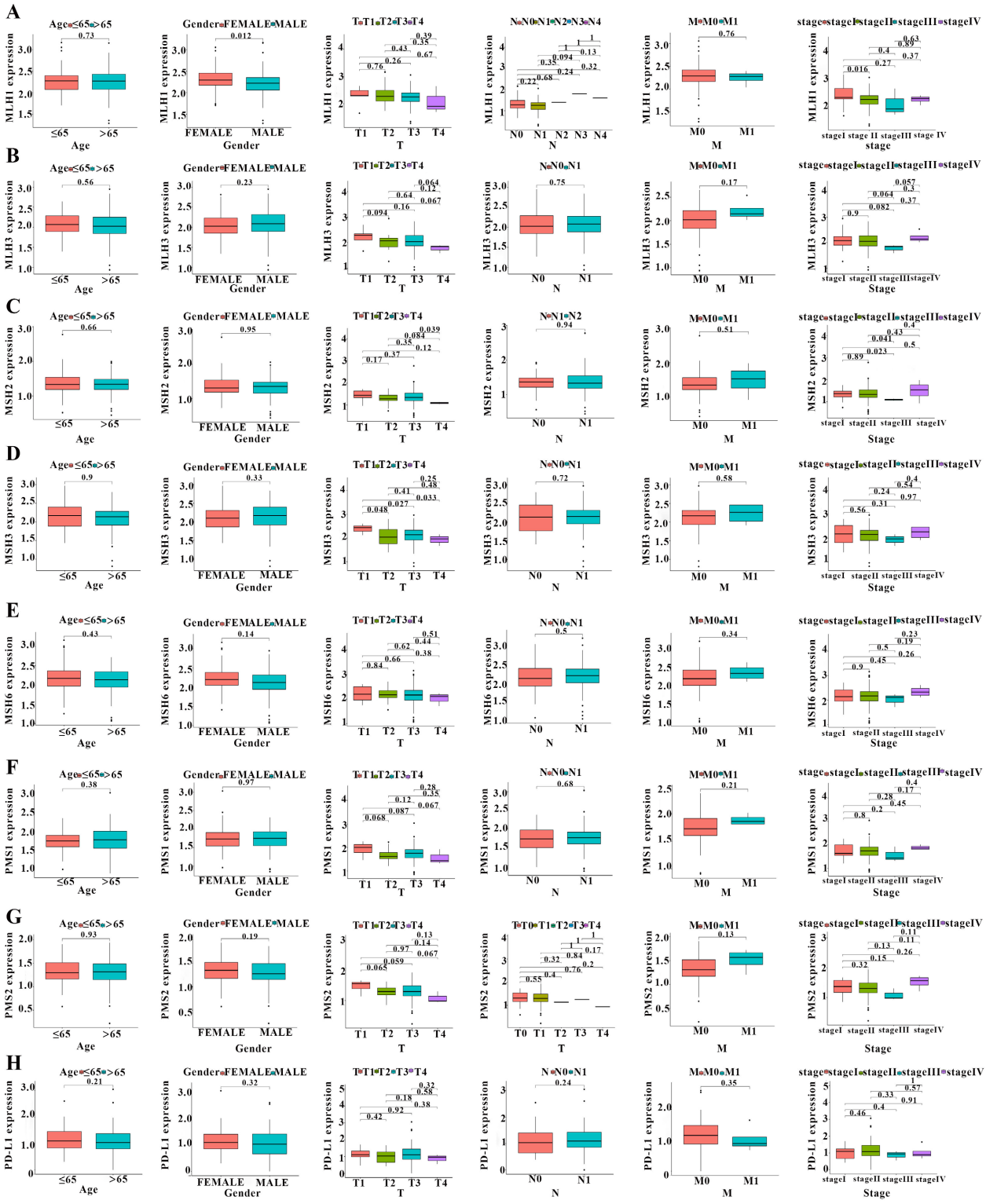

Figure 2. Correlation between MMR, PD-L1 and clinicopathological characteristics of PC in the TCGA. (A). MLH1; (B). MLH3, (C). MSH2, (D). MSH3, (E). MSH6, (F). PMS1, (G). PMS2, (H). PD-L1.

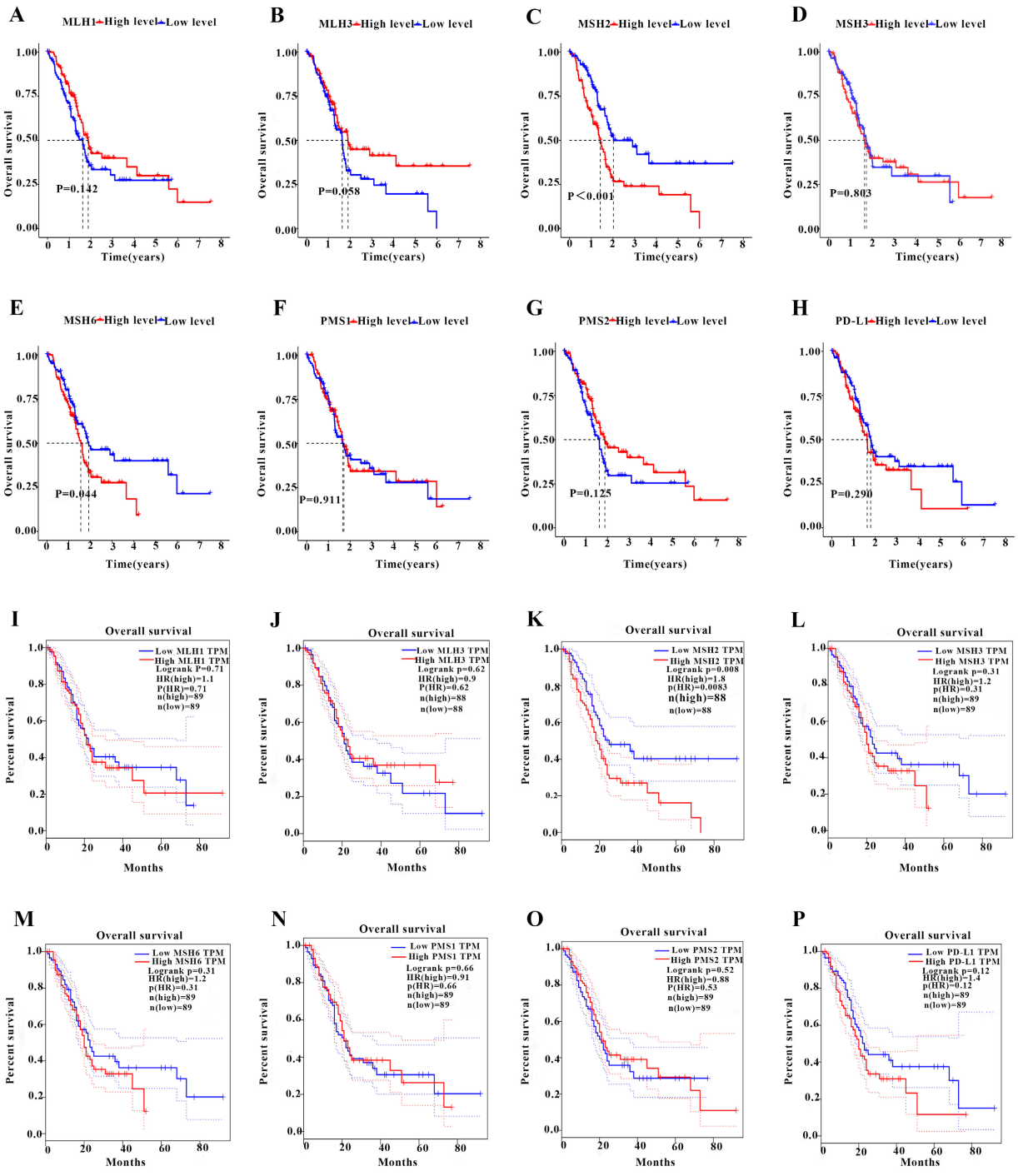

Figure 3. Correlation between MMR, PD-L1 and prognosis of PC in the TCGA and the GEPIA. (A)-(H). The relationship between MLH1 (A), MLH3 (B), MSH2 (C), MSH3 (D), MSH6 (E), PMS1 (F), PMS2 (G), and PD-L1 (H) with the survival in the TCGA database. (I)-(P). The relationship between MLH1 (I), MLH3 (J), MSH2 (K), MSH3 (L), MSH6 (M), PMS1 (N), PMS2 (O), and PD-L1 (P) with the survival in the GEPIA database.

3.7. Correlation between MLH1, MSH2, PMS2, PD-L1 and the Clinicopathological Characteristics, Prognosis in the Real-World

As shown in Table 2, both MLH1 ($P = 0.033$) and MSH2 ($P = 0.033$) had rela-

tionships with clinical stage, and MLH1 was related to age (P = 0.048). While MLH1 and MSH2 had no relationship with the other clinicopathological characteristics (P > 0.05). As for PMS2 and PD-L1, there was no correlation between the two genes and all the clinicopathological characteristics of the 41 PC (P > 0.05).

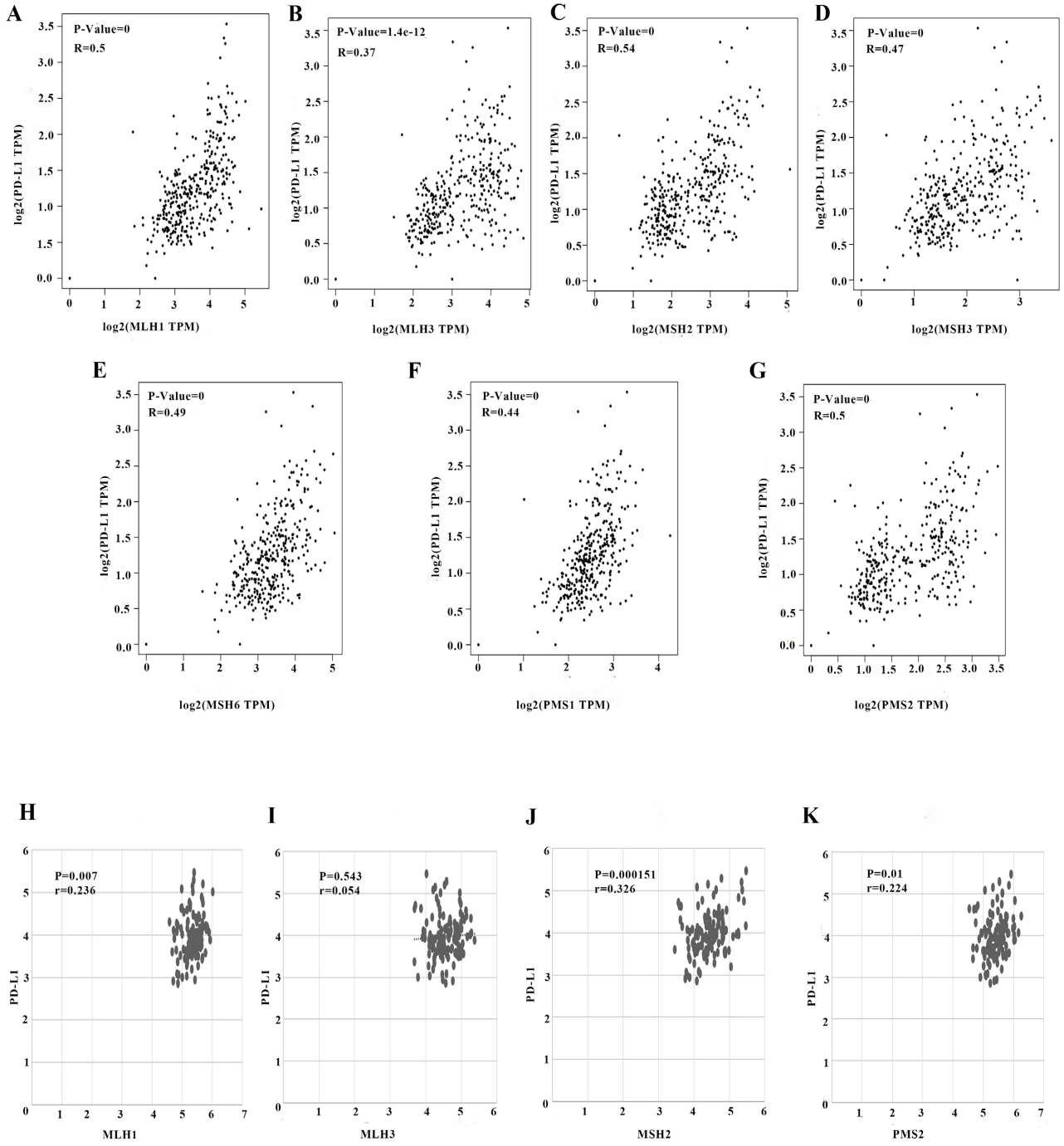

Figure 4. Correlation between MMR and PD-L1 in GEPIA and GEO databases. (A)-(G). The relationship between MLH1 (A), MLH3 (B), MSH2 (C), MSH3 (D), MSH6 (E), PMS1 (F), PMS2 (G) and PD-L1 in the GEPIA database. (H)-(K). The relationship between MLH1 (H), MLH3 (I), MSH2 (J), PMS2 (K) and PD-L1 in the GEO database.

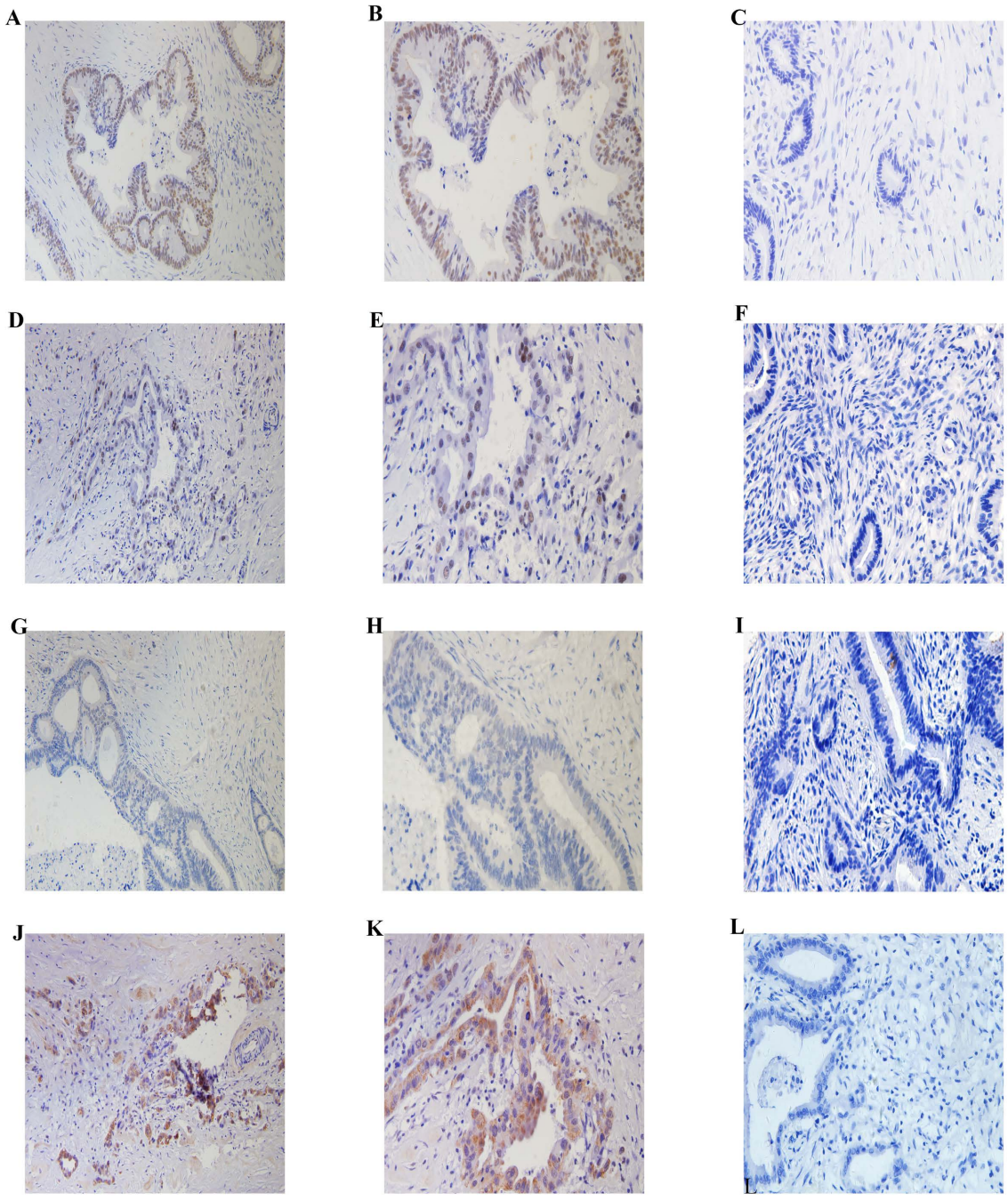

Figure 5. Expressions of MLH1, MSH2, PMS2 and PD-L1 were examined by immunohistochemistry method in 41 tissue samples from PC patients. (A)-(C). MLH1; (D)-(F). MSH2; (G)-(I). PMS2; (J)-(L). PD-L1. (A), (D), (G), (J). Positive results (×200). (B), (E), (H), (K). Positive results (×400); (C), (F), (I), (L). Negative result (×400).

Table 1. Correlation between MMR and PD-L1 in 41 PC patients.

group		MLH1		r	*P*	MSH2		r	*P*	PMS2		r	*P*
		−	+			−	+			−	+		
PD-L1	−	2 (40)	27 (75)	−0.252	0.112	3 (60)	26 (72.2)	−0.088	0.585	1 (25)	28 (75.7)	−3.30	0.035
	+	3 (60)	9 (25)			2 (40)	10 (27.8)			3 (75)	9 (24.3)		

Table 2. Correlations between the expressions of MMR, PD-L1 and clinicopathological features of 41 PC patients.

Feature	MLH1		χ^2	P	MSH2		χ^2	P	PMS2		χ^2	P	PD-L1		χ^2	P
	+[n (%)]	−[n (%)]			+[n (%)]	−[n (%)]			+[n (%)]	−[n (%)]			+[n (%)]	−[n (%)]		
Gender																
Male	16 (84.2)	3 (15.8)	0.031	0.861	15 (78.9)	4 (21.1)	1.282	0.258	16 (84.2)	3 (15.8)	0.465	0.495	6 (31.6)	13 (68.4)	0.000	1.000
Female	20 (90.9)	2 (9.1)			21 (95.5)	1 (4.5)			21 (95.5)	1 (4.5)			6 (27.3)	16 (72.7)		
Age																
≥63	20 (100)	0	—	0.048	19 (95)	1 (5)	0.804	0.370	19 (95)	1 (5)	0.226	0.635	4 (20)	16 (80)	0.864	0.353
<63	16 (76.2)	5 (23.8)			17 (81)	4 (19)			18 (85.7)	3 (14.3)			8 (38.1)	13 (61.9)		
Stage																
I + II	28 (96.6)	1 (3.4)	4.564	0.033	28 (96.6)	1 (3.4)	4.564	0.033	28 (96.6)	1 (3.4)	2.364	0.124	9 (31)	20 (69)	0.000	0.993
III + IV	8 (66.7)	4 (33.3)			8 (66.7)	4 (33.3)			9 (75)	3 (25)			3 (25)	9 (75)		
T Stage																
T1 + T2	31 (91.2)	3 (8.8)	0.672	0.412	31 (91.2)	3 (8.8)	0.672	0.412	31 (91.2)	3 (8.8)	0.000	1.000	10 (29.4)	24 (70.6)	0.000	1.000
T3 + T4	5 (71.4)	2 (28.6)			5 (71.4)	2 (28.6)			6 (85.7)	1 (14.3)			2 (28.6)	5 (71.4)		
N Stage																
N1	9 (75)	3 (25)	1.182	0.277	11 (91.7)	1 (8.3)	0.000	1.000	11 (91.7)	1 (8.3)	0.000	1.000	3 (25)	9 (75)	0.000	0.993
N0	27 (93.1)	2 (6.9)			25 (86.2)	4 (13.8)			26 (89.7)	3 (10.3)			9 (31)	20 (69)		
M Stage																
M1	8 (72.7)	3 (27.3)	1.557	0.212	8 (72.7)	3 (27.3)	1.557	0.212	9 (81.8)	2 (18.2)	0.257	0.612	2 (18.2)	9 (81.8)	0.311	0.577
M0	28 (93.3)	2 (6.7)			28 (93.3)	2 (6.7)			28 (93.3)	2 (6.7)			10 (33.3)	20 (66.7)		
Tumor locations																
The head of the pancreas	22 (95.7)	1 (4.3)	1.575	0.210	20 (87)	3 (13)	0.000	1.000	22 (95.7)	1 (44.3)	0.622	0.430	6 (26.1)	17 (73.9)	0.026	0.873
The Body and tail of pancreas	14 (77.8)	4 (22.2)			16 (88.9)	2 (11.1)			15 (83.3)	3 (16.7)			6 (33.3)	12 (66.7)		
source																
surgery	15 (93.8)	1 (6.3)	0.195	0.659	13 (81.3)	3 (18.8)	0.288	0.591	14 (87.5)	2 (12.5)	0.000	1.000	4 (25)	12 (75)	0.017	0.898
puncture	21 (84)	4 (16)			23 (92)	2 (8)			23 (92)	2 (8)			8 (32)	17 (68)		

Further, the correlations between MLH1, MSH2, PMS2, PD-L1 and the prognosis of 41 cases were explored. The follow-up time was up to September 30, 2022, with the median follow-up time of 9.2 months (range 1 - 25 months). A total of 12 cases (29.268%) were alive whereas 29 cases (70.732%) were dead. The survival analysis demonstrated that the expression of MLH1 (P = 0.697), MSH2 (P = 0.956), PMS2 (P = 0.341), and PD-L1 (P = 0.734) did not affect the overall survival of PC patients (**Figure 6**).

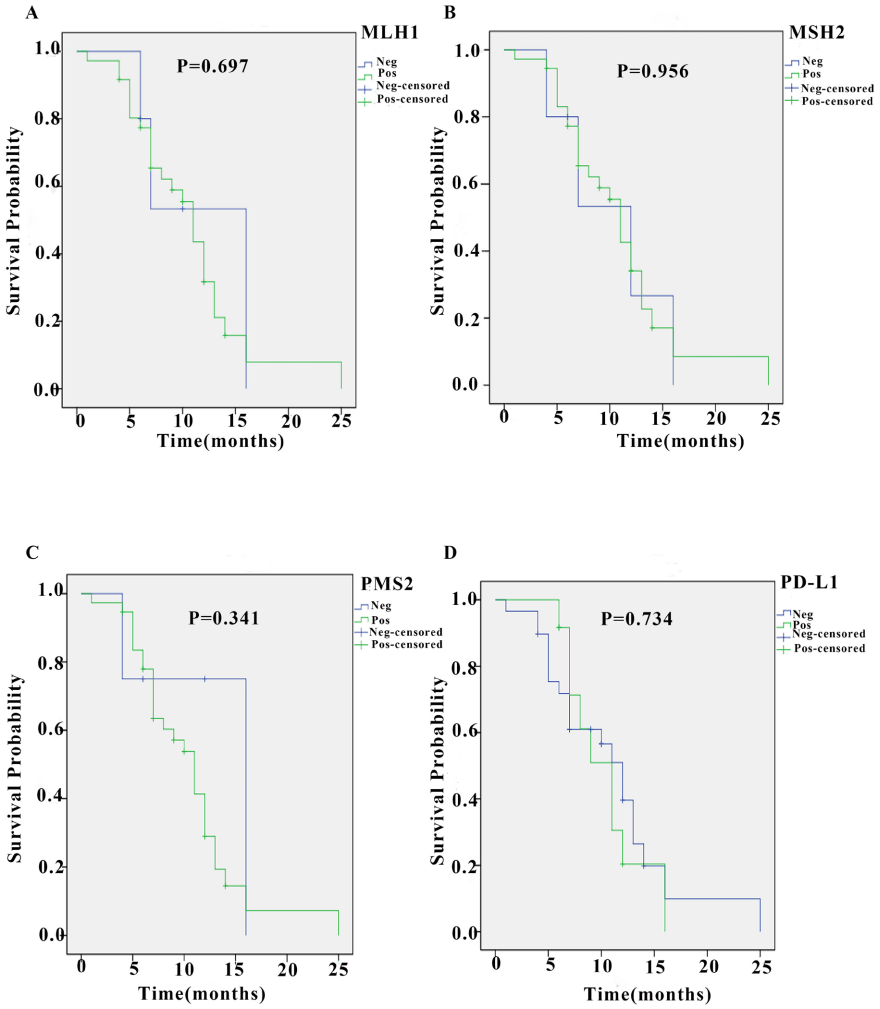

Figure 6. Kaplan-Meier survival curves for 41 PC patients. (A). MLH1; (B). MSH2; (C). PMS2; (D). PD-L1.

4. Discussion

The difficulty to detect in the early clinical stage and the lack of specific biomarkers to predict disease progression make the precise treatment for PC a conundrum. Therefore, the pathogenesis, treatment, and prognostic marker of PC will be the focus of future studies. With the development of precision medicine, immunotherapy has become a promising treatment method. Recent studies have shown that PD-L1 is up-regulated in various malignant tumors including non-small cell lung cancer, head and neck squamous cell carcinoma, breast cancer, urothelial carcinoma, ovarian cancer, colorectal cancer, gastric cancer, and PC, etc. [13], and PD-L1 can bind the PD-1 receptor on tumor-infiltrating lymphocytes to induce T-cell tolerance and promote cancer cell survival [14]. PD-1/PD-L1 inhibitor, as a blockade of the PD-1/PD-L1 pathway, restores effector T-cell function and enhances anti-tumor immune responses. Compared with traditional schemes, immune checkpoint inhibitor has provided significant clinical benefit for various types of cancer, and the survival rate of patients has been

significantly improved [15].

ESMO guidelines state that higher PD-L1 expression is correlated with MMR deficiency (dMMR) status in multiple cancers [16]. Kim *et al.* [17] evaluate both PD-L1 and MLH1/MSH2 expression in 365 patients with advanced gastrointestinal (GI) cancers, genitourinary (GU) cancers, or rare cancers, and found there was a significant association between the PD-L1 expression and MLH1/MSH2 loss. Svensson *et al.* [18] found that high PD-L1 expression on both tumor cells and tumor-infiltrating lymphocytes, but not PD-1 expression, was significantly associated with dMMR. Several clinical trials have demonstrated that the expression levels of PD-L1 protein in the patients with dMMR were significantly increased, moreover, these patients achieved higher clinical remission rates after immunotherapy than patients with intact MMR [19] [20] [21]. Böger *et al.* found that the expression of PD-L1 on tumor cells is related to dMMR, that is, PD-L1+/dMMR may be more likely to benefit from immunotherapy [22]. The present study also found that the loss of PMS2 was significantly associated with PD-L1 positive expression, suggesting that PMS2 may be a biomarker for PD-1/PD-L1 inhibitor treatment in PC.

Based on bioinformatics databases and 41 PC tissue samples in the real world, this study analyzed the correlations between MLH1, MSH2, PMS2, PD-L1 and clinicopathological characteristics, prognosis in PC patients. The analysis results showed that the PD-L1 expression was not related to clinicopathological characteristics and prognosis. Saif *et al.* [23] also found that there were no correlations between PD-L1 expression and age, gender, clinical stage, smoking status, and tumor histology in non-small cell lung cancer. A meta-analysis of 10,310 cancer patients concluded that overexpression of PD-L1 was associated with poor prognosis in multiple solid tumors [24]. However, research regarding the association between PD-L1 and prognosis in PC patients is limited and controversial. Birnbaum's study showed that PD-L1 upregulation was not associated with clinicopathological features such as patients' age and sex, pathological type, tumor size, lymph node status, and grade, but was associated with shorter disease-free survival and overall survival [25]. In a study including 68 patients with PC, Guo *et al.* [26] reported that PD-L1 expression level in poorly differentiated PC was higher than that in well or moderately differentiated ones, and there was no significant correlation between PD-L1 expression status and overall survival in patients with PC.

Besides, this study found that in the 41 cases of PC, there were 5 cases (12.195%) of MLH1 deletion, 5 cases (12.195%) of MSH2 deletion, and 4 cases (9.756%) of PMS2 deletion. Tomaszewska *et al.* [24] detected MMR genes in 30 tissues from PC patients by IHC, and the results showed that in all cases MMR gene expression was present [27]. Rizay *et al.* [28] and Eatrides *et al.* [29] detected tissue microarrays by IHC, comprising 265 and 109 PC patients, respectively, and found that the proportions of PC patients with dMMR were 15.5% and 22%, respectively.

In addition, the results of this study based on both bioinformatic and real-world

data analysis suggested there was a correction between MLH1 and clinical stage. Chen *et al.* [30] reported that the deletion of MMR was significantly different in pTNM stage, tumor differentiation degree and lymph node metastasis, and MMR deletion in young and middle-aged patients with sporadic gastric cancer was more common in patients with stage III and low differentiation. Jin *et al.* [31] found that the expression loss rate of MSH2 protein was 18. 6% in the FIGO stage III, and there was significant difference. Similar results have been found by Yoo *et al.*, who investigated the associations between tumoral MSH2 immunohistochemical expression and clinicopathological parameters, and the results demonstrated that T stage was significantly higher in the MSH2-negative group than in the MSH2 positive-group, suggesting that MSH2 protein expression may be a useful marker for predicting TNM stage [32].

In conclusion, PD-1/PD-L1 inhibitors display better effectiveness for PC patients with dMMR status, and the present study suggested that PMS2 deletion may be a biomarker of response for immunotherapy. In this study, we used bioinformatics analysis software and websites to analyze the relationships between the 7 MMR-related genes and PD-L1, as well as clinicopathological characteristics, prognosis in PC patients. Moreover, the protein expressions of three MMR genes (MLH1, MSH2, PMS2) and PD-L1 were detected in 41 tissue samples from PC patients, and the correlations between the three MMR genes and PD-L1, clinicopathological characteristics were analyzed. The common results from bioinformatics and the real world showed that there were correlations between PMS2 deficiency and PD-L1 expression, and correlations between MLH1, MSH2 and clinical stage. Due to the limited number of tissue samples and no relationship between MSH6 and PD-L1 based on the bioinformatics results, the expression of MSH6 was not involved in the real-world study, which needs to be researched further.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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