Tumor microenvironment classification based on T-cell infiltration and PD-L1 in patients with mismatch repair-proficient and -deficient colorectal cancer

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Abstract. The classification of tumor microenvironments according to the presence or absence of tumor infiltrating lymphocytes (TILs) and programmed death ligand-1 (PD-L1) expression has been used to predict the efficacy of immune checkpoint inhibitor antibodies in several cancer types, not including colorectal cancer (CRC). The current study investigated the TIL/PD-L1 status of patients with CRC, particularly patients who presented as mismatch repair-proficient (pMMR) and mismatch repair-deficient (dMMR). A total of 243 patients with CRC were enrolled and defined as pMMR (121 patients) or dMMR (122 patients). Using Pearson’s χ² test and multivariable multinomial logistic regression analysis, the associations between MMR status, TIL presence and PD-L1 expression were investigated, in addition to the association between TIL/PD-L1 status and clinicopathological features. The results demonstrated that the dMMR group more frequently exhibited TIL+ (85/122 vs. 61/121) and PD-L1+ (49/122 vs. 32/121) phenotypes compared with the pMMR group. PD-L1+ expression was identified in 42.4% of TIL+ cases in the dMMR group, while only 18.0% of TIL+ cases were PD-L1+ in the pMMR group.

High programmed death-1 expression and dMMR status were revealed as two independent risk factors for TIL+ PD-L1+ status. In conclusion, compared with the pMMR group, the dMMR group was more likely to present with a TIL+ PD-L1+ status, which suggests that a TIL+ PD-L1+ tumor microenvironment may partly contribute to the improved response of dMMR patients to anti-PD-1/L1 therapy.

Introduction

Cancer immunotherapy has gained interest due to the clinical efficacy of immune checkpoint inhibitors, including antibodies targeting programmed death-1 (PD-1), programmed death ligand-1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), in a number of cancer types, including melanoma, colorectal cancer (CRC), renal cell carcinoma and non-small cell lung carcinoma (1-4). Identification of factors that could predict the curative effect of anti-PD-1/L1 therapy is a major challenge. Mismatch repair protein (MMR) status has been reported to be a primary indicator for anti-PD-1/L1 therapy in patients with CRC, as only MMR-deficient (dMMR) patients are promising candidates (2). However, to the best of our knowledge, it is unknown why only dMMR patients are sensitive to anti-PD-1/L1 therapy. In addition, only certain dMMR patients benefit from the treatment (2), which suggests that other predictive factors should also be explored.

The tumor microenvironment is a complicated system that has the dual function of promoting and inhibiting tumor growth, which therefore influences drug response. The tumor microenvironment is composed of non-tumor cells, cytokines, chemokines and the extracellular matrix. Immune cells, particularly acquired immune cells, including T and B lymphocytes, are important non-tumor cells (5). The current study primarily focused on tumor infiltrating lymphocytes (TILs), crucial acquired immune cells, and PD-L1 expressed by tumor cells, which negatively affects the function of TILs. In human melanoma, the classification of tumor microenvironments based on TIL presence and PD-L1 expression has been proposed to...
The intensity of the lymphocyte infiltrate was scored as follows: Score 0, none; score 1, isolated (<5% of lymphocytes); score 2, moderate (5-50% of lymphocytes; and score 3, severe (>50% of lymphocytes). Populations with scores of 0 and 1 were considered as negative and ≥5% was considered as positive (3,14). PD-1 expression in TILs was scored as follows: Score 0, none (0% of lymphocytes); score 1, isolated (<5% of lymphocytes); score 2, moderate (5-50% of lymphocytes; and score 3, severe (>50% of lymphocytes). Scores of 0 and 1 were considered as low expression and score of 2 and 3 were considered as high expression (7,15).

Statistical analysis of the data. All statistical analyses were performed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Pearson's χ² test was used to assess the association between categorical variables. Multivariable multinomial logistic regression analysis was used for multivariate analysis to predict the odds ratio (OR) of individual factors for TIL-PD-L1 status. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. The clinicopathological characteristics of all 243 patients are presented in Table I. Patients were selected according to pMMR (121 patients) and dMMR (122 patients) status, including 189 patients with colon cancer (left, 90 patients; right, 99 patients) and 54 patients with rectum cancer. Table II presents the frequency of aberrant MMR protein expression in dMMR patients. The median age at the time of diagnosis was 60 years (range, 22-84 years),
Table I. Association analysis between MMR status and clinicopathological features using a $\chi^2$ test.

| Characteristic                    | pMMR, n (%)       | dMMR, n (%)       | C    | P-value |
|-----------------------------------|-------------------|-------------------|------|---------|
| Age at diagnosis, years           |                   |                   |      |         |
| <60                               | 54 (44.6)         | 78 (63.9)         | 0.190| 0.003   |
| ≥60                               | 67 (55.4)         | 44 (36.1)         |      |         |
| Sex                               |                   |                   | 0.22 |         |
| Male                              | 66 (54.5)         | 76 (62.3)         |      |         |
| Female                            | 55 (45.5)         | 46 (37.7)         |      |         |
| Primary tumor site                |                   |                   | 0.18 | 0.017   |
| Left colon                        | 55 (45.5)         | 35 (28.7)         |      |         |
| Right colon                       | 40 (33.1)         | 59 (48.4)         |      |         |
| Rectum                            | 26 (21.5)         | 28 (23.0)         |      |         |
| Stagea                            |                   |                   |      | 0.514   |
| I                                 | 22 (18.2)         | 26 (21.3)         | 0.204| 0.033   |
| II                                | 73 (60.3)         | 62 (50.8)         |      |         |
| III                               | 20 (16.5)         | 26 (21.3)         | 0.204| 0.033   |
| IV                                | 6 (5.0)           | 8 (6.6)           |      |         |
| Tumor stage                       |                   |                   | 0.204| 0.033   |
| T1                                | 6 (5.0)           | 5 (3.1)           |      |         |
| T2                                | 21 (17.4)         | 24 (14.7)         |      |         |
| T3                                | 82 (67.8)         | 63 (51.6)         |      |         |
| T4a                               | 9 (7.4)           | 22 (18.0)         |      |         |
| T4b                               | 3 (2.5)           | 8 (6.6)           |      |         |
| Node stage                        |                   |                   |      | 0.458   |
| Negative                          | 97 (80.2)         | 93 (76.2)         |      |         |
| Positive                          | 24 (19.8)         | 29 (23.8)         |      |         |
| Tumor histological grade          |                   |                   | 0.186| 0.033   |
| Well-differentiated               | 1 (0.8)           | 1 (0.8)           |      |         |
| Moderately differentiated         | 80 (66.1)         | 62 (50.8)         |      |         |
| Poorly differentiated             | 19 (15.7)         | 18 (14.8)         |      |         |
| Mucinous                          | 21 (17.4)         | 41 (33.6)         |      |         |
| Vascular invasion                 |                   |                   | 0.149| 0.019   |
| No                                | 106 (87.6)        | 117 (95.9)        |      |         |
| Yes                               | 15 (12.4)         | 5 (4.1)           |      |         |
| Nerve invasion                    |                   |                   | 0.179| 0.005   |
| No                                | 100 (82.6)        | 115 (94.3)        |      |         |
| Yes                               | 21 (17.4)         | 7 (5.7)           |      |         |
| NLRb                              |                   |                   |      | 0.699   |
| Low (≤5)                          | 109 (91.6)        | 110 (90.2)        |      |         |
| High (>5)                         | 10 (8.4)          | 12 (9.8)          |      |         |
| CRP, mg/l                         |                   |                   | 0.201| 0.001   |
| Low (≤5)                          | 85 (72.0)         | 63 (52.1)         |      |         |
| High (>5)                         | 33 (28.0)         | 58 (47.9)         |      |         |
| PD-1 expression                   |                   |                   | 0.805|         |
| Low                               | 43 (44.8)         | 40 (43.0)         |      |         |
| High                              | 53 (55.2)         | 53 (57.0)         |      |         |
| TIL expression                    |                   |                   | 0.193| 0.002   |
| TIL−                              | 60 (49.6)         | 37 (30.3)         |      |         |
| TIL+                              | 61 (50.4)         | 85 (69.7)         |      |         |
| PD-L1 expression                  |                   |                   | 0.144| 0.023   |
| PD-L1−                            | 89 (73.6)         | 73 (59.8)         |      |         |
| PD-L1+                            | 32 (26.4)         | 49 (40.2)         |      |         |

*According to the 7th edition of the American Joint Committee on Cancer staging system. bNLR data was missing for 2 patients. C, contingency coefficient; MMR, mismatch repair; pMMR, MMR-proficient; dMMR, MMR-deficient; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; PD-1, programmed death-1; TIL, tumor-infiltrating lymphocyte; PD-L1, programmed death ligand-1.
and 58.4% of patients were male and 41.6% were female. According to the American Joint Committee on Cancer (AJCC) 7th edition TNM staging system, pathologically confirmed stage I-IV disease was identified in 48 (19.8%), 135 (55.6%), 46 (18.9%) and 14 (5.8%) patients respectively. Moderately differentiated adenocarcinoma accounted for 58.4% of cases and well-differentiated adenocarcinoma accounted for 0.8% of all cases. A total of 20 (8.2%) and 28 (11.5%) patients exhibited vascular and nerve invasion, respectively.

Table II. Aberrant protein expression in MMR-deficient patients.

| Aberrant protein expression | Cases, n (%) |
|----------------------------|--------------|
| MMR (1)                    | 44 (36.1)    |
| MLH1                       | 9 (7.4)      |
| MSH2                       | 4 (3.3)      |
| MSH6                       | 25 (20.5)    |
| PMS2                       | 6 (4.9)      |
| MMR (2)                    | 68 (55.7)    |
| MMR (3)                    | 7 (5.7)      |
| MMR (4)                    | 3 (2.5)      |

MR (1), negative expression of 1 MMR protein; MMR (2), concurrent negative expression of 2 MMR proteins; MMR (3), concurrent negative expression of 3 MMR proteins; MMR (4), concurrent negative expression of 4 MMR proteins. MMR, mismatch repair; MLH1, MutL homolog 1; MSH2, MutS homolog 2; MSH6, MutS homolog 6; PMS2, PMS1 homolog 2.

Association between MMR status, TILs, PD-L1 expression and other clinicopathological features. Image obtained to determine TIL presence and PD-L1 expression are presented in Fig. 1. The presence of TILs was classified as positive or negative according to H&E staining. The degree of PD-L1 expression was based on IHC (negative, <5% positive tumor cells; positive, ≥5% positive tumor cells). The associations of MMR status with TIL presence and PD-L1 expression are presented in Table I. The dMMR group exhibited a significantly higher frequency of TIL* (85/122 vs. 61/121) and PD-L1+ expression (49/122 vs. 32/121) compared with the pMMR group (P<0.05). Other clinicopathological characteristics, including age, primary tumor site, tumor stage, histological grade, C-reactive protein (CRP) level, vascular invasion and nerve invasion were also significantly associated with MMR status (P<0.05).

Association between TIL/PD-L1 status and clinicopathological features. A total of 4 distinct groups were determined according to the presence of TILs and PD-L1: The presence of TILs and PD-L1 (TIL+ PD-L1+), the presence of TILs without PD-L1 (TIL+ PD-L1-), PD-L1 expression without TILs (TIL- PD-L1+) and the absence of TILs and PD-L1 expression (TIL- PD-L1-). The current study investigated whether TIL/PD-L1 status was associated with other clinicopathological features, particularly MMR status. As presented in Table IV, TIL/PD-L1 status was significantly associated with AJCC stage, tumor stage, node stage, tumor histological grade, nerve invasion, neutrophil lymphocyte ratio, CRP level, PD-1 expression and MMR status (P<0.05). These significantly associated features were then further analyzed by multivariable multinomial logistic regression analysis (Table V). The results demonstrated that PD-1 expression and MMR status were significantly associated with TIL/PD-L1 status. Taking TIL* PD-L1+ as a reference, low PD-1 expression compared with high expression was associated with a higher likelihood of presenting as TIL- PD-L1+ [OR, 10.473; 95% confidence interval (CI), 3.005-35.650; P<0.001] and TIL+ PD-L1- [OR, 3.443; 95% CI, 1.145-10.352; P=0.028]. PD-L1 expression status compared with dMMR status was associated with an increased likelihood of being TIL- PD-L1+ [OR, 11.536; 95% CI, 3.223-41.290; P<0.001] and TIL+ PD-L1- [OR, 4.718; 95% CI, 1.607-13.848; P=0.005]. Compared with the pMMR group, the dMMR group was more likely to present with a TIL* PD-L1+ status (76.6 vs. 23.4%; Table IV).

Discussion

Classification of tumor microenvironments according to the presence of TILs and PD-L1 expression has been established in certain cancer types, particularly human melanoma, and applied to predict the effect of anti-PD-1/L1 and select the optimum immunotherapy strategies (6,11). However, to the best of our knowledge, the occurrence of the four types of immune microenvironments in CRC and their association with MMR status remains to be investigated. The current study performed this investigation and revealed a difference in TIL/PD-L1 status between pMMR and dMMR groups, with dMMR patients more likely to exhibit a TIL* PD-L1+ tumor microenvironment.

Compared with the pMMR group, the dMMR group more frequently exhibited a TIL* PD-L1+ phenotype. Previous studies have also demonstrated that tumors from dMMR patients contain a greater TIL density and higher PD-L1 expression compared with tumors from pMMR patients (16-19). The extent of DNA mutation directly or indirectly correlates with the strength of immunogenicity in tumors (20); therefore, the higher density of TILs in dMMR tumors may be due to the accumulation of frameshift mutations and the production of neo-antigens, which activates the immune system of the host (21,22). TILs upregulate PD-L1 expression through the release of interferon-γ, which mediates an adaptive immune-resistance mechanism by inhibiting local effector T cell activity (6,23-25). This suggests that PD-L1 expression is an adaptive approach for tumor escape from cytokine-mediated T-cell killing.
accordance with previous studies, the current study revealed that PD-L1+ expression was significantly higher in the TIL+ dMMR group compared with that in the TIL+ pMMR group, which indicated that TILs may induce the expression of PD-L1 in the dMMR group. In addition, a negative association was identified between TILs and PD-L1 expression in the pMMR group, implying a complexity in the immune tolerance mechanism in this group that cannot be explained by the current study.

Previous studies have divided the tumor immune microenvironments into 4 groups namely TIL-PD-L1+, TIL+ PD-L1-, TIL- PD-L1+ and TIL- PD-L1-, according to PD-L1 expression and the presence or absence of TILs. The association of TILs with PD-L1 expression is considered to be more valuable for predicting the response to anti-PD-1/L1 therapy compared with TILs or PD-L1 expression alone (26). In TIL+ PD-L1+ tumors, a sufficient number of T cells exist inside the tumor that can induce adaptive expression of PD-L1, which in turn suppresses the function of T cells and may support an effective response to PD-1/L1 blockade therapy (6,7). TIL+ PD-L1- tumors account for ~20% of melanoma cases and contain TILs, but no PD-L1 expression, which indicates a lack of adaptive resistance and

Table III. Association analysis between TILs and PD-L1 expression in colorectal cancer cells.

| MMR status | TIL+, n (%) | TIL-, n (%) |
|------------|-------------|-------------|
|            | PD-L1+      | PD-L1-      | PD-L1+      | PD-L1-      | C   | P-value |
| pMMR       | 11 (18.0)   | 50 (82.0)   | 21 (35.0)   | 39 (65.0)   | 0.189 | 0.034   |
| dMMR       | 36 (42.4)   | 49 (57.6)   | 13 (35.1)   | 24 (64.9)   | 0.455 |         |
| All        | 47 (32.2)   | 99 (67.8)   | 34 (35.1)   | 63 (64.9)   | 0.643 |         |

MMR, mismatch repair; pMMR, MMR-proficient; dMMR, MMR-deficient; C, contingency coefficient; TIL, tumor-infiltrating lymphocyte; PD-L1, programmed death ligand-1.

Figure 1. Representative expression of TILs, PD-L1 and PD-1. (A) Absence and (B) presence of TILs at the central area of the tumor and the invasive margin. The presence of TILs was determined by hematoxylin and eosin staining. (C) Negative and (D) positive PD-L1 expression in tumor cells. (E) Low and (F) high expression of PD-1 by TILs. PD-L1 and PD-1 expression was determined by immunohistochemistry. Magnification, x200. TIL, tumor-infiltrating lymphocyte; PD-L1, programmed death ligand-1; PD-1, programmed death-1.
| Characteristic                  | TIL⁺ PD-L1⁺ | TIL⁺ PD-L1⁻ | TIL⁻ PD-L1⁺ | TIL⁻ PD-L1⁻ | C    | P-value |
|--------------------------------|-------------|-------------|-------------|-------------|------|---------|
| Age at diagnosis, years        |             |             |             |             | 0.751|         |
| <60                            | 28 (59.6)   | 51 (51.5)   | 20 (58.8)   | 33 (52.4)   |      |         |
| ≥60                            | 19 (40.4)   | 48 (48.5)   | 14 (41.2)   | 30 (47.6)   |      |         |
| Sex                            |             |             |             |             | 0.271|         |
| Male                           | 23 (48.9)   | 63 (63.6)   | 22 (64.7)   | 34 (54.0)   |      |         |
| Female                         | 24 (51.1)   | 36 (36.4)   | 12 (35.3)   | 29 (46.0)   |      |         |
| Primary tumor site             |             |             |             |             | 0.297|         |
| Left colon                     | 11 (23.4)   | 37 (37.4)   | 14 (41.2)   | 28 (44.4)   |      |         |
| Right colon                    | 26 (55.3)   | 37 (37.4)   | 13 (38.2)   | 23 (36.5)   |      |         |
| Rectum                         | 10 (21.3)   | 25 (25.3)   | 7 (20.6)    | 12 (19.0)   |      |         |
| Stageª                         |             |             |             |             | 0.389'| <0.001 |
| I                              | 13 (27.7)   | 33 (33.3)   | 1 (2.9)     | 1 (1.6)     |      |         |
| II                             | 29 (61.7)   | 47 (47.5)   | 20 (58.8)   | 39 (61.9)   |      |         |
| III                            | 4 (8.5)     | 17 (17.2)   | 8 (23.5)    | 17 (27.0)   |      |         |
| IV                             | 1 (2.1)     | 2 (2.0)     | 5 (14.7)    | 6 (9.5)     |      |         |
| Tumor stage                    |             |             |             |             | 0.396'| <0.001 |
| T1                             | 2 (4.3)     | 9 (9.1)     | 0 (0.0)     | 0 (0.0)     |      |         |
| T2                             | 12 (25.6)   | 30 (30.3)   | 1 (2.9)     | 2 (3.2)     |      |         |
| T3                             | 27 (57.4)   | 51 (51.5)   | 24 (70.6)   | 43 (68.3)   |      |         |
| T4a                            | 5 (10.6)    | 8 (8.1)     | 6 (17.6)    | 12 (19.0)   |      |         |
| T4b                            | 1 (2.1)     | 1 (1.0)     | 3 (8.8)     | 6 (9.5)     |      |         |
| Node stage                     |             |             |             |             | 0.227'| 0.004  |
| Negative                       | 43 (91.5)   | 82 (82.8)   | 23 (67.6)   | 42 (66.7)   |      |         |
| Positive                       | 4 (8.5)     | 17 (17.2)   | 11 (32.4)   | 21 (33.3)   |      |         |
| Tumor histological grade       |             |             |             |             | 0.279'| 0.015  |
| Well-differentiated            | 0 (0.0)     | 2 (2.0)     | 0 (0.0)     | 0 (0.0)     |      |         |
| Moderately differentiated      | 30 (63.8)   | 65 (65.7)   | 14 (41.2)   | 33 (52.4)   |      |         |
| Poorly differentiated           | 5 (10.6)    | 18 (18.2)   | 8 (23.5)    | 6 (9.5)     |      |         |
| Mucinous                       | 12 (25.5)   | 14 (14.1)   | 12 (35.3)   | 24 (38.1)   |      |         |
| Vascular invasion              |             |             |             |             | 0.166 |         |
| No                             | 46 (97.9)   | 92 (92.9)   | 29 (85.3)   | 56 (88.9)   |      |         |
| Yes                            | 1 (2.1)     | 7 (7.1)     | 5 (14.7)    | 7 (11.1)    |      |         |
| Nerve invasion                 |             |             |             |             | 0.256'| 0.001  |
| No                             | 45 (95.7)   | 94 (94.9)   | 28 (82.4)   | 48 (76.2)   |      |         |
| Yes                            | 2 (4.3)     | 5 (5.1)     | 6 (17.6)    | 15 (23.8)   |      |         |
| NLR                            |             |             |             |             | 0.235'| 0.003  |
| Low (≤5)                       | 41 (87.2)   | 98 (99.0)   | 27 (81.8)   | 53 (85.5)   |      |         |
| High (>5)                      | 6 (12.8)    | 1 (1.0)     | 6 (18.2%)   | 9 (14.5)    |      |         |
| CRP, mg/l                      |             |             |             |             | 0.311'| <0.001 |
| Low (≤5)                       | 23 (50.0)   | 80 (80.8)   | 15 (48.4)   | 30 (47.6)   |      |         |
| High (>5)                      | 23 (50.0)   | 19 (19.2)   | 16 (51.6)   | 33 (52.4)   |      |         |
| PD-1 expression                |             |             |             |             | 0.315'| <0.001 |
| Low                            | 7 (18.9)    | 33 (43.4)   | 9 (36.0)    | 34 (66.7)   |      |         |
| High                           | 30 (81.1)   | 43 (56.6)   | 16 (64.0)   | 17 (33.3)   |      |         |
| MMR status                     |             |             |             |             | 0.268'| <0.001 |
| pMMR                           | 11 (23.4)   | 50 (50.5)   | 21 (61.8)   | 39 (61.9)   |      |         |
| dMMR                           | 36 (76.6)   | 49 (49.5)   | 13 (38.2)   | 24 (38.1)   |      |         |

ªAccording to the 7th edition of the American Joint Committee on Cancer staging system. *n (%)*. C, contingency coefficient; MMR, mismatch repair; pMMR, MMR-proficient; dMMR, MMR-deficient; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; PD-1, programmed death-1; TIL, tumor-infiltrating lymphocyte; PD-L1, programmed death ligand-1.
Table V. Multivariate multinomial logistic regression analysis of risk factors for TIL+ PD-L1 status.

| Factor                                      | TIL+ PD-L1+ | TIL+ PD-L1+ | TIL+ PD-L1- |
|---------------------------------------------|-------------|-------------|-------------|
| Factor                                      | OR (95% CI) | P-value     | OR (95% CI) | P-value     | OR (95% CI) | P-value |
| Stage (reference, stage IV)                 | All >0.05   | All >0.05   | All >0.05   |
| Tumor stage (reference, T4b)                | All >0.05   | All >0.05   | All >0.05   |
| Negative lymph node metastasis              | 1.889 (0.030-120.105) 0.764 | 1.072 (0.014-83.342) 0.975 | 21.414 (0.093-4946.861) 0.764 |
| Tumor histological grade                    | All >0.05   | All >0.05   | All >0.05   |
| No nerve invasion (reference, nerve invasion)| 6.533 (0.935-45.646) 0.058 | 1.058 (0.100-11.239) 0.963 | 0.417 (0.036-4.826) 0.484 |
| Low NLR level (reference, high NLR level)   | 0.677 (0.100-4.569) 0.689 | 0.588 (0.082-4.214) 0.598 | N/A |
| Low CRP level (reference, high CRP level)   | 0.735 (0.209-2.585) 0.631 | 1.409 (0.343-5.786) 0.634 | 2.697 (0.871-8.351) 0.085 |
| Low PD-1 expression (reference, high expression) | 10.473 (3.005-36.503) <0.001 | 2.017 (0.490-8.295) 0.331 | 3.443 (1.145-10.352) 0.028 |
| No CRP level (reference, high CRP level)    | 11.536 (3.223-41.290) <0.001 | 14.523 (3.358-62.803) <0.001 | 4.718 (1.607-13.848) 0.005 |

TIL+ PD-L1+ status was taken as reference. OR, odds ratio; CI, confidence interval; TIL, tumor-infiltrating lymphocyte; PD-L1, programmed death ligand-1; NLR, neutrophil-lymphocyte ratio; CRP, C-reactive protein; PD-1, programmed death-1; pMMR, mismatch repair-proficient; dMMR, MMR-deficient.
safety, activity, and immune correlates of PD-L1 blockade is ineffective in this tumor type (11). No definite immunotherapy approaches have been applied in the clinic for patients with these tumors; therefore, novel immunosuppressive strategies should be developed in the future (11). In TIL+ PD-L1+ tumors, PD-L1 is expressed by tumor cells through oncogenic signaling instead of in response to TILs. It is unlikely that blocking PD-1 or PD-L1 alone is effective in this group due to a lack of T cell involvement (11). Radiotherapy or chemotherapy that induces cell death and the release of neo-antigens to induce a T-cell-mediated anti-tumor response has been used in combination with anti-PD-1 agents (8,27,28). Similar to that in TIL+ PD-L1+ tumors, single checkpoint blockade agents appear to be ineffective in TIL- PD-L1+ tumors due to the lack of T-cell infiltrates. Combination therapy strategies that aim to attract T cells into tumors and prevent inhibition of T cells should be considered. CTLA-4, an inducer of numerous T-cell responses, in combination with anti-PD-1, has been confirmed to effective in a clinical trial, regardless of PD-L1 expression (9,10). Based on the recognition that TIL+ PD-L1+ tumors exhibit the best response to PD-1/L1 blockade therapy, the current study assumed that dMMR patients contain a higher proportion of TIL+ PD-L1+ tumors compared with pMMR patients. The results confirmed this hypothesis and demonstrated that dMMR status is an independent risk factor for TIL+ PD-L1+ status. This could provide immunological evidence for an improved response to anti-PD-1/L1 therapy in dMMR patients compared with that in pMMR patients.

Immune checkpoint blockade is predominantly applied for patients with stage IV CRC (2,3), therefore, the current study attempted to investigate dMMR patients with an advanced stage of CRC. However, dMMR patients account for only a small percentage of patients with stage IV disease and obtaining pathological specimens from these patients was limited due to the loss of surgical opportunities. Although some immunological evidence could be provided, the current study would be more valuable if a higher number of stage IV patients were included. The current study aimed to collect samples from multiple centers to solve this problem, however, this was difficult due to objective factors. This is a notable limitation of the present study, therefore, further investigations are required in the future.

In conclusion, the current study indicated that dMMR patients are more likely to express TILs and PD-L1, and present with a TIL+ PD-L1+ status compared with pMMR patients. Therefore, the tumor type identified by this classification method can partially explain the improved response of dMMR patients to PD-1/L1 blockade. However, the response of each tumor type to PD-1/L1 blockade requires further investigation.

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Availability of data and materials

All data generated and analyzed during this study are included in this manuscript.

Authors’ contributions

SL, PK, CJ, QQ, QX and LX designed and performed the study. XW, LY, WH and JH collected and analyzed the data. SL, BZ and XX analyzed the results and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was performed according to the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of Sun Yat-sen University (Guangzhou, China). Written informed consent was obtained from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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