BASIC STUDY

B2M and JAK1/2–mutated MSI-H Colorectal Carcinomas Can Benefit From Anti-PD-1 Therapy

Chenzhi Zhang,*† Dandan Li,*† Binyi Xiao,*† Chi Zhou,*† Wu Jiang,*† Jinghua Tang,*† Yuan Li,*† Rongxin Zhang,*† Kai Han,*† Zhenlin Hou,*† Linjie Zhang,*† Qiaoqi Su,*† Leen Lia,*† Zhizhong Pan,*† Xiaoshi Zhang † † and Peirong Ding*†

Summary: β2-microglobulin (B2M) and Janus kinases 1 and 2 (JAK1/2) mutations have been suggested as genetic mechanisms of immune evasion for anti–programmed cell death protein 1 (PD-1) therapy. Whether B2M and JAK1/2 loss-of-function mutation can cause primary resistance to anti-PD-1 therapy in colorectal carcinoma (CRC) patients remains controversial. Here, we sought to compare the efficacy of anti-PD-1 therapy in DNA mismatch repair deficient/microsatellite instability–high CRC patients with or without B2M or JAK1/2 mutations. Thirty-Five CRC patients who received anti-PD-1 therapy were enrolled in this study. All tumor samples underwent next-generation sequencing. The clinical and molecular data from 110 CRC patients sequenced with the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay and accessed through cBioportal were also analyzed in this study. Of the 35 CRC patients from our center, 10 (28.6%) had a B2M loss-of-function mutation, and 8 (22.9%) had a JAK1/2 loss-of-function mutation. Compared with B2M wild-type CRCs, B2M-mutated CRCs did not show a higher frequency of resistance to anti-PD-1 therapy (P=0.71). There was even better response to anti-PD-1 therapy in patients with JAK1/2 mutation than in those without (P=0.015). Of the 110 CRC patients in the MSK-IMPACT datasets, 13 (11.8%) had a B2M mutation, and 15 (13.6%) had a JAK1/2 mutation. After analyzing the response to anti-PD-1 therapy in these 110 patients, we found similar results (P=0.438 and 0.071, respectively). Moreover, patients with B2M or JAK1/2 mutation had a lower tumor mutational burden score compared with those without. B2M and JAK1/2 loss-of-function mutations occur frequently in microsatellite instability–high CRC. Our study demonstrated that patients with CRC harboring B2M or JAK1/2 mutations should not be excluded from anti-PD-1 therapy.

Key Words: B2M, JAK1/2, colorectal carcinoma, anti-PD-1 therapy (J Immunother 2022;45:187–193)

Colorectal carcinoma (CRC) is a genetically heterogenous disease, and ~10%–15% of all CRCs are identified as DNA mismatch repair deficient (dMMR)/microsatellite instability–high (MSI-H).1,2 This feature can cause high mutational loads, high levels of tumor neoantigens, dense infiltration of CD8+ T cells, and upregulation of programmed cell death protein 1.

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TABLE 1. Baseline Characteristics

| Factors                  | N = 35 [n (%)] |
|--------------------------|----------------|
| Age (y)                  |                |
| ≤ 40                     | 20 (57.1)      |
| > 40                     | 15 (42.9)      |
| Sex                      |                |
| Male                     | 28 (73.6)      |
| Female                   | 7 (20.6)       |
| Lynch syndrome           |                |
| Lynch                    | 25 (71.4)      |
| Sporadic                 | 10 (28.6)      |
| Tumor location           |                |
| Rectum                   | 9 (25.7)       |
| Left-sided               | 12 (34.3)      |
| Right-sided              | 14 (40.0)      |
| RAS status               |                |
| Wild-type                | 15 (42.9)      |
| Mutant-type              | 20 (57.1)      |
| PIK3CA status            |                |
| Wild-type                | 17 (48.6)      |
| Mutant-type              | 18 (51.4)      |
| MLH1                     |                |
| Present                  | 18 (51.4)      |
| Absent                   | 17 (48.6)      |
| MSH2                     |                |
| Present                  | 19 (54.3)      |
| Absent                   | 16 (45.7)      |
| MSH6                     |                |
| Present                  | 18 (51.4)      |
| Absent                   | 17 (48.6)      |
| PMS2                     |                |
| Present                  | 17 (48.6)      |
| Absent                   | 18 (51.4)      |
| Clinical settings        |                |
| Neoadjuvant              | 30 (85.7)      |
| Metastatic               | 5 (14.3)       |
| B2M mutation             |                |
| Wild-type                | 25 (71.4)      |
| Mutant-type              | 10 (28.6)      |
| JAK1/2 mutation          |                |
| Wild-type                | 27 (77.1)      |
| Mutant-type              | 8 (22.9)       |
| PD-1 response            |                |
| Response                 | 23 (65.7)      |
| No response              | 12 (34.3)      |

B2M indicates β2-microglobulin; JAK1/2, Janus kinases 1 and 2; PD-1, programmed cell death protein 1.
death-ligand 1 (PD-L1), making dMMR/MSI-H CRC patient suitable for immunotherapy such as anti-programmed cell death protein 1 (PD-1) therapy. Recently, PD-1 inhibitors have been approved by the Food and Drug Administration (FDA) for dMMR/MSI-H metastatic CRC patients. However, nearly 40%–70% of these patients would not respond, with 10%–28% experiencing early progression. There are several mechanisms of resistance to anti-PD-1 therapy in microsatellite instability (MSI) cancer, among which mutations in the β2-microglobulin (B2M) and Janus kinases (JAK1 and JAK2) have been widely reported.

The B2M gene encodes the protein β2-microglobulin, an extracellular component of major histocompatibility complex (MHC) class I molecules that is present on every nucleated cell in the human body. MHC class I molecules are essential for proper and stable antigen presentation. Biomarker to identify patients who might not benefit from ant-PD-1 therapy, including dMMR/MSI-H CRCs.

These results are meaningful because they suggest that B2M and JAK loss-of-function mutations could be used as a biomarker to identify patients who might not benefit from an anti-PD-1/PD-L1 therapy. We acknowledge that the biology of these loss-of-function mutations is not simple and clear, as several well-documented cases of patients with B2M mutations at baseline were reported to respond to anti-PD-1 therapy, and pharmacological JAK1/2 inhibition can restore the response of immune checkpoint blockade-resistant tumors. The aim of the current study was to compare the efficacy of anti-PD-1 therapy in CRC patients with or without B2M or JAK1/2 mutations.

**METHODS**

Baseline Data Collection

We reviewed 78 patients who received anti-PD-1 therapy for CRC in Sun Yat-sen University Cancer Center. Of them, 40 underwent next-generation sequencing (NGS). Two identified as microsatellite stable and 3 receiving anti-PD-1 as adjuvant therapy after surgery were excluded. Finally, 35 patients who received anti-PD-1 therapy and underwent NGS were included in our study. Demographic and clinicopathologic data were collected from hospital records, including sex, age, and primary tumor site. RAS, PIK3CA, MLH1, MSH6, PMS2, B2M, and JAK1/2 status were tested through NGS.

Treatment and Response Assessment

All patients enrolled in this study received at least 2 courses of anti-PD-1 therapy with or without chemotherapy. Anti-PD-1 therapy used included pembrolizumab.
nivolumab, sintilimab, toripalimab, or camrelizumab. The recommended anti-PD-1 therapy dose was 200 mg for pembrolizumab, sintilimab, or camrelizumab, and 3 mg/kg for nivolumab or toripalimab. Tumor responses to anti-PD-1 therapy were evaluated after at least 2 courses. Response Evaluation Criteria in Solid Tumors (RECIST) scores were assessed via radiologic data and classified as follows: complete response (CR), disappearance of all target lesions; partial response (PR), &ge;30% decrease; progressive disease, &ge;20% increase over smallest sum observed; and stable disease, meeting none of the other criteria. Responders were defined as patients achieving PR or CR.

cBioportal Datasets

The frequency of B2M and JAK1/2 mutations was assessed using the cbioPortal for a Memorial Sloan Kettering Cancer Center (MSKCC) database, which included the details for &gt;1000 patients with various cancer types sequenced with the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay. Of them, 110 were CRC patients who received anti-PD-1 therapy. Further, we assessed the overall survival (OS) and tumor mutational burden (TMB) with the B2M and JAK1/2 gene status as queried with cbioPortal.

Statistical Analyses

The χ² test or Fisher exact test were employed to analyze the associations between the B2M or JAK1/2 mutation status and clinicopathologic variables. A 2-sided P-value &lt;0.05 was considered statistically significant. Statistical analysis was performed in SPSS (version 25.0). The key raw data have been uploaded to the Research Data Deposit public platform (www.researchdata.org.cn) with approval number RDDA2022997199.

RESULTS

Baseline Information

Table 1 shows the baseline information of the selected patients. All of the 35 patients were MSI-H/dMMR and received anti-PD-1 therapy. The median age was 39.0 years, and 7 (26.4%) were female. About two third of the patients (25/35) were Lynch syndrome carriers. Tumor sequencing was performed in all of the 35 patients, 10 (28.6%) of whom were B2M mutant-type, 8 (22.9%) were JAK1/2 mutant-type, and none of them had both B2M and JAK1/2 mutation. Twenty-three of the 35 patients responded to anti-PD-1 therapy.

Comparison of Baseline Information Between 2 B2M/JAK Status

Table 2 shows comparisons between patients with different B2M status. There were no significant differences in age, sex, and other basic information between B2M wild and mutational types. TMB score have been reported as a predictive biomarker for anti-PD-1 therapy. Here, we did not find a statistic difference in TMB score between the 2 groups (P = 0.54).

Similarly, basic clinical information was compared in JAK1/2 wild and mutational types (Table 3). There were also no significant differences in age, sex, histology, and other basic information between the 2 JAK1/2 status. We observed a low TMB score in patients with JAK1/2 mutation (P = 0.009). Other gene status like RAS and PIK3CA were not associated with B2M and JAK1/2 mutation status.

Association Between B2M and JAK1/2 Mutation and PD-1 Clinical Response

As for B2M status, the response to anti-PD-1 therapy had no significant difference between wild and mutational types (P = 0.53, Fig. 1A). As for JAK1/2 status, we even observed a better clinical response in patients with JAK1/2 mutation (P = 0.032, Fig. 1B), regardless of their low TMB score. Combined B2M and JAK1/2 status together, the better clinical response to anti-PD-1 therapy was also observed in patients with B2M or JAK1/2 mutation type (P = 0.035, Fig. 1C). These results are significant because it indicates that the resistance mechanism of anti-PD-1 therapy for CRC may be different from other solid tumors.

Verification With cBioPortal Datasets

To further verify the association between B2M and JAK1/2 status with clinical response to anti-PD-1 therapy, we used the cohort from a MSKCC research on cbioPortal. This cohort included 110 CRC patients who received anti-PD-1 therapy. Of the 110 patients, 13 (11.8%) had a B2M mutation, 15 (13.6%) had a JAK1/2 mutation, and 6 (5.5%) had both mutation (Fig. 2A). Patients with B2M mutation type showed no difference on OS compared with wild-type (69.2% vs. 73.5% at 2 years, P = 0.18).
57.7%, respectively, \( P = 0.438 \), Fig. 2B). While patients with \( JAK1/2 \) mutation seem to have a better OS compared with wild-type (73.3% vs. 56.8%, respectively, \( P = 0.0713 \), Fig. 2C).

Taken \( B2M \) and \( JAK1/2 \) status together, patients with \( B2M \) or \( JAK1/2 \) mutation also seem to have a better OS compared with wild-type (72.8% vs. 55.7%, respectively, \( P = 0.0836 \), Fig. 2D).
FIGURE 3. Tumor mutational burden score and β2-microglobulin (B2M) and Janus kinases 1 and 2 (JAK1/2) mutation status. A and B, Patients in this cohort with either B2M or JAK1/2 mutation type had a higher tumor mutational burden score.

Fig. 2D). It is interesting to note that, these patients had a higher TMB than patients without B2M or JAK1/2 mutations (Fig. 3). It seems that TMB rather than B2M or JAK1/2 status is predictive of the efficacy of anti-PD-1 therapy.

DISCUSSION

Although anti-PD-1 therapy has proven to be an ideal therapy for dMMR/MSI-H CRC, <50% of the patients could reach CR or PR. Primary resistance to anti-PD-1 therapy is one of the most important reasons for treatment failure, but the mechanism remains unclear. Our study reveals that dMMR/MSI-H CRCs with B2M and JAK1/2 mutations are responsive to PD-1 inhibitors. This finding is contrary to that in lung cancer and malignant melanoma, indicating that the mechanism of resistance to anti-PD-1 therapy in CRC may be different from that in other solid tumors. The results were echoed by those of MSKCC-IMPACT. In a MSKCC research, patients with B2M or JAK1/2 mutation could benefit from anti-PD-1 therapy.

Defects in antigen presentation and disturbance of IFN-γ signal pathway are thought to be possible mechanisms of resistance to anti-PD-1 therapy.15,19 B2M loss-of-function mutation cause the absence of MHC class I and JAK1/2 loss-of-function mutation lead to impairment of the IFN-γ receptors. In some preclinical models, it has been shown that B2M and JAK1/2 mutation can cause resistance to anti-PD-1 therapy. Moreover, Zaretsky et al10 found resistance-associated loss-of-function JAK1/2 mutation in 2 of the 4 patients with melanoma and a B2M truncating mutation in a third patient. In 2 patients with non–small cell lung cancer not responsive to anti-PD-1 therapy, Rizvi et al20 found one of them had a homozygous deleterious mutation. However, the sample sizes of these studies are small, so it remains debatable whether B2M and JAK1/2 mutations are a contraindication for anti-PD-1 therapy.

Unlike with melanoma and lung cancer, B2M and JAK1/2 loss-of-function mutations do not necessarily confer resistance to anti-PD-1 therapy in MSI-H CRC. Snahnicanova et al12 found no significant difference in TILs and peritumoral lymphoid reaction between patients with B2M mutation and patients without. It was also shown that the infiltration of CD3+ and CD8+ T cells was not significantly correlated with the presence of B2M mutations.21,22 Furthermore, Middha et al23 reported that most patients with B2M-mutant MSI-H CRC could still benefit from anti-PD-1 therapy. In our study, we did not observe any correlation between B2M or JAK1/2 mutations and a poor response. Consistent with our hypothesis, MSI status rather then B2M or JAK1/2 status contribute to clinical response to anti-PD-1 therapy.

Despite the fact that B2M loss-of-function mutation can lead to the absence of MHC I class molecules and that JAK1/2 loss-of-function mutation can cause disturbance of IFN-γ signal pathway, the absence of B2M mutation still cannot be used as a negative predictor of immune checkpoint therapy. One possible reason is that not all the B2M mutations cause T-cell ignorance. Middha et al23 reported that B2M protein loss was not correlated with loss of MHC class I expression. Janikovits et al21 established B2M mutation as a consequence of high PD-1-positive T-cell counts in MSI cancers. In addition, studies have shown that the activation of innate and adaptive immunity mediated by natural killer (NK) T cells, CD4+ T cells, and CD8+ T cells could overcome the influence of B2M/JAK knockout in vivo.13 Germano et al24 reported that the efficacy of immunotherapy against dMMR B2M null tumors did not require CD8+ T cells but relied on the presence of CD4+ T cells. In fact, CD1 family is required for NK recognition, and it is reported that CD1a, CD1b, and CD1c were absent from the surfaces of B2M-deficient cells.25 Therefore, it seems that CD4+ T cells rather than NK and CD8+ T cells influence the immunotherapy response in B2M/JAK mutation CRC. However, the potential molecular mechanism needs to be further clarified.

KRAS and PIK3CA mutations are frequently found in CRC, which are present in 20%–35% and 14%–25% of CRC
patients, respectively. KRAS and PIK3CA mutations have been associated with a worse clinical outcome and with a negative prediction of response to targeted therapy by anti-EGFR monoclonal antibodies. However, whether the mutations of KRAS and PIK3CA influence the response to anti-PD-1 therapy have not been confirmed. Song et al found that the mutation of KRAS gene in non–small cell lung cancer tissues was an independent predictor of the long-term benefit of immunotherapy. Mishima et al found that patients with PIK3CA mutations had a higher overall response rate in advanced gastric cancer. However, in subgroup analysis of the PIK3CA mutations had a higher overall response rate in that the mutation of PIK3CA and RAS status influence the response to anti-PD-1 therapy. Therefore, the influence of RAS and PIK3CA status on the response of anti-PD-1 therapy need to be further verified.

There are some limitations in our study. First, receiving chemotherapy or not is associated with prognosis, but due to the scarcity of cases (n = 35), it was not analyzed in this study. Second, the radiologic assessment of tumor response to anti-PD-1 therapy is based mainly on the subjective judgment of the radiologist, and subjective volume estimation provides poor results. Last, some factors were dispensed with in the study due to a lack of samples, including the state of POLE mutation, the carcinomembrany antigen level, and the dose of PD-1 inhibitors. Therefore, larger, prospective studies are needed to clarify if the response rate and duration of response vary by B2M and JAK12 mutation status.

CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

Supported by the National Natural Science Foundation of China (No. 81871971) and the Guangdong Basic and Applied Basic Research Foundation (No. 2020A1515110048).

All authors have declared that there are no financial conflicts of interest with regard to this work.

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