Cite as: Gu JH, Wang M, Li YT. Molecular characteristics of patients with microsatellite instability colorectal cancer in China [J]. Chin J Clin Res, 2024, 37(9):1374-1378,1385.

DOI: 10.13429/j.cnki.cjcr.2024.09.013

Molecular characteristics of patients with microsatellite instability colorectal cancer in China

GU Jianhui, WANG Meng, LI Yuntao

Department of General and Gastrointestinal Surgery, The Third People's Hospital of Chengdu, Sichuan, Chengdu 610031, China

Corresponding author: WANG Meng, E-mail: wm17313085773@163.com

Abstract: Objective To analyze their molecular characteristics of microsatellite instability (MSI) by sequencing colorectal cancer patients in Sichuan region. **Methods** Next Generation Sequencing (NGS) was used to test 14 239 colon cancer patients from 2017 to 2022 in The Third People's Hospital of Chengdu for single nucleotide variation (SNV), Insertion-deletion (Indel), copy number variation (CNV), fusion, MSI, tumor mutational burden (TMB), and Epstein-Barr virus (EBV). The clinical and molecular characteristics of highly microsatellite unstable (MSI-H) patients (MSI-H group, n=1 018) and microsatellite stable (MSS) patients (MSS group, n=13 221) were compared. **Results** The incidence rate of MSI in colorectal cancer was 7.15%, and detection rate of MSI varied in terms of age at diagnosis, tumour location and sample type. TMB was significantly higher in patients with MSI-H than in patients with microsatellite stable (MSS) (92.8 mutations/Mb *vs* 12.7 mutations/Mb, P<0.05). EBV positivity in patients with colorectal cancer was 0.4%, but no patients with positive EBV were detected in MSI-H. ERBB2, and PIK3CA all had significantly higher mutation frequencies in the MSI-H group, however, the positive rates of *APC* (51.26% *vs* 70.76%, χ2=168.823, P<0.01), TP53(27.76% *vs* 69.54%, $\chi 2$=739.882, P<0.01) and NRAS (1.86% *vs* 3.98%, $\chi 2$=11.445, P<0.01) were higher in the MSS group. Furthermore, MSI-H patients had a higher proportion of carrying a mismatch repair (MMR) gene variant (P<0.05). **Conclusion** MSI-H and MSS types of colorectal cancer in Chinese population have different molecular characteristics and differ in TMB and EBV infections.

Keywords: Colorectal cancer; Next generation sequencing; Unstable microsatellites; Microsatellite stability; DNA mismatch repair; Tumor mutation burden; Epstein-Barr virus; Copy number variation; System mutation; Germline mutation

Fund program: Medical Science and Technology Plan of Sichuan Provincial Health Commission (21PJ147)

Colorectal cancer (CRC) is the third most common malignant tumor globally, and in China, it ranks second after lung cancer, with a mortality rate in fourth place [1]. Recent advances in molecular diagnostics have significantly improved the treatment of CRC, particularly in the realm of immunotherapy. The 2020 KEYNOTE-177 study [2] established programmed cell death-1 (PD-1) inhibitors as the foundation for first-line treatment of mismatch repair deficient (dMMR)/microsatellite instability-high (MSI-H) advanced colorectal cancer, further increasing interest in MSI-H colorectal cancer. Microsatellite instability (MSI) was first reported in CRC in 1993, where microsatellites refer to short tandem repeat DNA sequences composed of up to six nucleotides in the human genome. When DNA mismatch repair (MMR) mechanisms are defective, errors in DNA insertion and deletion are not corrected, resulting in changes in the length of microsatellite repeats, which characterizes MSI [3]. The incidence of MSI in colorectal cancer varies between studies, with reports ranging from 6.3% to 20.3% [4-5].

In addition to MSI-H, other immunotherapy-related biomarkers include programmed cell death 1 ligand 1 (PD-L1) and tumor mutational burden (TMB). Kim *et al.* [6] reported that in MSI-H colorectal cancer, PD-L1 positivity in tumor cells and immune cells was associated with sporadic high methylation subtypes and immune cell-rich subtypes, respectively. Trabucco *et al.* [7] also reported that MSI-H was usually accompanied by high TMB in solid tumors, though high TMB did not necessarily indicate MSI-H. Currently, data on the role of Epstein-Barr virus (EBV) in colorectal cancer are scarce and contradictory, making research into the relationship between EBV and MSI-H colorectal cancer necessary [8].

Previous studies have reported differences in gene mutations between microsatellite stable (MSS) and MSI-H colorectal cancer patients [4,7,9], but research on Chinese populations is limited and may be biased. MSI represents a unique mechanism of tumor development [3], and evidence regarding the molecular characteristics of MSI-H colorectal cancer in large Chinese cohorts, especially concerning MSI-H in relation to TMB and EBV, remains insufficient. This study therefore aimed to conduct a large-scale exploratory analysis of such patients in China.

1 Materials and methods

1.1 General information

Data were retrospectively collected from 14,247 bowel cancer patients who underwent next generation sequencing (NGS) testing at the Third People's Hospital of Chengdu City, Sichuan Province, from 2017 to 2022. All

samples were processed by a certified clinical laboratory with Clinical Laboratory Improvement Amendments (CLIA), International Organization for Standardization (ISO), and College of American Pathologists (CAP) accreditation (Shanghai Siduedi Medical Laboratory). Written informed consent was obtained from all patients for sample testing, the use of NGS results, and clinical information (including age and sex).

1.2 NGS detection method

NGS was performed on tissue or plasma samples, targeting 500 or 100 MSI loci and the entire exome and partial intron of 733 tumor-related genes. All tissue specimens underwent quality control by pathologists to ensure a tumor cell content of ⩾20%. Captured DNA libraries were uploaded to the NovaSeq 6000 platform (Illumina) for 100 bp paired-end sequencing. The average sequencing depth for tissue samples was ⩾500x, for blood samples was ⩾5000x, and for prognostic and resistance-related genes was ⩾30,000x. The analysis included single nucleotide variant (SNV), small insertions and deletions (indels), copy number variation (CNV), gene fusions, MSI status, TMB, and EBV.

1.3 TMB detection method

TMB was defined as the number of synonymous and non-synonymous SNVs and indels in the coding regions of the examined areas, excluding driver mutations. All SNVs and indels in target gene coding regions were considered, including missense, silent, and translation-termination mutations, as well as in-frame and out-of-frame mutations. For blood samples, known germline single nucleotide polymorphisms (SNPs) with a population frequency ⩾ 0.015 in dbSNP, 1000 Genomes, and ESP6500 databases were excluded [10-11].

1.4 MSI detection method

MSI status was calculated using internally developed R scripts [12]. This involved assessing the distribution of repeat lengths for each microsatellite locus in each sample. Coverage of 100 microsatellite loci in blood and 500 microsatellite loci in tissue was analyzed. MSI score or bMSI was defined as the percentage of unstable loci. Tissue samples with an MSI score ⩾0.4 were classified as MSI-H, otherwise microsatellite stable (MSS). Samples with a bMSI score ⩾0.2 were classified as bMSI-H, otherwise bMSS.

1.5 Statistical methods

Data analysis was performed using SPSS 25.0. Continuous variables were expressed as median (minimum, maximum). Categorical and count variables were described with absolute values and percentages and compared using Pearson's χ^2 test. All tests were two-sided, with $P < 0.05$ considered statistically significant.

2 Results

2.1 Comparison of clinical features between MSI-H and MSS colorectal cancer patients

Data from 14,239 colorectal cancer patients were analyzed (**Table 1**). Colorectal cancer comprised 62.9% colon cancer and 37.1% rectal cancer. Among these, 7.15% were MSI-H. The incidence of MSI-H was significantly higher in colon cancer than in rectal cancer ($P < 0.05$). No significant gender differences were observed among MSI-H patients ($P = 0.099$). The median age at diagnosis for MSI-H colorectal cancer patients was significantly younger than that for MSS patients (52 years *vs* 58 years, $P < 0.05$).

MSI-H was most frequently detected in primary lesions, with lower detection rates in metastatic lesions. Additionally, TMB was assessed in 8,360 patients and EBV in 6,339 patients; the median TMB was 15.76 mutations/Mb, with MSI-H patients showing significantly higher TMB compared to MSS patients (90.38 mutations/Mb *vs* 11.46 mutations/Mb, $P < 0.05$).

6,339 patients were tested for EBV (339 in the MSI-H group and 6,000 in the MSS group), and 27 were found to be positive (0 positive in the MSI-H group and 27 in the MSS group), with an overall EBV positivity rate of 0.43%, and the difference in EBV positivity between the MSI-H group and the MSS group was not statistically significant (0.45% *vs.* 0, $\chi^2 = 0.650$, P=0.418).

Tab.1 Characteristics of colorectal cancer patients with MSI/MSS status [case (%)]

Features	MSI-H (*n*=1018)	MSS (*n*=13 221)	χ^2 value	*P* value
Tumor type			287.778	<0.001
Colon cancer	892(87.62)	8060(60.96)		
Rectal cancer	126(12.38)	5161(39.04)		
Gender			2.728	0.099
Male	601(59.04)	8151(61.65)		
Female	417(40.96)	5070(38.35)		
Age(year)[a]	52(19,92)	58(10,94)		<0.05
Sample Type			76.791	<0.001
Blood	82(8.06)	1831(13.85)		
Primary Focus	904(88.8)	10212(77.24)		
Metastatic Focus	32(3.14)	1178(8.91)		

2.2 Comparison of molecular characteristics between MSI-H and MSS colorectal cancer patients

MSI-H and MSS colorectal cancers exhibited significant differences in gene mutations, as listed in **Figure 1** for genes with a positivity rate greater than 10% and common driver genes. The most common mutated genes in colorectal cancer included *APC* (69.34%), *TP53* (66.49%), *KRAS* (49.88%), and *PIK3CA* (16.63%). Other genes included: *BRAF* (6.39%), *NRAS* (3.83%), *GNAS* (3.32%), *ERBB2* (5.23%), *BRCA2* (3.42%), and *BRCA1* (1.34%).

The KRAS positivity rate in MSI-H groups was similar to that in MSS groups ($P = 0.781$). Compared to

MSS groups, the positivity rates for *PIK3CA*, *BRAF*, and *ERBB2* were higher in MSI-H groups ($P < 0.01$), while the positivity rates for *APC*, *TP53*, and *NRAS* were lower ($P < 0.01$). In addition, genes involved in DNA damage response (DDR) pathways, such as *ARID1A*, *TGFBR2*, *BRCA2*, *ATR*, and *BRCA1*, were more frequently altered in MSI-H colorectal cancer compared to MSS. Other mismatch repair (MMR) genes, such as *MSH6*, *MLH1*, *MSH2*, and *PMS2*, also had significantly higher mutation frequencies in MSI-H than MSS groups.

Notably, at least 70.1% of MSI-H colorectal cancer patients harbored a mutation in an MMR gene (*MSH6/MSH2/MLH1/PMS2*) (70.1% *vs*. 2.2%, $P < 0.05$). Among germline mutations, MSI-H patients also had a significantly higher frequency of MMR gene mutations, including *MSH, MLH1, MSH2*, and *PMS2* compared to MSS patients. Additionally, *MLH1, MSH2, MSH6,* and *PMS2* mutations appeared to be mutually exclusive. **See Table 2.**

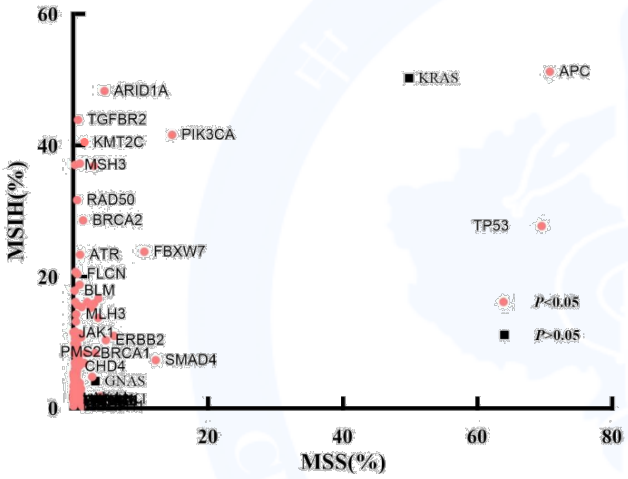

Fig.1 Gene mutation frequency map of MSI-H/MSS colorectal cancer patients

Tab. 2 Molecular characteristics of MSI-H and MSS colorectal cancer patients

Gene	MSI-H	MSS	χ^2 value	*P* value
KRAS	50.27	49.86	0.071	0.789
PIK3CA	41.63	14.67	496.806	<0.001
BRAF	11.04	0.89	607.871	<0.001
ERBB2	10.38	4.83	59.342	<0.001
APC	51.26	70.76	168.823	<0.001
TP53	27.76	69.54	739.882	<0.001
NRAS	1.86	3.98	11.455	0.001
DDR				
ARID1A	48.31	4.61	2532.852	<0.001
TGFBR2	43.93	0.71	4825.888	<0.001
BRCA2	28.63	1.44	2134.412	<0.001
ATR	23.39	1.101	1787.246	<0.001
BRCA1	8.31	0.8	406.913	<0.001
MMR				
MSH6	37.27	0.97	3619.481	<0.001
MLH1	20.77	0.31	2266.428	<0.001
MSH2	15.74	0.58	1322.818	<0.001
PMS2	8.09	0.32	655.498	<0.001
MMR				
MSH6	11.90	0.30	1134.500	<0.001
MLH1	22.00	0.20	2605.959	<0.001
MSH2	17.00	0.10	2092.984	<0.001
PMS2	5.30	0.30	360.614	<0.001

2.3 The relationship between MSI-H and TMB in different sample types

This study further analyzed the relationship between MSI-H and TMB in various sample types of colorectal cancer. For MSI-H, the median TMB values in primary tumors, metastases, and blood were 99.6, 97.5, and 97.5 mutations/Mb, respectively ($P = 0.468$). In contrast, for MSS, the median TMB values in primary tumors, metastases, and blood were 12.3, 9.4, and 10.1 mutations/Mb, respectively ($P = 0.0207$), with the TMB in primary tumors being higher than in metastases.

Similarly, in MSI-H colorectal cancer, the TMB in primary tumors and metastases was significantly higher than in MSS (99.6 vs 12.3 mutations/Mb, $P < 0.001$; 97.5 vs 9.4 mutations/Mb, $P < 0.01$), and the difference in TMB between MSI-H and MSS in blood was statistically significant (97.5 vs 10.1 mutations/Mb, $P < 0.01$).

3 Discussion

MSI-H colorectal cancer is a prominent group of patients receiving PD-1 antibody therapy. In most cases, the MSI phenotype is more common in colon cancer. The MSI-H incidence detected in this study was 7.15%, lower than the 15% reported in European and American populations [5], but similar to the 6.31% reported in East China [4] and consistent with the 7.6% dMMR incidence reported by Liang *et al* [13]. The median age at onset for MSI-H colorectal cancer patients was significantly younger than that of MSS patients (52 years vs 58 years, $P < 0.05$), which was consistent with previous reports in the Chinese population [4]. However, this study observed inconsistencies in the MSI-H incidence between primary tumors, blood, and metastases. Specifically, the incidence of MSI-H tumors in metastases was notably lower compared to primary tumors and blood. This had also been confirmed in previous studies and may be related to the better biological characteristics of MSI-H tumors [14]. Moreover, detection rates can vary across different regions, populations, sample types, and detection methods. Given the wide distribution of samples in this study, the observed MSI frequency may better reflect the overall characteristics of the Sichuan region.

The mutation frequencies of other genes, such as *KRAS* (49.88%), *BRAF* (6.93%), and *NRAS* (3.83%), were consistent with previous reports from both Western and Eastern studies, which were 40%-50% [15], 5.4%-6.7% [16], and 3.8% [17], respectively. In MSI-H tumors, mutation rates for *PIK3CA*, *BRAF*, *ERBB2*, and *DDR* genes were significantly higher, whereas the mutation rates for *APC*, *TP53*, and *NRAS* were lower. Trabucco *et al.* [7] also reported that some gene mutations were significantly enriched in MSI-H or MSS tumors, and these variations were associated with pathway enrichment in MSI-H or MSS tumors. The analysis of *RAS* and *BRAF* mutations in this study showed that *KRAS* and *BRAF* p.V600E mutations seem to be mutually exclusive, which was consistent with previous reports [4], although mutations at other BRAF sites can coexist. The results of this study also

indicated that MSI-H patients were relatively younger compared to MSS patients, which can provide diagnostic reference for clinicians.

MMR gene mutations lead to dMMR and subsequently to MSI [18]. In MSI-H colorectal cancer, 70.1% of patients carry an MMR gene mutation, showing a high consistency between MSI-H and MMR gene mutations. Compared to the 59.7% reported by Salem *et al.* [19], the data from this study was higher. The frequencies of *MSH6* (37.3%), *MLH1* (20.8%), *MSH2* (15.7%), and *PMS2* (8.1%) were consistent with those reported by Salem *et al.* [19] (MSH6: 38.1%, MLH1: 22%, MSH2: 14%, PMS2: 8%).

Besides somatic mutations in MMR genes, germline alterations in MMR genes can lead to Lynch syndrome [18]. This study showed that 56.2% of MSI-H colorectal cancers had germline MMR gene mutations. Unlike systemic mutations, MLH1 and MSH2 germline mutations were more common than *MSH6*, and *MLH3* germline mutations are less frequent. Although *MMR* genes can be detected by NGS, it is not yet possible to directly infer MMR protein expression/MSI status solely based on pathogenic mutations. In clinical practice, about 1%-10% of patients may have discrepancies between MMR and MSI test results [13, 19-20], primarily due to MMR staining heterogeneity [21], MMR gene missense mutations [22], and compensatory mechanisms in the MMR system [23]. For instance, besides *MSH6/MSH2/MSH3/MLH1/MLH3/PMS2* genes, MSI can also be caused by other genes (*POLE, POLD*), so MMR gene negativity might still indicate MSI-H; some MMR protein deficiencies could be functionally compensated, thus MSS might still occur despite MMR mutations.

TMB is an important independent biomarker for immunotherapy. Studies in solid tumors have found that TMB is highly correlated with the objective response rate (ORR) to anti-PD-1/PD-L1 therapy [24]. Recent research by Schrock *et al.* [25] found that 4.5% of patients were MSI-H, with a median TMB of 46.1 mutations/Mb in MSI-H patients and 3.5 mutations/Mb in MSS patients. Kabbarah's *et al.* reported a median TMB of 52 mutations/Mb in MSI-H patients and 6 mutations/Mb in MSS patients [26]. In contrast, the median TMB in MSI-H patients in this study was 80 mutations/Mb, while in MSS patients, it was 6.7 mutations/Mb. The MSI-H TMB data in this study were evidently higher, which may relate to differences in TMB algorithms and populations. The TMB values for MSI-H in primary tumors and metastases were higher than in MSS, but the trend was not significant in blood due to the smaller number of MSI-H blood samples. There was no significant difference in TMB values among different sample types in MSI-H tumors; however, in MSS tumors, TMB was higher in primary tumors than in metastases. Puccini *et al.* [14] reported that high TMB and MSI-H were more easily observed in primary tumors compared to distant metastases. Tumor heterogeneity may exist among different sample types, and blood TMB testing can overcome this issue, but whether blood TMB analysis is more advantageous than tissue TMB analysis still requires prospective trials for verification.

EBV infection was associated with the development of various tumors, including nasopharyngeal carcinoma, gastric cancer, and lymphomas, but there was limited research on its role in colorectal cancer. Chen et al [27] reported that EBV was associated with the pathology and clinical progression of colorectal cancer and promoted its advancement. In various reported studies, the proportion of EBV-positive cases ranges widely (1.4%-46%: PCR and IHC) [8]. The data from this study showed an EBV positivity rate of 0.4%, which was much lower than reported figures. However, some studies have reported extremely low or undetectable EBV positivity in invasive colorectal cancer [28, 29]. Additionally, the relationship between EBV positivity and MSI-H seems to be mutually exclusive, suggesting that further exploration of the relationship between MSI-H colorectal cancer and EBV is warranted.

This study compares MSI-H and MSS phenotypes in Chinese colorectal cancer patients using a large sample size. Compared to MSS, MSI-H tumors have distinct molecular features. DDR genes and MMR genes exhibit higher mutation frequencies in MSI-H, TMB-H is more common in MSI-H, and EBV prevalence is low in colorectal cancer. However, this study has some limitations, such as potential implicit selection bias due to its retrospective design. Currently, there is a lack of effective targeted therapies for most colorectal cancer genomic mutations, and understanding the molecular characteristics of MSI-H as a biomarker for immunotherapy will help guide more precise treatment for colorectal cancer in the future.

The authors report no conflict of interest

References

[1] Zheng RS, Chen R, Han BF, et al. Cancer incidence and mortality in China, 2022[J]. Chin J Oncol, 2024, 46(3): 221-231. **[In Chinese]**

[2] André T, Shiu KK, Kim TW, et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer[J]. N Engl J Med, 2020, 383(23): 2207-2218.

[3] Nojadeh JN, Behrouz Sharif S, Sakhinia E. Microsatellite instability in colorectal cancer[J]. EXCLI J, 2018, 17: 159-168.

[4] Song YL, Wang LL, Ran WW, et al. Effect of tumor location on clinicopathological and molecular markers in colorectal cancer in Eastern China patients: an analysis of 2, 356 cases[J]. Front Genet, 2020, 11: 96.

[5] Shubin V, Shelygin Y, Achkasov S, et al. Microsatellite instability in Russian patients with colorectal cancer[J]. Int J Mol Sci, 2022, 23(13): 7062.

[6] Kim JH, Park HE, Cho NY, et al. Characterisation of PD-L1-positive subsets of microsatellite-unstable colorectal cancers[J]. Br J Cancer, 2016, 115(4): 490-496.

[7] Trabucco SE, Gowen K, Maund SL, et al. A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1000 microsatellite instability-high cases in 67, 000 patient samples[J]. J Mol Diagn, 2019, 21(6): 1053-1066.

[8] Bedri S, Sultan AA, Alkhalaf M, et al. Epstein-Barr virus (EBV) status in colorectal cancer: a mini review[J]. Hum Vaccin Immunother, 2019, 15(3): 603-610.

[9] Myer PA, Lee JK, Madison RW, et al. The genomics of colorectal cancer in populations with African and European ancestry[J]. Cancer Discov, 2022, 12(5): 1282-1293.

[10] Zhuang W, Ma JX, Chen XD, et al. The tumor mutational burden of Chinese advanced cancer patients estimated by a 381-cancer-gene panel[J]. J Cancer, 2018, 9(13): 2302-2307.

[11] Wang ZJ, Duan JC, Cai SL, et al. Assessment of blood tumor mutational burden as a potential biomarker for immunotherapy in patients with non-small cell lung cancer with use of a next-generation sequencing cancer gene panel[J]. JAMA

Oncol, 2019, 5(5): 696-702.
[12] Wang ZH, Zhao XC, Gao C, et al. Plasma-based microsatellite instability detection strategy to guide immune checkpoint blockade treatment[J]. J Immunother Cancer, 2020, 8(2): e001297.
[13] Li XZ, Liu HJ, Liang MY, et al. Clinicopathological features and types of microsatellite instability in 1394 patients with colorectal cancer[J]. Nan Fang Yi Ke Da Xue Xue Bao, 2020, 40(11): 1645-1650.
[14] Puccini A, Seeber A, Xiu J, et al. Molecular differences between lymph nodes and distant metastases compared with primaries in colorectal cancer patients[J]. NPJ Precis Oncol, 2021, 5(1): 95.
[15] Schirripa M, Cohen SA, Battaglin F, et al. Biomarker-driven and molecular targeted therapies for colorectal cancers[J]. Semin Oncol, 2018, 45(3): 124-132.
[16] Yoshino T, Arnold D, Taniguchi H, et al. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS[J]. Ann Oncol, 2018, 29(1): 44-70.
[17] Wang YC, Loree JM, Yu C, et al. Distinct impacts of KRAS, NRAS and BRAF mutations on survival of patients with metastatic colorectal cancer[J]. J Clin Oncol, 2018, 36(15_suppl): 3513.
[18] Sinicrope FA. Lynch syndrome-associated colorectal cancer[J]. N Engl J Med, 2018, 379(8): 764-773.
[19] Salem ME, Bodor JN, Puccini A, et al. Relationship between MLH1, PMS2, MSH2 and MSH6 gene-specific alterations and tumor mutational burden in 1057 microsatellite instability-high solid tumors[J]. Int J Cancer, 2020, 147(10): 2948-2956.
[20] Debniak T, Kurzawski G, Gorski B, et al. Value of pedigree/clinical data, immunohistochemistry and microsatellite instability analyses in reducing the cost of determining hMLH1 and hMSH2 gene mutations in patients with colorectal cancer[J]. Eur J Cancer, 2000, 36(1): 49-54.
[21] McCarthy AJ, Capo-Chichi JM, Spence T, et al. Heterogenous loss of mismatch repair (MMR) protein expression: a challenge for immunohistochemical interpretation and microsatellite instability (MSI) evaluation[J]. J Pathol Clin Res, 2019, 5(2): 115-129.
[22] Zhang LY. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing[J]. J Mol Diagn, 2008, 10(4): 301-307.
[23] Stelloo E, Jansen AML, Osse EM, et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer[J]. Ann Oncol, 2017, 28(1): 96-102.
[24] Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition[J]. N Engl J Med, 2017, 377(25): 2500-2501.
[25] Schrock AB, Ouyang C, Sandhu J, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer[J]. Ann Oncol, 2019, 30(7): 1096-1103.
[26] Innocenti F, Ou FS, Qu XP, et al. Mutational analysis of patients with colorectal cancer in CALGB/SWOG 80405 identifies new roles of microsatellite instability and tumor mutational burden for patient outcome[J]. J Clin Oncol, 2019, 37(14): 1217-1227.
[27] Meng QT, Sun H, Wu SS, et al. Epstein-Barr virus-encoded microRNA-BART18-3p promotes colorectal cancer progression by targeting de novo lipogenesis[J]. Adv Sci, 2022, 9(35): e2202116.
[28] Sarvari J, Mahmoudvand S, Pirbonyeh N, et al. The very low frequency of Epstein-Barr JC and BK viruses DNA in colorectal cancer tissues in Shiraz, southwest Iran[J]. Pol J Microbiol, 2018, 67(1): 73-79.
[29] Mehrabani-Khasraghi S, Ameli M, Khalily F. Demonstration of herpes simplex virus, cytomegalovirus, and Epstein-Barr virus in colorectal cancer[J]. Iran Biomed J, 2016, 20(5): 302-306.

Submission received: 2023-10-21/ **Revised:** 2024-01-11

·论　著·

微卫星不稳定性结直肠癌 14 239 例患者的分子特征

古建辉，　王蒙，　李云涛

成都市第三人民医院 西南交通大学附属医院普外科胃肠外科，四川 成都 610031

摘要：目的　对四川地区结直肠癌患者进行基因测序，以分析其微卫星不稳定（MSI）的分子特征。**方法**　采用第二代测序技术（NGS）方法对 2017 年至 2022 年四川省成都市第三人民医院 14 239 例结直肠癌患者的进行检测，包含单核苷酸位点变异（SNV）、插入和缺失（InDel）、拷贝数变异（CNV）、基因融合（fusion）、MSI、肿瘤突变负荷（TMB）、EB 病毒（EBV）等。比较高度微卫星不稳定（MSI-H）患者（MSI-H 组，$n=1\ 018$）和微卫星稳定（MSS）患者（MSS 组，$n=13\ 221$）的临床及分子特征。**结果**　MSI 在结直肠癌中的发生率为 7.15%，且 MSI 检出率在诊断年龄、肿瘤位置、样本类型等方面均存在差异。MSI-H 组 TMB 显著高于 MSS 组（92.8 突变/Mb *vs* 12.7 突变/Mb，$P<0.05$）。结直肠癌患者 EBV 阳性率为 0.43%（27 例），而 MSI-H 未检测到有 EBV 阳性的患者。*ERBB2*、*PIK3CA* 和 *BRAF* 等在 MSI-H 组中的频率都明显更高，但 *APC*（51.26% *vs* 70.76%，$\chi^2=168.823$，$P<0.01$）、*TP53*（27.76% *vs* 69.54%，$\chi^2=739.882$，$P<0.01$）、*NRAS*（1.86% *vs* 3.98%，$\chi^2=11.445$，$P<0.01$）在 MSS 组中的阳性率更高。此外，携带一个 DNA 错配修复基因（MMR）变异的患者在 MSI-H 型中占比更高（$P<0.05$）。**结论**　四川地区结直肠癌 MSI-H 型与 MSS 型具有不同的分子特征，且在 TMB 及 EBV 感染也有所差异。

关键词：结直肠癌；下一代测序技术；微卫星不稳定；微卫星稳定；DNA 错配修复；肿瘤突变负荷；EB 病毒；拷贝数变异；体系突变；胚系突变

中图分类号：R735.3　**文献标识码：**A　**文章编号：**1674-8182(2024)09-1374-06

Molecular characteristics of 14 239 patients with microsatellite instability colorectal cancer

GU Jianhui, WANG Meng, LI Yuntao

Department of General and Gastrointestinal Surgery, The Third People's Hospital of Chengdu, Affiliated Hospital of Southwest Jiaotong University, Chengdu, Sichuan 610031, China

Corresponding author: WANG Meng, E-mail: wm17313085773@163.com

Abstract: Objective　To analyze their molecular characteristics of microsatellite instability (MSI) by sequencing colorectal cancer patients in Sichuan region. **Methods**　Next generation sequencing (NGS) was used to test 14 239 colon cancer patients from 2017 to 2022 in the Third People's Hospital of Chengdu for single nucleotide variation (SNV), insertion-deletion (InDel), copy number variation (CNV), fusion, MSI, tumor mutational burden (TMB), and Epstein-Barr virus (EBV). The clinical and molecular characteristics of highly microsatellite unstable (MSI-H) patients (MSI-H group, $n=1\ 018$) and microsatellite stable (MSS) patients (MSS group, $n=13\ 221$) were compared. **Results**　The incidence rate of MSI in colorectal cancer was 7.15%, and detection rate of MSI varied in terms of age at diagnosis, tumour location and sample type. TMB was significantly higher in patients with MSI-H than in patients with microsatellite stable (MSS) (92.8 mutations/Mb *vs* 12.7 mutations/Mb, $P<0.05$). EBV positivity in patients with colorectal cancer was 0.43%, but no patient with positive EBV was found in MSI-H. *ERBB2*, *PIK3CA* and *BRAF* all had significantly higher mutation frequencies in the MSI-H group, however, the positive rates of *APC* (51.26% *vs* 70.76%, $\chi^2=168.823$, $P<0.01$), *TP53* (27.76% *vs* 69.54%, $\chi^2=739.882$, $P<0.01$) and *NRAS* (1.86% *vs* 3.98%, $\chi^2=$

DOI: 10.13429/j.cnki.cjcr.2024.09.013

基金项目：四川省卫生健康委员会医学科技计划项目（21PJ147）

通信作者：王蒙，E-mail: wm17313085773@163.com

出版日期：2024-09-20

QR code for English version

11.445, $P<0.01$) were higher in the MSS group. Furthermore, MSI-H patients had a higher proportion of carrying a mismatch repair (MMR) gene variant ($P<0.05$). **Conclusion** MSI-H and MSS types of colorectal cancer in Sichuan have different molecular characteristics and differ in TMB and EBV infections.

Keywords: Colorectal cancer; Next generation sequencing; Microsatellite instability; Microsatellite stability; DNA mismatch repair; Tumor mutation burden; Epstein-Barr virus; Copy number variation; System mutation; Germline mutation

Fund program: Medical Science and Technology Plan of Sichuan Provincial Health Commission (21PJ147)

结直肠癌是全球第三大常见的恶性肿瘤,而在中国结直肠癌占比仅次于肺癌,高居第二位,死亡率排第四位[1]。近些年随着分子诊断的发展,结直肠癌的治疗也取得了重大进展,尤其是免疫治疗方面。2020 年的 KEYNOTE-177 研究[2]确立了程序性死亡受体 1(programmed cell death-1, PD-1)抑制剂作为错配修复缺陷(different mismatch repair, dMMR)/微卫星高度不稳定型(microsatellite instability-high, MSI-H)晚期肠癌一线治疗的基础[3]。1993 年微卫星不稳定(microsatellite instability,MSI)首次在结直肠癌中被报道,其中的微卫星是指人体内基因组中由至多 6 个核苷酸组成的短串联重复 DNA 序列,而当 DNA 错配修复功能出现异常时,不能对 DNA 错误的插入和缺失进行修复,造成微卫星串联重复长度发生改变的现象即为 MSI[4]。不同的研究中结直肠癌患者中 MSI 的发生率有所不同,从 6.3%~20.3%均有报道[5-6]。

免疫治疗相关生物标志物除了 MSI-H,还有细胞程序性死亡配体 1(programmed cell death 1 ligand 1, PD-L1)、肿瘤突变负荷(tumor mutational burden, TMB)。Kim 等[7]报道了在 MSI-H 结直肠癌中,肿瘤细胞中的 PD-L1 阳性和免疫细胞中的 PD-L1 阳性分别与散发的高甲基化亚型和免疫细胞丰富亚型相关。Trabucco 等[8]也报道了在实体瘤中,MSI-H 通常伴随着高 TMB,而高 TMB 却不一定表现出 MSI-H。目前,关于 EB 病毒(Epstein-Barr virus, EBV)在结直肠癌中作用的资料稀少且相互矛盾,因此探索 EBV 与结直肠癌 MSI-H 之间关系的研究也是必要的[9]。既往一些研究报道微卫星稳定(mocrosatellite stable MSS)和 MSI-H 这两种表型的结直肠癌患者的基因突变之间的差异[5,8]。在中国,大样本的 MSI-H 型结直癌肠患者分子特征的证据还不充足,尤其是 MSI-H 与 TMB、EBV 的关系,因此本研究对这类中国结直肠癌患者进行了大样本探索分析。

1　资料与方法

1.1　一般资料　回顾性收集 2017 至 2022 年期间四川省成都市第三人民医院进行过第二代测序(next generation sequencing, NGS)检测的 14 247 例肠癌患者的数据,所有样本由经过认证的临床实验室改进修正案(clinical laboratory improvement amendments, CLIA)、国际标准化组织(International Organization for Standardization, ISO)和美国病理学家学会(College of American Pathologists, CAP)认证的实验室(上海思路迪医学检验所)进行 NGS 检测。在采集样本时,所有患者都对检查其样本、使用 NGS 检测结果和临床信息(包括年龄、性别)提供了书面知情同意。本研究通过医院伦理委员会审查。

1.2　NGS 检测方法　利用 NGS 对组织或血浆样本进行测序,该测序的靶区是选定的 500 或 100 个 MSI 位点和 733 个肿瘤相关基因的整个外显子和部分内含子。所有组织标本均由病理学家进行质量控制,确保肿瘤细胞含量≥20%。将捕获的 DNA 文库上传到 NovaSeq 6 000 平台(Illumina)上,进行 100 bp 的对端测序,组织样本平均测序深度为≥500X,血液样本平均测序深度≥5 000X,预后和耐药相关基因平均测序深度≥30 000X。分析单核苷酸位点变异(single nucleotide variation, SNV)、小片段插入和缺失(insertion-deletion, InDel)、拷贝数变异(copy number variation, CNV)、基因融合(fusion)、MSI 状态、TMB 和 EBV。

1.3　TMB 检测方法　TMB 被定义为被检查的编码区的同义和非同义体细胞 SNV 和 InDel 的数量,不包括驱动突变。目标基因编码区的所有 SNV 和 InDel 都被考虑在内,包括错义、沉默、翻译提前终止,终止密码子缺失、框内和框外突变。其中血液 TMB 排除了 dbSNP 数据库、1 000 基因组和 ESP 6 500 数据库中群体频率≥0.015 的已知胚系单核苷酸多态性(single nucleotide polymorphism, SNP)[10-11]。

1.4　MSI 检测方法　MSI 状态是通过使用内部开发的 R 脚本进行 DNA 分析而计算得出的[12]。即对每个样品的每个微卫星位点的各种重复长度分布进行评估。对血液中 100 个微卫星位点和组织中 500 个微卫星位点的同时覆盖率进行分析。MSI 评分或血液

MSI(blood MSI, bMSI)被定义为不稳定位点的百分比。MSI 评分≥0.4 的组织样本被归类为 MSI-H,否则为 MSS,而 MSI 评分≥0.2 的样本被归类为 MSI-H,否则为 bMSS。

1.5　统计学方法　使用 SPSS 25 进行统计分析。其中连续变量用中位数(最小值,最大值)表示。分类变量以绝对值和百分比描述,并使用 Pearson χ^2 检验进行比较。所有检测采用双侧检验,P<0.05 表示差异有统计学意义。

2　结　果

2.1　MSI-H 与 MSS 结直肠癌患者临床特征对比　本研究分析了 14 239 例结直肠癌患者的数据(表 1),结肠癌占比为 62.87%,直肠癌占比为 37.13%;其中 MSI-H 的肠癌患者有 7.15%,结肠癌中的 MSI-H 占比显著高于直肠癌(P<0.05)。发生 MSI-H 的患者性别占比差异无统计学意义(P=0.099)。MSI-H 结直肠癌患者发病年龄为 52(19,92)岁,MSS 患者为 58(10,94)岁,两者比较差异有统计学意义(P<0.05)。结直肠癌患者的原发灶中 MSI-H 的检出率最高,转移灶中检出率较低。此外,8 360 例患者检测了 TMB,TMB 中位值为 15.76 突变/Mb,MSI-H 患者 TMB 显著高于 MSS 患者(90.38 突变/Mb *vs* 11.46突变/Mb,P<0.05);6 339 例患者检测了 EBV(MSI-H 组检测 339 例,MSS 组检测 6 000 例),发现阳性 27 例(MSI-H 组阳性 0 例,MSS 组阳性 27 例),EBV 总阳性率为0.43%,MSI-H 组和 MSS 组 EBV 阳性率比较差异无统计学意义(0.45% *vs* 0,χ^2=0.650,P=0.418)。

2.2　MSI-H 与 MSS 结直肠癌患者分子特征差异比较结果　MSI-H 和 MSS 结直肠癌表现出显著的基因突变差异,图 1 中列出在 MSI-H 中阳性率大于 10%和一些常见的驱动基因。结直肠癌中最常见的突变基因为 *APC*(69.34%),*TP53*(66.49%),*KRAS*(49.88%),*PIK3CA*(16.63%)等,其他分别为:*BRAF*(6.39%)、*NRAS*(3.83%)、*GNAS*(3.32%)、ERBB2(5.23%)、*BRCA2*(3.42%)以及 *BRCA1*(1.34%)。MSI-H 组的 *KRAS* 阳性率与 MSS 组相近(P=0.789)。与 MSS 组相比,MSI-H 组中 *PIK3CA*、*BRAF* 和 *ERBB2* 的阳性率更高(P<0.01),但 *APC*、*TP53*、*NRAS* 阳性率较低(P<0.01)。并且 MSI-H 肠癌中 DNA 损伤修复途径基因(DNA damage response, DDR)改变,如 *ARID1A*、*TGFBR2*、*BRCA2*、*ATR*、*BRCA1* 等的频率高于 MSS(P<0.01)。至于其他 DNA 错配修复(mismatch repair, MMR)基因,如 *MSH6*、*MLH1*、*MSH2*、*PMS2* 的突变频率也显著高于 MSS 组(P<0.01)。至少有 70.1%的 MSI-H 结直肠癌患者携带一个 MMR 基因(*MSH6/MSH2/MLH1/PMS2*)变异(70.1% *vs* 2.2%,P<0.05)。而在胚系突变中,MSI-H 型携带一个 MMR 基因(*MSH6*、*MLH1*、*MSH2*、*PMS2*)变异频率同样显著高于 MSS 型(P<0.01)。另外,*MLH1*、*MSH2*、*MSH6*、*PSM2* 之间似乎也是互斥的。见表 2。

2.3　不同样本类型中 MSI-H 与 TMB 的关系　进一步分析肠癌中不同的样本类型 MSI-H 与 TMB 关系发现在 MSI-H 中,原发灶、转移灶及血液中的 TMB 中位值分别为 99.6 突变/Mb、97.5 突变/Mb、97.5 突变/Mb,差异无统计学意义(P=0.468),而在 MSS 原发灶、转移灶及血液中的 TMB 中位值分别为 12.3 突变/Mb、9.4 突变/Mb、10.1 突变/Mb 差异有统计学意义(P=0.021),其中原发灶中检测的 TMB 高于转移灶。同样 MSI-H 型肠癌的原发灶、转移灶中 TMB 均高于 MSS(99.6 突变/Mb *vs* 12.3 突变/Mb,P<0.01;97.5 突变/Mb *vs* 9.4 突变/Mb,P<0.01),血液中 MSI-TMB 与 MSS-TMB 差异有统计学意义(97.5 突变/Mb *vs* 10.1 突变/Mb,P<0.01)。

表 1　MSI/MSS 状态结直肠癌 14 239 例患者的特征　[例(%)]

Tab. 1　Characteristics of colorectal cancer 14 239 patients with MSI/MSS status　[case (%)]

特征	MSI-H (n=1 018)	MSS (n=13 221)	χ^2 值	P 值
肿瘤类型				
结肠癌	892(87.62)	8 060(60.96)	287.778	<0.001
直肠癌	126(12.38)	5 161(39.04)		
性别				
男	601(59.04)	8 151(61.65)	2.728	0.099
女	417(40.96)	5 070(38.35)		
样本类型				
血液	82(8.06)	1 831(13.85)	76.791	<0.001
原发灶	904(88.8)	10 212(77.24)		
转移灶	32(3.14)	1 178(8.91)		

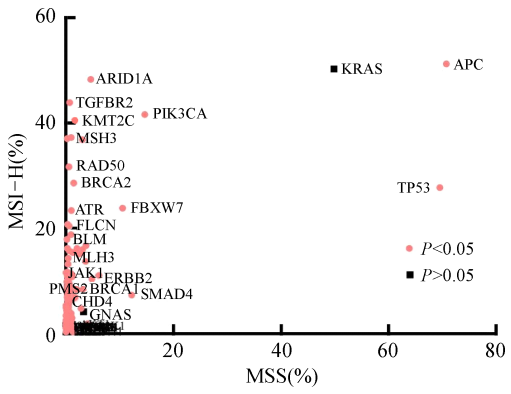

图 1　MSI-H/MSS 结直肠癌患者的基因突变频率图

Fig. 1　Gene mutation frequency map of MSI-H/MSS colorectal cancer patients

表 2　MSI-H 与 MSS 结直肠癌患者常见基因阳性率　(%)

Tab. 2　Common gene positivity rate of MSI-H and MSS colorectal cancer patients　(%)

基因	MSI-H	MSS	χ^2 值	*P* 值
KRAS	50.27	49.86	0.071	0.789
PIK3CA	41.63	14.67	496.806	<0.001
BRAF	11.04	0.89	607.871	<0.001
ERBB2	10.38	4.83	59.342	<0.001
APC	51.26	70.76	168.823	<0.001
TP53	27.76	69.54	739.882	<0.001
NRAS	1.86	3.98	11.455	0.001
DDR				
ARID1A	48.31	4.61	2 532.852	<0.001
TGFBR2	43.93	0.71	4 825.888	<0.001
BRCA2	28.63	1.44	2 134.412	<0.001
ATR	23.39	1.101	1 787.246	<0.001
BRCA1	8.31	0.8	406.913	<0.001
MMR(体系)				
MSH6	37.27	0.97	3 619.481	<0.001
MLH1	20.77	0.31	2 266.428	<0.001
MSH2	15.74	0.58	1 322.818	<0.001
PMS2	8.09	0.32	655.498	<0.001
MMR(胚系)				
MSH6	11.90	0.30	1 134.500	<0.001
MLH1	22.00	0.20	2 605.959	<0.001
MSH2	17.00	0.10	2 092.984	<0.001
PMS2	5.30	0.30	360.614	<0.001

3　讨　论

MSI-H 结直肠癌是接受 PD-1 抗体治疗的一类优势人群。大多数情况下,MSI 表型更常见于结肠癌中。本研究检测的 MSI-H 在结直肠癌中发生率为7.15%,低于欧美人群的 15%[5],但与报道的华东地区 MSI 频率6.31%[4]相当,与梁莉团队[13]报道的dMMR 在结直肠癌的发生率7.6%一致。MSI-H 结直肠癌患者的发病中位年龄显著低于 MSS 的患者(52 岁 *vs* 58 岁),与既往中国人群中报道的结果一致[4]。然而,本研究观察到原发性肿瘤、血液和转移灶之间的 MSI-H 发生率不太一致,与原发肿瘤和血液相比,转移灶的 MSI-H 肿瘤的发生率明显更低,这在以往的研究中也得到证实,可能与 MSI-H 肿瘤中更好的生物学特性有关[14]。同时可以发现不同的地区、人群、样本类型及检测方法得出的检测率是不完全一致的,本研究的样本分布较广泛,因此得到的 MSI 频率也许更能反映四川地区的总体特征。

而其他如 *KRAS*(49.88%)、*BRAF*(6.93%)、*NRAS*(3.83%)突变频率与东西方既往报道数据一致,分别为 40%~50%[15]、5.4%~6.7%[16]、3.8%[17]。而在 MSI-H 中,*PIK3CA*、*BRAF*、*ERBB2* 和 DDR 基因的突变率显著更高,但 *APC*、*TP53*、*NRAS* 的突变率却更低。Trabucc 等[7]报道也显示一些基因突变在 MSI-H 或 MSS 中明显富集,并且这些变异与 MSI-H 或 MSS 肿瘤中的通路富集相关。本研究分析 *RAS* 和 *BRAF* 的突变状态,*KRAS* 与 *BRAF* p.V600E 似乎是互斥的,这与之前的报道一致[4],但与 *BRAF* 其他位点突变能够共存。本研究结果还显示,MSI-H 相对比 MSS 患者要更年轻些,这也能为临床医生提供诊断参考。

MMR 基因变异会导致 dMMR,进而导致 MSI[18]。在 MSI-H 型肠癌中,有 70.1%的患者携带一个 MMR 基因变异,显示 MSI-H 与 MMR 基因变异存在高度一致性。对比 Salem 等[19]报道的 59.7%,本研究数据会偏高;其他 *MSH6*(37.27%)、*MLH1*(20.77%)、*MSH2*(15.74%)、*PMS2*(8.09%),与 Salem 等[19]发表的 *MSH6*(38.1%),*MLH1*(22%)、*MSH2*(14%)、*PMS2*(8%)均一致。除了 MMR 基因的体细胞突变外,MMR 基因种系改变会导致 Lynch 综合征的发生[18]。本研究的结果显示在 56.20%的 MSH 结直肠癌中发现了胚系的 MMR 基因变异。不同于体系变异,MLH1 和 MSH2 胚系突变高于 MSH6,且 MLH3 胚系突变频率较低。虽然 MMR 基因可以通过 NGS 进行检测,但还不能直接根据致病变异情况来判断 MMR 蛋白的表达/MSI 状态。实际临床中存在 1%~10%的患者 MMR 与 MSI 检测结果不一致的情况[13,19-20],主要原因有 MMR 染色异质性[21]、MMR 基因错义突变[22]、MMR 系统的功能补偿机制[23]等。比如除了 *MSH6/MSH2/MSH3/MLH1/MLH3/PMS2* 基因以外,MSI 还能由其他基因(*POLE*、*POLD*)引起,那么 MMR 基因阴性也可能是 MSI-H;如某些 MMR 蛋白的缺失会被功能代偿那么即使存在 MMR 突变也可能是 MSS。

TMB 是免疫治疗的一个重要的独立生物标志物,在实体瘤研究中发现,TMB 与抗 PD-1/PD-L1 治疗的客观缓解率(objective response rate, ORR)高度相关[24]。Schrock 等[25]近期研究显示,4.5%患者为 MSI-H,MSI-H 患者的中位 TMB 为 46.1 个突变/Mb,而 MSS 患者为 3.5 个突变/Mb; Kabbarah 团队报道的中位 TMB 为 52 个突变/Mb,MSS 的患者中位 TMB 为 6 个突变/Mb[26],而本研究数据显示在 MSI-H 的患者中,中位 TMB 为 80 个突变/Mb,MSS 的患者中位 TMB 为 6.7 个突变/Mb。本研究的 MSI-H 中 TMB 数据显然偏高,可能与不同 TMB 算法及人群不一样相关。原发灶和转移灶中 MSI 的 TMB 值高于 MSS,然而血液 MSI-H 样本较少,这一趋势是不显著的。在 MSI-H 型中,不同样本类型中检测的 TMB 值差异无统计学意义;在 MSS 型中,原发灶的 TMB 高于转

移灶。Puccini 等[14]报道相比于远端转移,高 TMB 及 MSI-H 在原发肿瘤中更容易观察到。不同的样本可能存在肿瘤异质性,血液检测 TMB 能克服这一问题,但血液 TMB 是否比组织 TMB 分析更有优势,仍需进行前瞻性研究来验证。

EBV 感染与鼻咽癌、胃癌及淋巴瘤等多种肿瘤的发生发展有关,但在结直肠癌中的研究数据较少。Meng 等[27]研究中显示 EBV 与结直肠癌的病理和临床进展有关,能够促进结直肠癌进展。在已报道的各种研究中,EBV 阳性比例的范围相当大(1.4% ~ 46%)[8]。本研究的数据显示 EBV 阳性率为 0.43%,远低于已报道的数据。但也有些研究报道浸润性结直肠癌中 EBV 阳性率[28]极低,甚至未发现 EBV 表达[29]。此外 EBV 阳性与 MSI-H 的关系,似乎也是相互排斥的,因此对于结直肠癌 MSI-H 与 EBV 的关系仍值得进一步的探讨。

本研究以大样本对比中国结直肠癌患者 MSI-H 与 MSS 两种表型的分子差异。与 MSS 相比,MSI-H 型具有不同的分子特征。DDR 基因及 MMR 基因在 MSH 型中变异频率更高,高 TMB 在 MSI-H 更常见,EBV 在肠癌中的发生率低。本研究还存在一些局限性,回顾性研究设计可能导致隐性的选择偏差。目前大多数结直肠癌的基因组变异缺乏有效的靶向治疗方案,而 MSI-H 作为免疫治疗的生物标志物,充分了解其分子特征有助于在未来更好的指导结直肠癌的精准治疗。

利益冲突　无

参考文献

[1] 郑荣寿,陈茹,韩冰峰,等.2022 年中国恶性肿瘤流行情况分析[J].中华肿瘤杂志,2024,46(3):221-231.
Zheng RS, Chen R, Han BF, et al. Cancer incidence and mortality in China, 2022[J]. Chin J Oncol, 2024, 46(3): 221-231.

[2] André T, Shiu KK, Kim TW, et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer[J]. N Engl J Med, 2020, 383(23): 2207-2218.

[3] 郭梦雅,彭炜惟,方瑛,等.早中期微卫星高度不稳定及稳定型结直肠癌的免疫治疗进展[J].中国临床研究,2023,36(12):1886-1889.
Guo MY, Peng WW, Fang Y, et al. Advances in immunotherapy of early-middle stage colorectal cancer with microsatellite instability-high and stability[J]. Chin J Clin Res, 2023, 36(12): 1886-1889.

[4] Nojadeh JN, Behrouz Sharif S, Sakhinia E. Microsatellite instability in colorectal cancer[J]. EXCLI J, 2018, 17: 159-168.

[5] Song YL, Wang LL, Ran WW, et al. Effect of tumor location on clinicopathological and molecular markers in colorectal cancer in Eastern China patients: an analysis of 2, 356 cases[J]. Front Genet, 2020, 11: 96.

[6] Shubin V, Shelygin Y, Achkasov S, et al. Microsatellite instability in Russian patients with colorectal cancer[J]. Int J Mol Sci, 2022, 23(13): 7062.

[7] Kim JH, Park HE, Cho NY, et al. Characterisation of PD-L1-positive subsets of microsatellite-unstable colorectal cancers[J]. Br J Cancer, 2016, 115(4): 490-496.

[8] Trabucco SE, Gowen K, Maund SL, et al. A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1,000 microsatellite instability-high cases in 67, 000 patient samples[J]. J Mol Diagn, 2019, 21(6): 1053-1066.

[9] Bedri S, Sultan AA, Alkhalaf M, et al. Epstein-Barr virus (EBV) status in colorectal cancer: a mini review[J]. Hum Vaccin Immunother, 2019, 15(3): 603-610.

[10] Zhuang W, Ma JX, Chen XD, et al. The tumor mutational burden of Chinese advanced cancer patients estimated by a 381-cancer-gene panel[J]. J Cancer, 2018, 9(13): 2302-2307.

[11] Wang ZJ, Duan JC, Cai SL, et al. Assessment of blood tumor mutational burden as a potential biomarker for immunotherapy in patients with non-small cell lung cancer with use of a next-generation sequencing cancer gene panel[J]. JAMA Oncol, 2019, 5(5): 696-702.

[12] Wang ZH, Zhao XC, Gao C, et al. Plasma-based microsatellite instability detection strategy to guide immune checkpoint blockade treatment[J]. J Immunother Cancer, 2020, 8(2): e001297.

[13] 黎相照,刘焕娇,梁敏仪,等.微卫星不稳定性类型及临床病理特征:基于 1 394 例结直肠癌患者的标本检测[J].南方医科大学学报,2020,40(11):1645-1650.
Li XZ, Liu HJ, Liang MY, et al. Clinicopathological features and types of microsatellite instability in 1394 patients with colorectal cancer[J]. J Southern Med Univ, 2020, 40(11): 1645-1650.

[14] Puccini A, Seeber A, Xiu J, et al. Molecular differences between lymph nodes and distant metastases compared with primaries in colorectal cancer patients[J]. NPJ Precis Oncol, 2021, 5(1): 95.

[15] Schirripa M, Cohen SA, Battaglin F, et al. Biomarker-driven and molecular targeted therapies for colorectal cancers[J]. Semin Oncol, 2018, 45(3): 124-132.

[16] Yoshino T, Arnold D, Taniguchi H, et al. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS[J]. Ann Oncol, 2018, 29(1): 44-70.

[17] Wang YC, Loree JM, Yu C, et al. Distinct impacts of *KRAS*, *NRAS* and *BRAF* mutations on survival of patients with metastatic colorectal cancer[J]. J Clin Oncol, 2018, 36(15_suppl): 3513.

[18] Sinicrope FA. Lynch syndrome-associated colorectal cancer[J]. N Engl J Med, 2018, 379(8): 764-773.

[19] Salem ME, Bodor JN, Puccini A, et al. Relationship between *MLH*1, *PMS*2, *MSH*2 and *MSH*6 gene-specific alterations and tumor mutational burden in 1 057 microsatellite instability-high solid tumors[J]. Int J Cancer, 2020, 147(10): 2948-2956.

(下转第 1385 页)

PARP1 and caspase-3[D].Nanjing: Nanjing University of Chinese Medicine, 2017.

[17] 张瑞华,张静文,刘玲,等.黄芪及其有效组分药理作用与临床应用现状[J].陕西中医,2021,42(8):1138-1141,1146.
Zhang RH, Zhang JW, Liu L, et al. The pharmacological effects and current situation of clinical application of *Astragalus* and its effective components[J]. Shaanxi J Tradit Chin Med, 2021, 42(8): 1138-1141, 1146.

[18] 付远虹,吴敏.黄芪多糖通过 ANXA3 诱导人结肠癌 HT-29 细胞凋亡[J].遵义医科大学学报,2020,43(2):202-207.
Fu YH, Wu M. Astragalus polysaccharide induces the apoptosis of human colon cancer HT-29 cells through ANXA3[J]. J Zunyi Med Univ, 2020, 43(2): 202-207.

[19] 李梓盟,张佳彦,李菲,等.白花蛇舌草抗肿瘤化学成分及药理作用研究进展[J].中医药信息,2021,38(2):74-79.
Li ZM, Zhang JY, Li F, et al. Research progress on chemical components and pharmacological action rof *Hedyotis diffusa* in treating cancer[J]. Inf Tradit Chin Med, 2021, 38(2): 74-79.

[20] 赵亚兰.白花蛇舌草在肿瘤治疗中的应用[J].甘肃科技,2018,34(19):141-142,109.
Zhao YL. Application of *Hedyotis diffusa* in tumor treatment[J]. Gansu Sci Technol, 2018, 34(19): 141-142, 109.

[21] 靳祎祎.复方白花蛇舌草减轻大肠癌 5-FU 化疗肠道损伤的作用机制研究[D].福州:福建中医药大学,2018.
Jin YY. Study on the mechanism of compound *Hedyotis diffusa* in alleviating intestinal injury caused by 5-FU chemotherapy for colorectal cancer[D].Fuzhou: Fujian University of Traditional Chinese Medicine, 2018.

收稿日期:2024-02-24　**修回日期**:2024-04-30　**编辑**:王娜娜

(上接第 1378 页)

[20] Debniak T, Kurzawski G, Gorski B, et al. Value of pedigree/clinical data, immunohistochemistry and microsatellite instability analyses in reducing the cost of determining *hMLH*1 and *hMSH*2 gene mutations in patients with colorectal cancer[J]. Eur J Cancer, 2000, 36(1): 49-54.

[21] McCarthy AJ, Capo-Chichi JM, Spence T, et al. Heterogenous loss of mismatch repair (MMR) protein expression: a challenge for immunohistochemical interpretation and microsatellite instability (MSI) evaluation[J]. J Pathol Clin Res, 2019, 5(2): 115-129.

[22] Zhang LY. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing[J]. J Mol Diagn, 2008, 10(4): 301-307.

[23] Stelloo E, Jansen AML, Osse EM, et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer[J]. Ann Oncol, 2017, 28(1): 96-102.

[24] Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition[J]. N Engl J Med, 2017, 377(25): 2500-2501.

[25] Schrock AB, Ouyang C, Sandhu J, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer[J]. Ann Oncol, 2019, 30(7): 1096-1103.

[26] Innocenti F, Ou FS, Qu XP, et al. Mutational analysis of patients with colorectal cancer in CALGB/SWOG 80405 identifies new roles of microsatellite instability and tumor mutational burden for patient outcome[J]. J Clin Oncol, 2019, 37(14): 1217-1227.

[27] Meng QT, Sun H, Wu SS, et al. Epstein-Barr virus-encoded microRNA-BART18-3p promotes colorectal cancer progression by targeting *de novo* lipogenesis[J]. Adv Sci, 2022, 9(35): e2202116.

[28] Sarvari J, Mahmoudvand S, Pirbonyeh N, et al. The very low frequency of Epstein-Barr JC and BK viruses DNA in colorectal cancer tissues in Shiraz, Southwest Iran[J]. Pol J Microbiol, 2018, 67(1): 73-79.

[29] Mehrabani-Khasraghi S, Ameli M, Khalily F. Demonstration of herpes simplex virus, cytomegalovirus, and Epstein-Barr virus in colorectal cancer[J]. Iran Biomed J, 2016, 20(5): 302-306.

收稿日期:2023-10-21　**修回日期**:2024-01-11　**编辑**:叶小舟