Clinicopathologic features and prognostic value of DNA mismatch protein expression patterns in stage II/III colorectal cancer.

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Abstract

Background

MSI CRCs were associated with better prognosis and limited predictive value for adjuvant chemotherapy. However, whether the same is true in Northeastern China is still unclear. The aim of the present study was to evaluate the association of clinicopathologic features and MMR/MSI status determined with immunohistochemistry analysis in Northeast China patients with stage II/III CRCs. Particularly, we sought to detect the relationship between MMR/MSI status and efficacy of oxaliplatin and fluoropyrimidine based adjuvant chemotherapy.

Methods

In total, 476 pathological specimens from eligible stage II/III CRCs were analyzed with IHC between 2016 and 2018, of which 63 CRCs were diagnosed with MMR protein deficiency. Clinicopathological features and overall survival (OS) were compared between these above two groups.

Result

The incidence of dMMR CRCs in our cohort was 13.2% (63/476). Immunohistochemistry (IHC) revealed two common dMMR IHC patterns in 63 dMMR CRCs. And dMMR type1 showed a higher proportion of women (P=0.001) and earlier pathological N stage (P=0.075). In the multivariate Cox regression model, POC (Postoperative chemotherapy) and dMMR were associated with a favor prognosis in CRC patients with stage II/III (HR 0.47, 95%CI 0.30-0.74, P=.001; HR 0.34, 95%CI 0.14-0.79, P=.013). However, adjuvant chemotherapy based on oxaliplatin and fluorouracil cannot prolong the OS of dMMR CRCs (P=0.182).

Conclusions

MMR protein appeared distinct associations with tumor staging, serum CEA level and tumor size. And MMR protein was an independent prognostic marker in patients with stage II CRC, whereas dMMR CRC patients did not seem to benefit from oxaliplatin combined with fluorouracil-based adjuvant chemotherapy.

Take Home Messages

- In this work, IHC was used to analyze the MMR protein status of surgical specimens obtained from CRC patients, and the clinicopathological characteristics and prognosis were compared between dMMR and pMMR CRCs.
In addition, our results suggest that the deficiency of MMR protein in tissue of color rectal cancer was associated with a better prognosis in stage III patients, but not to benefit from oxaliplatin/5-FU based adjuvant chemotherapy.

This study is of reference for the chemotherapy treatment of dMMR CRCs.

**Background**

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer-related death in the United States, with approximately 78,500 new cases in males and 67,100 new cases in females, and it is estimated that 27,640 males and 23,380 females died from CRC in 2019[1].

Currently, it was wildly accepted that chromosomal instability (CIN) and microsatellite instability (MSI) were the major pathways for CRC development[2]. CIN carcinomas, the most commonly pathway, characterized by gene mutations, which leading to chromosomal aneuploidy, deficiency of heterozygosity and chromosomal rearrangements. However, MSI tumors were usually caused by the deficiency of mismatch repair (MMR) genes including MSH1, PMS2, MSH2 and MSH6 with the morbidity of approximately 15% of all sporadic CRCs[3]. Compared with CIN CRCs, dMMR/MSI colorectal malignancy tend to be proximal and histologically exhibit poor differentiation, a mucinous cell type and a better prognosis[4 5]. Therefore, it’s crucial to identify the MMR/MSI status of CRCs.

Although there were currently three approaches for detection including immunohistochemistry (IHC), polymerase chain reaction (PCR) based methods and next generation sequencing (NGS), the sensitivity and specificity among the three methods were with high concordance rate (92-97%)[6].

And the sensitivity to detect dMMR/MSI carcinomas with IHC is nearly 95% with the specificity of almost 100% in most reports.[7 8].

It is well known that dMMR/MSI is associated with a better prognosis in colorectal malignancy. However, whether MSI CRCs can benefit from postoperative adjuvant chemotherapy remains controversial. Multiple previous studies had demonstrated that 5-FU-based adjuvant chemotherapy did not prolong 5-year overall survival (OS) in dMMR/MSI colorectal carcinomas[9 10]. Recently, several researches considered that the addiction of oxaliplatin to fluoropyrimidine adjuvant chemotherapy can significantly improve OS and DFS compared to fluoropyrimidine alone treatment in dMMR/MSI CRCs[11 12]. Therefore, further study is needed to verify the predictive significance of MMR/MSI status for adjuvant chemotherapy, especially in Northeast China. After all, few relevant reports were conducted here.

The aim of the present research was to evaluate the association of clinicopathologic features and MMR/MSI status determined with immunohistochemistry analysis in Northeast China patients with stage II/III CRCs. Particularly, we sought to detect the relationship between MMR/MSI status and efficacy of oxaliplatin and fluoropyrimidine based adjuvant chemotherapy.

**Materials And Method**
Patients and materials

The approval has been obtained by the ethics committee of Jilin University Second hospital before the study proceeded, and we conducted the research complying with Helsinki Declaration of World Medicine Association. The detection of the four MMR proteins with immunohistochemistry has been routinely performed since 2016 in our institution, therefore, colorectal carcinoma patients diagnosed from December 1st, 2016 to December 1st, 2018 were enrolled, eventually, 476 patients were considered eligible after rigorous screening. The clinical data of the eligible CRCs (such as gender, age etc.) was obtained from medical records. The pathologic data (such as T category, pathologic N category, differentiation, tumor pathologic type and vascular invasion) was collected from pathological examination result, and IHC was used to evaluated MMR status in CRC patients. The overall survival (OS) of the CRCs was defined as the time from radical surgical operation to death. There are two main approaches to obtain the follow-up information, outpatient clinic system and telephone questionnaire respectively. Inspired by Lee’s article, we defined the proximal colon as cecum, ascending colon or transverse colon[13]. Pathological stage (TNM) depended on depth of infiltration, lymphatic metastasis and distant metastasis in accordance with the American Joint Committee on Cancer (AJCC) cancer staging manual. Adjuvant chemotherapy was performed postoperatively was determined by the TNM stage and the patient’s willingness with the regimen of oxaliplatin combined with capecitabine.

IHC

The immunohistochemistry analyses for surgical specimens obtained from CRC patients who underwent radical resection were performed in Department of Pathology, Second Affiliated Hospital of Jilin University. The pre-programmed autostainer (YZB/USA 2016-2012) was used to perform IHC on paraffin-embedded tissues. Here are the brief steps. 2-μm-thick formalin-fixed and paraffin-embedded tumor tissue sections were heating for two hours at 70 °C. After that, we used a PT link machine(California, America) to perform HIER(heat induced epitope retrieval) after the sections were deparaffinized and rehydrated. The slides were incubated after adding primary antibodies for PMS2 (clone: ZA-0542; 1:1; Wuxi), MLH1(clone: ZM-0154; 1:1; Wuxi), MSH2(clone: ZA-0622; 1:1; Wuxi), MSH6(clone: G24072; 1:1; Ventana). Finally, hematoxylin was used to counterstain the slides, and then sealed with neutral balsam. In all the runs, the negative and positive controls were existed. The tissue sections omitting the primary antibodies were regarded as negative controls, and the tissues which were known to express the proteins were positive controls.

Evaluation of IHC

Two pathologists (Z.Y. Wang and D.W. Huang) were responsible for reviewing the IHC strains of colorectal carcinoma samples. The MMR protein status was considered of expression loss when nuclear straining of carcinoma cells was absent whereas the surrounding stromal cells performed positive nuclear straining. Therefore, proficient mismatch repair (pMMR) is considered only if all the four MMR proteins
expression in tumor tissues, while nuclear staining absence at least one MMR proteins was regarded as deficient mismatch repair (dMMR) (Figure 1).

The inclusion and exclusion criteria

The following inclusion criteria were: 1) Patients were in the 18-75 age ranges. 2) The pathological diagnosis was colorectal carcinoma. 3) TNM / / . 4) Radical surgery was applied. The exclusion criteria were showed as follows: 1) History of malignant carcinoma. 2) Poor physical condition(such as severe liver, respiratory tract, cardiovascular or kidney disease). 3) Underwent preoperative neoadjuvant therapy, which was considered likely to affect MMR protein expression[14]. 4) Clinicopathological data cannot be collected accurately.

Statistical analysis

Statistical analyses were performed using SPSS for MAC, version 26.0 (IBM Corporation). Continuous variables were performed using Mann–Whitney U test or t test, while $\chi^2$ test or Fisher exact test was used for comparing categorical data. For multivariate analyses, Cox proportional hazards model was applied, and survival curves were created by the Kaplan-Meier method. The distinction was considered statistically significant if P values were less than 0.05.

Result

Initially, we enrolled a total of 670 CRC patients who underwent radical surgery between December 1st, 2016 and December 1st in the Second Affiliated Hospital of Jilin University. After that, 194 CRCs were excluded according to the exclusion criteria, of which 39 patients with history of malignant carcinoma, 14 cases in poor physical conditions, 28 CRCs undergoing preoperative neoadjuvant therapy, and 113 patients’ clinicopathological data cannot be collected accurately (Figure 2).

MMR protein expression in CRCs

Among the 476 patients, 63(13.2%) CRCs were identified as defective expression of MMR protein. The loss of PMS2 was observed in 37 of 476(7.9%) CRCs and was the most frequent, followed by 31(6.6%) with the loss of MLH1, 29(6.2%) with the loss of MSH6, 29(6.2%) with the loss of both MSH1 and PMS2, 16(3.4%) with the isolated loss of MSH2, 11(2.4%) with the loss of both MSH2/MSH6, and 3(0.1%) CRCs with MLH1-/PMS2-/MSH6-. Patients with at least one loss of MLH1 and PMS2 but no MSH2 or MSH6 deficiency were assigned to group type 1, while patients with at least one loss of MSH2 and MSH6 but no MLH1 or PMS2 deficiency were assigned to group type 2.

MMR expression and patient characteristics

The clinicopathological features between pMMR CRCs and dMMR CRCs were shown in Table 1. There was a significant difference in tumor size, tumor site, CEA level, pN status, TNM stage, differentiation, and
pathological type between the two groups (P<0.05 for all comparisons). Moderate mucinous carcinoma, stage ☵, pN0, and proximal colon cancer were more common in dMMR CRCs compared with pMMR CRCs. The CEA level was significantly higher in pMMR group (4.05 vs. 2.75, P=0.004), whereas bigger tumor size was observed in dMMR group (5.8 vs. 4.7, P= 0.000). No difference in gender, age, BMI, pT status, and vascular invasion was witnessed between patients with dMMR CRC and pMMR CRC. Among the 63 dMMR CRCs, we identified 53 type1 and type 2 dMMR CRCs.

Table 1 Patients’ clinicopathologic characteristics in regard to mismatch repair protein status.

| Characteristic              | Patients, No. |          |          | P value |
|-----------------------------|---------------|----------|----------|---------|
|                             | pMMR (n=413)  | dMMR (n=63) |         |         |
| gender                      |               |          |          |         |
| male                        | 264           | 42       |          | 0.672<sup>a</sup> |
| female                      | 149           | 21       |          |         |
| age, y                      | 61.4(±9.8)    | 60.7(±10.5) |          | 0.602<sup>c</sup> |
| BMI, Kg/m<sup>2</sup>       | 23.0(±3.5)    | 22.3(±3.0) |          | 0.114<sup>c</sup> |
| Tumor site                  |               |          |          |         |
| Proximal colon              | 114           | 38       |          |         |
| Distal colon or rectum      | 299           | 25       |          |         |
| Tumor size, cm              | 4.7(±1.7)     | 5.8(±2.1) |          | 0.000<sup>a</sup> |
| CEA, ng/ml                  | 4.05(2.26-7.54) | 2.75(1.33-4.40) |          | 0.000<sup>c</sup> |
| pT status                   |               |          |          |         |
| pT1                         | 6             | 0        |          | 0.004<sup>d</sup> |
| pT2                         | 15            | 1        |          |         |
| pT3                         | 366           | 55       |          |         |
| pT4                         | 26            | 7        |          |         |
| pN status                   |               |          |          |         |
| pN0                         | 192           | 45       |          | 0.469<sup>b</sup> |
| pN1                         | 154           | 13       |          |         |
| pN2                         | 67            | 5        |          |         |
| TNM stage                   |               |          |          |         |
| ☵                           | 192           | 44       |          | 0.001<sup>a</sup> |
| ☾                           | 221           | 19       |          |         |
| Differentiation             |               |          |          |         |
| Well                        | 10            | 0        |          | 0.001<sup>a</sup> |
| Moderate                    | 383           | 55       |          |         |
| Poor                        | 20            | 8        |          |         |
| Pathological type           |               |          |          |         |
| Tubular                     | 389           | 47       |          | 0.040<sup>b</sup> |
| Mucinous                    | 11            | 16       |          |         |
| Mixed                       | 13            | 0        |          |         |
| Vascular invasion           |               |          |          |         |
| Yes                         | 160           | 18       |          | 0.000<sup>b</sup> |
| No                          | 253           | 45       |          |         |

Abbreviations: dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; BMI, Body Mass Index; CEA, Carcinoma Embryonic Antigen.

a, χ<sup>2</sup> test; b, Fisher exact test; c, t test; d, Mann–Whitney U test.

The clinicopathological features of 29 type 1 and 24 type 2 dMMR CRCs are presented in Table 2. The MSH2/MSH6 protein expression loss was associated with gender, and which was more common in male.
CRC patients (P=0.011). However, there was no significant differences between the two groups among the other clinicopathological features (P>0.05).

Table 2 Clinicopathological features of 29 type 1 and 24 type 2 dMMR CRCs

| Characteristic            | Type 1 PMS2/MLH1 (n=29) | Type 2 MSH2/MSH6 (n=24) | P value |
|---------------------------|--------------------------|--------------------------|---------|
| gender                    |                          |                          |         |
| male                      | 16                       | 21                       | 0.011<sup>a</sup> |
| female                    | 13                       | 3                        |         |
| age, y                    | 60.14(±11.26)            | 60.33(10.21)             | 0.948<sup>c</sup> |
| BMI, Kg/m<sup>2</sup>     | 22.35(3.43)              | 22.66(2.45)              | 0.722<sup>c</sup> |
| Tumor site                |                          |                          |         |
| Proximal colon            | 18                       | 15                       | 0.974   |
| Distal colon or rectum    | 11                       | 9                        |         |
| Tumor size, cm            |                          |                          |         |
| CEA, ng/ml                | 6.09(2.41)               | 5.54(1.89)               | 0.372<sup>c</sup> |
| pT status                 |                          |                          |         |
| pT3                       | 27                       | 21                       | 0.308<sup>d</sup> |
| pT4                       | 2                        | 3                        |         |
| pN status                 |                          |                          |         |
| pN0                       | 22                       | 16                       | 0.649<sup>b</sup> |
| pN1                       | 7                        | 4                        |         |
| pN2                       | 0                        | 4                        |         |
| TNM stage                 |                          |                          |         |
| Moderate                  | 21                       | 16                       | 0.075<sup>b</sup> |
| Poor                      | 8                        | 8                        |         |
| Pathological type         |                          |                          |         |
| Tubular                   | 25                       | 21                       | 0.650<sup>a</sup> |
| Mucinous                  | 4                        | 3                        |         |
| Vascular invasion         |                          |                          |         |
| Yes                       | 20                       | 19                       | 1.000<sup>b</sup> |
| No                        | 9                        | 5                        |         |
| Differentiation           |                          |                          |         |
| Moderate                  | 8                        | 8                        | 0.459<sup>a</sup> |
| Poor                      | 25                       | 21                       |         |
| Pathological type         |                          |                          |         |
| Tubular                   | 4                        | 3                        |         |
| Mucinous                  | 20                       | 19                       |         |
| Vascular invasion         |                          |                          |         |
| Yes                       | 9                        | 5                        |         |
| No                        | 22                       | 16                       |         |

Abbreviations: BMI, Body Mass Index; CEA, Carcinoma Embryonic Antigen.
<sup>a</sup>, χ<sup>2</sup> test; <sup>b</sup>, Fisher exact test; <sup>c</sup>, t test; <sup>d</sup>, Mann–Whitney U test.

**MMR expression and clinical outcomes**

In the present research, 89 CRCs patients had died (dMMR CRCs, N=82; pMMR CRCs, N=7). To further analyze the association of MMR status and prognosis in patients suffering from colorectal carcinoma, Kaplan-Meier analyses were performed (Figure 3). The survivorship analysis (Kaplan-Meier) showed 86% OS rate in dMMR group and a 68% OS rate in pMMR group after 5 years (P=.004, Kaplan-Meier log-rank). In stage <i>+</i> CRC patients, the estimated OS rate for patients with loss of MMR protein was 89% and patients without deficiency was 74% after 5 years, which indicated that the loss expression of MMR protein preformed a more favorable prognosis in stage <i>+</i> colorectal carcinoma patients (P=.014,
Kaplan-Meier log-rank). However, OS did not differ from the two groups in patients with stage II colorectal carcinomas (P=.353, Kaplan-Meier log-rank).

To determine whether dMMR was independent prognostic factor associated with CRC clinical outcomes, Cox proportional hazard model was used to performed univariate and multivariate analysis (table 3). The risk variables were consisted of age, gender, tumor location, tumor size, pathological N stage, pathological type, CEA level, differentiation, vascular invasion, TNM stage, MMR status, and postoperative chemotherapy(POC), which were generally considered to be associated with prognosis of CRC. In the univariate analysis, TNM stage (HR 2.25, 95%CI 1.43-3.54, P=.000), CEA level (HR 2.08, 95%CI 1.37-3.16, P=.001), and dMMR (HR 0.32, 95%CI 0.14-0.72, P=.006) were significantly associated with survival, while vascular invasion, gender, age, tumor size, POC, tumor location, pathological type, and differentiation were not. In the final multivariate analyses, POC and dMMR independent of other factors were associated with a favor prognosis in CRC patients with stage II/III (HR 0.47, 95%CI 0.30-0.74, P=.001; HR 0.34, 95%CI 0.14-0.79, P=.013).

Table 3 Univariate and multivariate associations between covariates and the composite primary endpoint of dead in stage II/III CRC patients
| Characteristic       | Univariate HR (95% CI) | P value | Multivariate HR (95% CI) | P value |
|---------------------|------------------------|---------|--------------------------|---------|
| **Gender**          |                        |         |                          |         |
| Female              | 1.0 (Reference)        |         |                          |         |
| Male                | 1.39 (0.88-2.19)       | 0.161   |                          |         |
| **Age**             |                        |         |                          |         |
| <50                 | 1.0 (Reference)        |         |                          |         |
| ≥50                 | 1.78 (0.82-3.85)       | 0.143   |                          |         |
| **Tumor site**      |                        |         |                          |         |
| Proximal            | 1.0 (Reference)        |         |                          |         |
| Distal              | 1.33 (0.84-2.11)       | 0.223   |                          |         |
| **Tumor size**      |                        |         |                          |         |
| <5 cm               | 1.0 (Reference)        |         |                          |         |
| ≥5 cm               | 1.14 (0.75-1.73)       | 0.539   |                          |         |
| **TNM stage**       |                        |         |                          |         |
| I                   | 1.0 (Reference)        |         | 1.0 (Reference)          |         |
| II                  | 2.25 (1.43-3.54)       | 0.000   | 2.52 (1.50-4.24)         | 0.001   |
| **Pathological type** |                      |         |                          |         |
| Tubular             | 1.0 (Reference)        |         | 1.0 (Reference)          |         |
| Mucinous            | 1.24 (0.57-2.70)       | 0.585   | 2.58 (1.14-5.85)         | 0.023   |
| Mixed               | 1.59 (0.50-5.04)       | 0.435   |                          |         |
| **Differentiation** |                        |         |                          |         |
| Well                | 1.0 (Reference)        |         |                          |         |
| Moderate            | 1.55 (0.21-11.17)     | 0.663   |                          |         |
| Poor                | 1.43 (0.17-12.29)     | 0.745   |                          |         |
| **Vascular invasion** |                      |         |                          |         |
| No                  | 1.0 (Reference)        |         |                          |         |
| Yes                 | 1.26 (0.83-1.92)       | 0.285   |                          |         |
| **CEA**             |                        |         |                          |         |
| <5.2 ng/ml          | 1.0 (Reference)        |         | 1.0 (Reference)          |         |
| ≥5.2 ng/ml          | 2.08 (1.37-3.16)       | 0.001   | 1.93 (1.25-2.98)         | 0.003   |
| **POC**             |                        |         |                          |         |
| Did not receive     | 1.0 (Reference)        |         | 1.0 (Reference)          |         |
| Receive             | 0.68 (0.45-1.04)       | 0.075   | 0.47 (0.30-0.74)         | 0.001   |
| **MMR**             |                        |         |                          |         |
| pMMR                | 1.0 (Reference)        |         | 1.0 (Reference)          |         |
| dMMR                | 0.32 (0.14-0.72)       | 0.006   | 0.34 (0.14-0.79)         | 0.013   |

**Abbreviations:** dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; CEA, Carcinoma Embryonic Antigen; POC, postoperative adjuvant chemotherapy.

**The predictive value of MMR protein for efficacy of chemotherapy**

Further assessment was performed to analysis the effect of POC in both pMMR and dMMR CRC patients using Kaplan-Meier analyses (Figure 4). Among the 413 pMMR CRCs, the overall 5 years survival rates of patients with POC and without POC were 78.1% and 57.2% respectively (P=.026, Kaplan-Meier log-rank). In the subgroup of 192 stage I CRCs, POC didn't seem to make any sense to promote a better prognosis (P=.254, Kaplan-Meier log-rank), whereas did in subgroup of 221 stage II CRCs (P=.000, Kaplan-Meier log-rank). However, among 63 dMMR CRCs, POC did not improve the outcome of patients with either stage I or II.

**Discussion**
The present study elicited three main findings. First, in Northeast China, dMMR CRCs were commonly located in the proximal colon, had poorly differentiation histology with mucinous features, and appeared to have bigger tumor size, lower CEA level, and earlier TNM stage. Second, dMMR was an independent prognostic marker with a favorable impact on survival in stage III CRCs. Finally, postoperative adjuvant chemotherapy, based on oxaliplatin capecitabine, cannot prolong the overall 5 years survival in patients with dMMR CRC.

The morbidity of dMMR in the present study was 13.2%, which was similar with Western countries[15]. Several researches comparing the clinicopathological features between dMMR and pMMR CRCs have been published over recent decades. The study[16] of Evaluation of Concordance Between Deficient Mismatch Repair and Microsatellite Instability Testing and Their Association with Clinicopathological Features in Colorectal Cancer enrolled 738 patients, of which 131 patients possessed deficiency of MMR protein. There was a significant difference between two groups in age, tumor location, differentiated degree. These results were almost congruent with the result in the present research. However, the rate of mucinous carcinoma was higher and TNM stage was earlier in dMMR group in our study. Only stage III/IV CRC patients were enrolled in our study, whereas Bai et.al’ research included colorectal carcinoma patients with all pathological stage, which may account for the difference. The tumor size of dMMR CRC was considered bigger in the present study, which was coincident with the report of Batur’s[17]. The serum CEA level was significant lower in dMMR CRC in our study.

As far as we know, no literature has reported the present finding. This result can probably be explained by the fact that serum CEA level was associated with carcinoma pathological stage[18], and the rate of stage III CRC was higher in the dMMR group in the present research. In addition, male patients were more frequently seen in type 2 CRCs among 53 dMMR colorectal cancer patients, which may imply a potential ethnic difference in the molecular pathogenesis of CRC.

For the past few years, a great quantity of studies had found that the deficiency of MMR protein for CRC was associated with a favor prognosis[19-21]. However, researches evaluating the association between MMR protein and prognosis in CRC patients with stage III were quite rare. The prognostic impact of dMMR/MSI was studied in 1254 patients with stage III colon cancer who participated in the PETACC-3 trial[22]. Among the 1254 patients, 190 patients had dMMR tumors, and of which, and 104 patients of which were diagnosed with stage III colorectal cancer. The study revealed that dMMR/MSI was associated with better OS (HR 0.47, 95% CI: 0.31–0.72, p < 0.001), however, the prognostic effect was mainly driven by the benefits seen in stage III disease since no significant difference was witnessed in OS between stage III CRCs with dMMR and pMMR. The result was almost concurrent with the findings in the present study.

Why is dMMR/MSI irrelevant to prognosis in stage III CRCs? It is considered that the prognostic benefits from dMMR rely on the immunological reaction associated with dMMR/MSI tumors, which can to increase host anti-tumor immunity to suppress tumor metastasis[23]. However, with disease progression
and tumor metastasis, mechanisms of immune evasion develop that enable dMMR/MSI tumors to evade immune surveillance with loss of a prognostic advantage[24].

It's generally believed that fluorouracil (5-FU)-based adjuvant chemotherapy does not improve 5-year OS in patients with dMMR tumors, and lack of benefit seems to be similar in both stage I and II dMMR cancers[9-25]. However, interestingly, several studies revealed that oxaliplatin combined with fluorouracil-based adjuvant chemotherapy was associated better DFS and OS. A small retrospective study[26] enrolling 32 stage II dMMR CRC patients who had either 5-FU (n = 20) or FOLFOX (n = 12) as adjuvant treatment showed that the addition of oxaliplatin was associated with improved DFS in dMMR/MSI patients compared to 5-FU only treatment (HR 0.17, 95% CI: 0.04–0.68, p = 0.01). In an update of the MOSAIC study[27], 95 dMMR/MSI stage II/III CRC cases were identified among 1008 patients. FOLFOX as adjuvant treatment was associated with a trend toward improved OS (HR 0.41, 95% CI: 0.16–1.07, p = 0.069) among patients with dMMR/MSI tumors. However, addition of oxaliplatin did not appear to prolong 5-year overall survival for dMMR CRC patients either with stage I or stage II in the present study. Although only 63 dMMR CRC patients were enrolled in our study, the patients were screened strictly according to inclusion and exclusion criteria, and the survive curve may reveal tumor-related deaths more really because of elderly patients and Frail patients were excluded. Therefore, the result of our study may imply a potential ethnic distinction in responsiveness to oxaliplatin-based chemotherapy among dMMR CRC patients.

In the present study, IHC was used to detect the MMR status. IHC directly evaluates the MMR protein presence/absence in the tumor cells while PCR-based tests use a set of primers to check for PCR products size differences between normal and tumor tissues. These two approaches are sensitive and specific with high concordance rate (97%)[6]. Therefore, IHC detection for CRC tumors to evaluate the MMR status was acceptable if PCR-based test was not available. The chemotherapy regimens of oxaliplatin and capecitabine was performed in patients after radical surgery. Capecitabine, an oral fluorouracil, was designed to preferentially produce 5-FU at tumor sites. Studies showed that the regimen has fewer side effects with the same efficiency compared with the traditional oxaliplatin combined with 5-FU regimen, and patient compliance was good due to oral route of administration.

The present study had several limitations. First, MMR gene analyses were not performed in the present research, and that may be meaningful for the mechanism. Second, confounding factors were remained in the retrospective study. Third, the number of patients included was still small because the detection of MMR protein using IHC has been routinely performed after December 2016. But 467 patients enrolled in the present study were also acceptable. Fours, there was no differences in the adjuvant chemotherapy regimen among CRC patients. Therefore, the effect of different adjuvant chemotherapy regimens for dMMR CRC patients should be put forward through further study. In addition, it was necessary to carry out a prospective and multi-center study with a large sample size in the future.

**Conclusion**
In the present study, we found that expression of MMR protein appeared distinct associations with tumor staging, serum CEA level and tumor size. The expression of MMR protein was an independent prognostic marker in patients with stage II CRC, whereas dMMR CRC patients did not seem to benefit from oxaliplatin combined with fluorouracil-based adjuvant chemotherapy.

**Declarations**

**Availability of data and materials**

The datasets generated and analyzed during the current study available from the corresponding author on reasonable request.

**Abbreviations**

CRC: Colorectal cancer;
CIN: chromosomal instability;
MMR: mismatch repair;
MSI: microsatellite instability;
IHC: immunohistochemistry;
PCR: polymerase chain reaction;
NGS: next generation sequencing;
OS: overall survival;
CEA: Carcinoembryonic antigen;
POC: postoperative adjuvant chemotherapy;
AJCC: American Joint Committee on Cancer.

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**Contributions**
AS and MW conceived the study design. ZZ and DL acquired the data for the study. YG, YY, and RQ analyzed and interpreted the data. ZW read the pathological section. AS drafted the manuscript. YY and ZZ revised the manuscript critically. The authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

The institutional review board of The Second Hospital of Jilin University had approved the research. And the ethics approval number was 2021111. The patient informed consent was not necessary because of the retrospective design, which had been confirmed by the local ethic committee.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Figures

**Figure 1**

The evaluation for immunohistochemistry (IHC) analyses of dMMR CRCs. The nuclear staining loss of MLH1 (A), PMS2 (B), MSH2 (C), and MSH6 (D) in cancer cells.
CRC patients underwent R0 resection between December 1st, 2016 to December 1st, 2018 (n=942).

Inclusion criteria
1) Age between 18-75 y.
2) Pathologically diagnosed as CRC.
3) TNM II/III.
4) colorectal R0 resection was applied.

670 patients were identified.

39 patients with history of malignant carcinoma.

28 CRCs undergoing preoperative neoadjuvant therapy.

14 cases with severe respiratory tract, liver, kidney or cardiovascular disease.

113 patients’ clinicopathological data cannot be collected accurately.

476 patients included in the present study.

476 patients included in the present study.

dMMR CRC (n=63).

pMMR CRC (n=413).

Clinicopathological characteristics, OS, and benefit from POC were analyzed.

Figure 2

CONSORT Diagram of Patient Flow
Figure 3

prognosis value for MMR protein status. Notes: (A) the association of OS and MMR protein status in all patients. (B) the association of OS and MMR protein status in patients with stage II. (C) the association of OS and MMR protein status in patients with stage III. The red line is pMMR. The blue line is dMMR. Abbreviation: pMMR, mismatch repair proficient. dMMR, mismatch repair deficient. OS, overall survival.
Figure 4

prognosis value for MMR protein status. Notes: (A, B and C) the association of OS and MMR protein status in patients with pMMR CRCs. (D, E and F) the association of OS and MMR protein status in patients with pMMR CRCs. The red line is POC. The blue line is without POC. Abbreviation: pMMR, mismatch repair proficient. dMMR, mismatch repair deficient. POC, postoperative adjuvant chemotherapy. OS, overall survival.