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梅北, 慎也

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Original article

Clinicopathological significance of microsatellite instability and immune escape mechanism in patients with gastric solid-type poorly differentiated adenocarcinoma

Shinya Umekita, MD[a], Daisuke Kiyozawa, MD, PhD[a], Kenichi Kohashi, MD, PhD[b], Shinichiro Kawatoko, MD, PhD[c], Taisuke Sasaki, MD, PhD[d], Eikichi Ihara, MD, PhD[d], Eiji Oki, MD, PhD[e], Masafumi Nakamura, MD, PhD[f], Yoshihiro Ogawa, MD, PhD[d] and Yoshinao Oda, MD, PhD[a]

[a]*Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

[b]*Department of Pathology, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan*

[c]*Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

[d]*Department of Medicine and Bioregulatory, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

[e]*Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

[f]*Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*

Corresponding author and requests for reprints: Yoshinao Oda, Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan

E-mail: oda.yoshinao.389@m.kyushu-u.ac.jp

Tel: +81-92-642-6061, FAX: +81-92-642-5968

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Abstract

Background In gastric solid-type poorly differentiated adenocarcinoma (PDA), the role of microsatellite instability and immune escape mechanism remains unclear. The current study aimed to elucidate the clinical significance of mismatch repair (MMR) status, genome profile, C-X-C motif chemokine receptor 2 (CXCR2) expression, and myeloid-derived suppressor cell (MDSC) infiltration in solid-type PDA.

Methods In total, 102 primary solid-type PDA cases were retrieved, and classified into 46 deficient-MMR (dMMR) and 56 proficient-MMR (pMMR) cases based on immunohistochemistry (IHC) and polymerase chain reaction-based molecular testing results. The mRNA expression profiles (NanoString nCounter Assay) of stage-matched dMMR (n = 6) and pMMR (n = 6) cases were examined. The CXCR2 expression and MDSC infiltration (CD11b- and CD33-positive cells) were investigated via IHC in all solid-type PDA cases.

Results mRNA analysis revealed several differentially expressed genes and differences in biological behavior between the dMMR (n = 46) and pMMR (n = 56) groups. In the multivariate analysis, the dMMR status was significantly associated with a longer disease-free survival (hazard ratio = 5.152, $p = 0.002$) and overall survival (OS) (hazard ratio = 5.050, $p = 0.005$). CXCR2-high expression was significantly correlated with a shorter OS in the dMMR group ($p = 0.018$). A high infiltration of CD11b- and CD33-positive cells was significantly correlated with a shorter OS in the pMMR group ($p = 0.022$, 0.016, respectively).

Conclusions dMMR status can be a useful prognostic predictor, and CXCR2 and MDSCs can be novel therapeutic targets in patients with solid-type PDA.

Mini abstract

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Keywords: PDA, MSI, CXCR2, MDSC

Introduction

Solid-type poorly differentiated adenocarcinoma (PDA) is a subtype of gastric cancer (GC) based on the Japanese Classification of GC [1]. Microscopically, solid-type PDA exhibits a sheet-like proliferation of polygonal tumor cells arranged in a solid and expansive growth pattern with scanty stroma. Solid-type PDA is often associated with prominent tumor-infiltrating lymphocytes and Crohn's-like lymphoid reaction [2]. Solid-type PDA has a relatively indolent clinical course [3-5]. However, the prognostic factors of solid-type PDA remain unclear.

According to recent studies, solid-type PDA is frequently associated with microsatellite instability (MSI) [6-8]. MSI GC is a molecular subtype based on The Cancer Genome Atlas (TCGA) classification of GC [9]. Its clinical features (including older age, female sex, and tumoral location [predominant lower third of the stomach]) differ from those of microsatellite-stable (MSS) GC [10]. MSI GC is associated with a lower risk of lymph node metastasis and pathological T stage compared with MSS GC. Hence it has a favorable prognosis [10,11]. Based on these clinicopathological differences, MSI GC and MSS GC are distinct from each other. In addition, several studies have reported that MSI GC has different genetic characteristics from MSS GC [12,13]. However, in patients with solid-type PDA, the clinical significance of MSI, and differentially expressed genes (DEGs) between the MSI and MSS group has not been identified.

In the tumor immune microenvironment of MSI-high tumors, the role of the immune escape mechanism in predicting tumor behavior and prognosis has gained attention [14-16]. C-X-C motif

chemokine receptor 2 (CXCR-2) and myeloid-derived suppressor cells (MDSCs) play an important role in immune escape mechanism. CXCR2 is a chemokine receptor, and expressed in various immune cells including neutrophils, mast cells, monocytes and macrophages [17]. CXCR2 promotes immune escape and chemoresistance by promoting tumor cell growth, angiogenesis and infiltration of other immunosuppressive cells [18,19]. MDSCs, a heterogeneous population of immunosuppressive cells including precursors for granulocytes, macrophages or dendritic cells [20], inhibit the activity of T cells and induce the production of immunosuppressive cells, such as Tregs. This mechanism results in escape from antitumor immune surveillance and the promotion of tumor growth [21]. A high expression of CXCR2 and increased MDSC infiltration were an independent predictor of worse prognosis in GC [19,20]. However, the clinical significance of CXCR2 and MDSCs in solid-type PDA should still be evaluated.

The current study aimed to elucidate the clinical significance of MSI, DEGs, CXCR2, and MDSCs in solid-type PDA.

Materials and methods

Case selection

We reviewed 3115 surgically resected GC cases, which were diagnosed from 2006 to 2021 at the Department of Anatomic Pathology. Among them, 136 presented with primary solid-type PDA. Cases with tumors containing signet ring cells and special histological types such as hepatoid

adenocarcinoma and neuroendocrine carcinoma, those pretreated with neoadjuvant chemotherapy and/or radiotherapy, and those with Lynch syndrome, were excluded from the analysis. Finally, 102 cases with primary solid-type PDA were selected.

Molecular subtyping according to the TCGA classification

According to the TCGA molecular classification, we classified 102 primary solid-type PDA cases into the MSI (deficient-mismatch proteins; dMMR) subtype, MSS (proficient-MMR; pMMR)/chromosomal instability (CIN) subtype, and Epstein-Barr virus-infected (EBV) subtype [6]. To identify the MSI subtype, immunohistochemistry (IHC) of the MMR proteins (MLH1, PMS2, MSH2 and MSH6), which are sensitive surrogate markers of MSI, was performed. Polymerase chain reaction (PCR)-based molecular testing was also conducted to assess for microsatellite status. PCR was conducted on cases involving the loss of MMR protein on IHC. For PCR analysis, DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue sections using the MSI Analysis Kit (FALCO Biosystems), as described in a previous study [11]. Analysis of EBV infection was performed via in situ hybridization (ISH), according to the manufacturer's instructions. The EBER probe (#Y5200, Dako) was detected using the PNA ISH Detection Kit (#K5201, Dako). Identifiable nuclear staining of EBER in > 95% of tumor cell nuclei was interpreted as EBV positive. Finally, 102 cases of solid-type PDA were classified into 46 dMMR- and 56 pMMR-solid-type PDA. None of the cases were classified as EBV subtype.

Gene expression analysis

To determine differences in gene expression between the dMMR and pMMR subtypes, mRNA analysis of stage-matched dMMR (n = 6) and pMMR (n = 6) cases was performed using the NanoString nCounter Assay. In both dMMR and pMMR groups, two cases were pStage I, two cases were pStageII, and two cases were pStageIII. Total RNA was isolated from the FFPE section using the CELLDATA RNAstorm FFPE RNA Extraction Kit (Cell Data Sciences, Fremont, CA, the USA), according to the manufacturer's instructions. The gene expression levels were assessed using 100 ng of the total RNA based on the manufacturer's protocol (NanoString Technologies, Seattle, WA, the USA) using the nCounter® Tumor Signaling 360 Panel, which included 760 genes covering the core pathways and processes of the tumor, tumor microenvironment, and tumor immune response, and 20 internal reference genes for data normalization (NanoString Technologies). Data were analyzed using the nSolver Analysis Software 4.0 (NanoString Technologies) and GeneSpring GX 14.8 (Agilent Technology, the USA) and compared to the controls. The expression values were log2-transformed for statistical analysis. The GeneSpring GX 14.8 was used to create the heat map and scatter plot. The differential expression analysis identified the genes with the most statistically significant increase or decrease in expression between the dMMR and pMMR groups. Genes with an adjusted p value of < 0.05 and a log2 fold change (log2FC) of > 1.5 were upregulated in pMMR. Genes with a log2FC of < 0.67 upregulated in

dMMR.

Functional enrichment analysis

Metascape (http://metascape.org) was used to perform functional enrichment analysis. The functional process and pathway, following the default, included the Canonical Pathway (MSigDB), Hallmark Gene Sets (MSigDB), Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway and Gene Oncology (GO). The parameters were set as follows: p value of < 0.01, minimum count of 3, and enrichment factor of > 1.5.

Immunohistochemical analysis and scoring

For immunohistochemical analysis, the FFPE tumor tissues of 102 solid-type PDA were sliced into 3 μm-thick sections. Immunohistochemical staining was performed using the universal immunoperoxidase polymer method (Envision Kit and EnVision Flex Kit; Dako, Tokyo). Antigen retrieval was conducted by heating the slides in 10-mM sodium citrate (pH 6.0) or Target Retrieval Solution (Dako, Carpinteria, CA) or ethylenediaminetetraacetic acid. After subtyping according to MMR expression (MLH1, PMS2, MSH2 and MSH6), immunohistochemical staining of HER2, CXCR2, CD11b and CD33 was performed. Supplementary Table 1 shows the primary antibodies and staining conditions utilized in this study. Four MMR proteins (MLH1, PMS2, MSH2 and MSH6) were considered as “complete loss” when nuclear staining in tumor cells was completely

absent. The internal positive control was stromal cells. dMMR was defined as one or more MMR proteins with complete loss in tumor cells. pMMR was defined as retention of all four MMR proteins [11]. HER2 expression with strong complete or basolateral membranous staining in ≥ 10% of the neoplastic cells was considered as positive. CXCR2-positive stromal cells were scanned at a magnification of ×400. The immunohistochemical score of CXCR2 was calculated by multiplying the percentage of positive cells (P) with intensity (I) (H = P × I). The ranges for P and I were 0 %–100 % and 0–3 (0, no staining; 1, weak; 2, moderate; and 3, strong), respectively. Figure 1a–d presents the representative images of CXCR2. To identify intratumor location with the highest CXCR2 expression, the expression status of CXCR2 at the center of the tumor, invasive front of the tumor, and peritumoral normal tissues, was examined individually. To analyze MDSC infiltration, anti-CD11b and anti-CD33 antibodies were selected based on previous studies [22, 23]. The number of CD11b-positive (Fig. 1e) and CD33-positive (Fig. 1f) cells at five different high-power fields (×400) was counted. Next, the mean number was calculated. Each median value (CXCR2 score at the tumor center: 80, CD11b count: 82, and CD33 count: 10) was used as the cutoff point to categorize high and low groups. The immunohistochemical staining results of each sample were independently evaluated by three pathologists (S.U., D.K., and Y.O.) without knowledge of the clinical data.

Statistical analysis

All statistical analyses were performed using the JMP Statistical Discovery Software (version Pro 16; SAS Institute, Cary, NC, the USA). Statistical analyses were performed using the Pearson χ^2, Fisher's exact test or Wilcoxon's test. Disease-free-survival (DFS) was defined as the time from surgery to the date when a new lymph node or distal metastasis was detected or the last follow-up, excluding four cases diagnosed as pathological Stage IV. Overall survival (OS) was defined as the time from surgery to the time of the last follow-up or death from GC. Survival curves were calculated based on the Kaplan-–Meier method and were tested statistically using the log-rank test. The Cox proportional hazard regression model was used to perform univariate and multivariate analyses of several factors associated with DFS and OS. A p value of < 0.05 was considered statistically significant.

Results

Molecular subtyping of solid-type PDA according to the TCGA classification

Via IHC of the four MMR proteins (MLH1/PMS2/MSH2/MSH6), multiplex PCR, and EBER-ISH, all 102 cases were classified into two subtypes (dMMR-solid-type-PDA [n = 46] and pMMR-solid-type-PDA [n = 56]) based on the MMR expression status. None of the 102 cases were classified as the EBV-subtype. Supplementary Table 2 shows the results of the IHC of MMR proteins and EBER-ISH. All 46 dMMR cases had a concurrent loss of MLH1/PMS2. The

expressions of MSH2 and MSH6 were preserved in all cases. Via multiplex PCR analysis, 37 of 46 dMMR cases were confirmed to be MSI-high. In total, 9 of 46 cases could not be analyzed via multiplex PCR because of insufficient DNA quality or the level of PCR amplification.

Association between MMR status and clinicopathological characteristics/prognosis

To identify the clinical significance of MMR status, we analyzed the association between MMR status and clinicopathological characteristics/prognosis. The status of dMMR was significantly correlated with older age ($p = 0.021$), female sex ($p < 0.001$), lower third location ($p < 0.001$), absence of vascular invasion ($p = 0.017$), and HER2-negativity ($p = 0.031$) (Table 1). Some cases were treated using adjuvant chemotherapy such as TS-1 in both dMMR and pMMR groups (dMMR 13/46, pMMR 31/56, $p < 0.009$); however, no cases received immune checkpoint inhibitors. Based on the log-rank test, dMMR status cases had significantly longer DFS ($p = 0.006$) and OS ($p = 0.008$) than cases with pMMR status (Fig. 2a, 2b).

Gene expression analysis

Among the 760 genes in the Tumor Signaling 360 Panel, 55 genes were significantly upregulated in dMMR. Meanwhile, 20 genes were significantly upregulated in pMMR. The scatter plot showed upregulated DEGs in dMMR (x-axis) and pMMR (y-axis) (Supplementary Fig. 1a). The 20 upregulated DEGs in pMMR were plotted on the upper left, and the 55 upregulated DEGs in

dMMR were plotted on the lower right. The heat maps generated using the 20 upregulated DEGs in pMMR and 55 DEGs in dMMR showed a separation between the pMMR and dMMR groups (Supplementary Fig. 1b). The gene involved in preventing immune destruction and tumor-promoting inflammation were mainly upregulated in the dMMR group (Supplementary Table 3). In the pMMR-group, the gene involved in sustaining proliferative signaling, and activating invasion and metastasis was mainly upregulated (Supplementary Table 4).

Functional enrichment analysis

Enriched Ontology Clusters by Metascape showed that the top five most significant biological processes in dMMR were the regulation of lymphocyte activation, cytokine signaling in the immune system, leukocyte activation, PID IL12 2PATHWAY, and regulation of immune effector process (Fig. 3). The top three most significant biological processes in pMMR were the pathways in cancer, regulation of muscle cell differentiation, and NABA CORE MATRISOME (Fig. 3).

Differences in CXCR2 immunohistochemical score according to intratumor location

To identify intratumor location with the highest CXCR2 expression, each CXCR2 immunohistochemical score at the center of the tumor, in the invasive front of the tumor, and peritumoral normal tissues, was calculated. The CXCR2 score at the center of the tumor (range: 1–210, median score: 80) was significantly higher than that at the invasive front of the tumor (range:

1–180, median score: 15) and peritumoral normal tissues (range: 0–20, median score: 2) in both the dMMR (Supplementary Fig. 2a) and pMMR (Supplementary Fig. 2b) groups. Therefore, the CXCR2 score of the center of the tumor was used.

Differences in CXCR2 immunohistochemical score according to MMR status

The CXCR2 score between the dMMR and pMMR groups was compared (Supplementary Fig. 3). The dMMR group (range: 2–210, median score: 95) had a significantly higher CXCR2 score than the pMMR group (range: 1–180, median score: 70) ($p = 0.035$).

Association between CXCR2 status and clinicopathological characteristics/prognosis

The association between CXCR2 status and clinicopathological characteristics/prognosis was analyzed. The median CXCR2 score (80) was used as the cutoff point for categorizing high and low groups.

CXCR2-high status was associated with a larger tumor size ($p = 0.003$), higher pathological tumor stage ($p = 0.002$), pathological node stage ($p < 0.001$), and pathological stage ($p < 0.001$), and presence of lymphatic invasion ($p = 0.032$) (Supplementary Table 5). Based on the log-rank test, CXCR2-high status cases had significantly shorter DFS ($p = 0.044$, Supplementary Fig. 4a) and OS ($p = 0.011$, Supplementary Fig. 4b) than CXCR2-low status cases.

Differences in the mean number of CD11b and CD33 according to MMR status

The mean number of CD11b- and CD33-positive cells between the dMMR and pMMR groups was compared. In CD11b, there was no significant difference between the dMMR (range: 19–198, median number: 87) and pMMR (range: 8–190, median number: 77) groups ($p = 0.135$) (Supplementary Fig. 5a). The dMMR group (range: 1–47, median number: 15) had a significantly higher mean CD33 count than the pMMR group (range: 1–28, median number 9) ($p = 0.041$) (Supplementary Fig. 5b).

Association between CD11b and CD33 status and clinicopathological characteristics/prognosis

The association between CD11b and CD33 status and clinicopathological characteristics/prognosis was analyzed. Each median number (CD11b: 82, CD33: 10) was used as the cutoff point for categorizing high and low groups.

CD11b-high status was associated with a higher pathological tumor stage ($p = 0.008$) and the presence of lymphatic invasion ($p = 0.021$) (Supplementary Table 6). Based on the log-rank test, CD11b-high status cases had significantly shorter DFS ($p = 0.033$, Supplementary Fig. 6a) and OS ($p = 0.009$, Supplementary Fig. 6b) than CD11b-low status cases.

CD33-high status was associated with a higher pathological tumor stage ($p = 0.011$), and pathological stage ($p = 0.006$), and the presence of lymphatic invasion ($p = 0.005$) (Supplementary Table 7). According to the log-rank test, CD33-high status cases had significantly shorter OS ($p =$

0.043, Supplementary Fig. 7b) than CD33-low status cases.

Survival analysis according to the combination of CXCR2 and CD11b or CD33 status in patients with solid-type PDA

The association between the combination of CXCR2/CD11b or CD33 status and prognosis was analyzed. The OS of CXCR2-high/CD11b-high status cases was significantly shorter than CXCR2-low/CD11b-low status cases ($p = 0.016$) (Supplementary Fig.8a). Additionally, the OS of CXCR2-high/CD33-high status cases was significantly shorter than that CXCR2-low/CD33-low status cases ($p = 0.012$) (Supplementary Fig. 8b).

Univariate and multivariate analysis in patients with solid-type PDA

To analyze the independent predicters of solid-type PDA, univariate and multivariate analyses were conducted. The univariate analysis revealed a significant association between a shorter DFS and a larger tumor size, higher pathological tumor stage, pathological node stage, and pathological stage, presence of lymphatic invasion, pMMR status, and CXCR2-high and CD11b-high status (Table 2). According to the multivariate analysis, pMMR status had a significant effect on DFS (HR = 5.152, $p = 0.002$) (Table 2). The univariate analysis revealed a significant association between a shorter OS and a larger tumor size, higher pathological tumor stage, pathological node stage, and pathological stage, presence of lymphatic invasion, pMMR status, and CXCR2-high, CD11b-high

and CD33-high status (Table 3). Based on the multivariate analysis, pMMR status had a significant effect on OS (HR = 5.050, $p = 0.005$) and tumor size (HR: 4.041, $p = 0.018$) (Table 3).

Survival analysis of the CXCR2, CD11b and CD33 status in the dMMR and pMMR group

The prognostic significance of the CXCR2, CD11b and CD33 status in the dMMR and pMMR group was analyzed individually. Based on the log-rank test, CXCR2-high status was significantly correlated with a shorter OS than CXCR2-low status in the dMMR group ($p = 0.018$), but not in the pMMR group ($p = 0.086$) (Fig. 4a, 4b). CD11b- and CD33-high status was significantly correlated with a shorter OS in the pMMR group, respectively ($p = 0.022$ vs $p = 0.016$) (Fig. 4d, 4f), but not in the dMMR group ($p = 0.170$ vs $p = 0.403$) (Fig.4c, 4e).

Discussion

This study showed the clinical significance of microsatellite instability and immune escape mechanism in solid-type PDA via molecular subtyping, gene expression analysis and IHC. dMMR status was detected in 45% (46/102) of solid-type PDA, and was correlated with older age, female sex and lower third location. Several DEGs and significant biological processes between the dMMR and pMMR groups were identified. The immune escape mechanism, particularly, CXCR2-high in the dMMR group, and MDSC-high in the pMMR group, was correlated with unfavorable prognosis. Multivariate analysis showed that dMMR status was an independent prognostic factor of

favorable prognosis.

To the best of our knowledge, this is the first report showing that dMMR status was an independent prognostic factor of favorable prognosis in patients with solid-type PDA. A growing body of evidence has revealed that dMMR status can be a predictor of better survival in patients with GC compared with MSI-negative (pMMR) status [11, 24]. Based on our study, the subclassification according to MMR status could be useful for predicting prognosis in patients with solid-type PDA.

In this study, the frequency of dMMR in solid-type PDA was 45%. Moreover, it was correlated with older age, female sex and lower third location, and this finding was almost consistent with that of previous studies [2, 3-7, 25, 26]. Other studies have shown that solid-type PDA has peculiar clinicopathological and molecular characteristics such as lower third location [4, 7], expanding growth pattern [5-7], tumor-infiltrating lymphocytes (TILs) [5, 6], venous invasion [3, 4, 7], and frequently MSI [5-7, 10, 25]. The reported frequencies of dMMR in solid-type PDA are 40.1% (7/17 cases) [5], 51.6% (16/31 cases) [2] and 40% (23/57 cases) [26]. Further, there is a significant association between dMMR and older age, female sex, lower third location and TILs compared to pMMR-solid-type PDA [2, 6].

We identified DEGs between the dMMR and pMMR groups in solid-type PDA. In the dMMR group, the expression of genes preventing immune destruction and tumor-promoting inflammation was significantly upregulated. Functional enrichment analysis revealed significant biological

processes including the regulation of lymphocyte activation and cytokine signaling in the immune system in the dMMR group. These findings were almost consistent with those of a previous report [14]. In addition, the IL12 pathway was a significant biological process in dMMR. To the best of our knowledge, the relevance of the IL12 pathway in dMMR-solid-type PDA has not been assessed. The IL12 pathway had anti-proliferative and pro-apoptotic effects by increasing interferon-gamma production [27]. These mechanisms can be associated with the favorable clinical course of dMMR-solid-type PDA. In the pMMR group, the expression of genes sustaining proliferative signaling, and activating invasion and metastasis was significantly upregulated. The significant biological processes included the core matrisome, which is composed of extracellular matrix proteins, and can be associated with biological processes involving EMT, angiogenesis, hypoxia, inflammation and poor prognosis in several cancers including GC [28]. Therefore, the core matrisome can be associated with the aggressiveness of pMMR-solid-type PDA.

The current study focused on the prognostic impact of immune escape mechanism (CXCR2 and MDSCs) in solid-type PDA. Moreover, it first showed that CXCR2-high in the dMMR group, and MDSC-high in the pMMR group, were correlated with an unfavorable prognosis in patients with solid-type PDA. Previous studies have reported the association between tumor cell CXCR2 expression and poor prognosis [18, 19, 29]. Other studies have revealed that the CXCR2 expression in stromal cells at the invasive front of the tumor was an independent prognostic factor of GC [30]. Chemokine/CXCR2 signaling could enhance the recruitment of MDSCs [31, 32]. Moreover,

MDSCs inhibit the anticancer activity of immune checkpoint inhibitors [15]. Therefore, the combination of ICIs and target therapy for CXCR2 or MDSCs may improve the prognosis of solid-type PDA.

The current study had several limitations in addition to its retrospective nature and small-sized cohort. First, the MSI status of 56 pMMR cases was not analyzed, and the MSI status could not be obtained from nine cases in the dMMR group due to poor sample quality. Second, we could not accurately examine whether the gene expression reflected tumor or stromal cells, since we could not separate tumor and stromal cells in FFPE tumor tissues.

Conclusions

This study first revealed that dMMR status was an independent factor of favorable prognosis in patients with solid-type PDA. Several DEGs and the significant biological processes of dMMR- or pMMR-solid-type PDA were identified. The expression of CXCR2 and MDSC infiltration (CD11b and CD33) were poor prognostic factors of dMMR- and pMMR-solid-type PDA, respectively.

These findings may pave the way for developing predictive factors and novel therapeutic strategies for patients with solid-type PDA.

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Author contributions

SU performed the research and wrote the paper. DK and KK contributed to the research design and slide review. SK, TS, EI, EO, MN and YO contributed to the sample collection and research design. YO designed the research and provided the final approval of the manuscript. All authors critically reviewed and approved the manuscript. YO, who is the guarantor, is responsible for the overall content of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. It was approved by the Medical Human Investigation Committee of Kyushu University (institutional Review Board no. 2020-476). Informed consent was obtained from all patients.

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Figure legend

Fig. 1 Representative immunohistochemical images of CXCR2 (a, negative; b, weak; c, moderate; and d, strong), CD11b+ (e), and CD33+ (f) in solid-type poorly differentiated adenocarcinoma (PDA).

Fig. 2 Kaplan-–Meier curves of disease-free survival (a) and overall survival (b) according to MMR status in patients with solid-type PDA. Patients with dMMR status had a significantly longer DFS ($p = 0.006$) and OS ($p = 0.008$) than those with pMMR status.

Fig. 3 Enriched Ontology Clusters by Metascape. After all statistically enriched terms (such as GO/KEGG terms, canonical pathways, and hall mark gene sets) were identified, accumulative hypergeometric p-values and enrichment factors were calculated and used for filtering. The remaining significant terms were then hierarchically clustered into a tree based on Kappa-statistical similarities among the gene memberships. Then a kappa score of 0.3 was applied as the threshold to cast the tree into term clusters.

Fig. 4 Kaplan-–Meier curves of overall survival according to CXCR2 (a, b), CD11 (c, d) and CD33 (e, f) status in the dMMR (a, c, e) and pMMR (b, d, f) groups in solid-type PDA.

a. In the dMMR group, CXCR2-high status was significantly correlated with a shorter OS

compared with CXCR2-low status ($p = 0.018$).

b. In the pMMR group, there was no significant association between CXCR2-high and CXCR2-low status ($p = 0.086$).

c. In the dMMR group, there was no significant association between CD11b-high and CD11b-low status ($p = 0.170$).

d. In the pMMR group, CD11b-high status was significantly more correlated with a shorter OS compared with CD11b-low status ($p = 0.022$).

e. In the dMMR group, there was no significant association between CD33-high and CD33-low status ($p = 0.403$).

f. In the pMMR group, CD33-high status was significantly more correlated with a shorter OS compared with CD33-low status ($p = 0.016$).