Clinic-pathological characteristics of MMR protein, KRAS-NRAS-BRAF gene mutation, HER-2 and PD-L1 status in 3822 Chinese colorectal cancer patients

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Research

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Abstract

Objectives

Colorectal cancer is one of the most common cancers. It is a heterogeneous tumor. The aim of this study was to investigate MMR protein expression, KRAS-NRAS-BRAF gene mutation, HER-2 and PD-L1 status in large Chinese CRC patients.

Methods

We reviewed all CRC patients who underwent surgery in Peking University Cancer Hospital between 2010 and 2018 from a prospective collected medical database. We analyzed the clinic-pathological factors associated with immunohistochemistry profiles. We analyzed KRAS-NRAS-BRAF gene changes through ARMS-PCR.

Results

In our study, d-MMR frequency of CRC was 8%. d-MMR was more common in poorly differentiated colorectal cancer ($p = 0.000$), and d-MMR was more likely to occur in stage T3 + T4($p = 0.026$), and p-MMR was more likely to have lymph node metastasis($p = 0.000$). d-MMR was more likely to occur in the right colon, and p-MMR patients usually occur in the rectum sigmoid colon($p = 0.000$). p-MMR patients were more likely to have distant metastasis ($p = 0.000$). We analyzed all 3822 patients with-BRAF status available by IHC, the frequency of BRAF positive expression ration was 1.4%. A total of 1068 colorectal cancer patients had gene analysis by ARMS-PCR. 484 of them had KRAS gene mutation, 28 NRAS gene mutation, 20 BRAF mutation and 536 patients had no mutation. The percentage was 45%, 2.6%, 2%, and 50%, respectively. d-MMR patients usually had BRAF gene mutation ($p < 0.001$). KRAS and NRAS gene mutations were not associated with MMR status ($p = 0.846$, $p = 0.438$). PD-L1 expression was higher in the female group ($p = 0.034$), poorly differentiated adenocarcinoma group($p = 0.026$), and d-MMR group ($p = 0.005$). The ratio of HER-2 3 + was 1.9%, and HER-2 3 + was usually found in p-MMR group($p = 0.043$).

Conclusion

Our data can improve deeper understanding of colorectal cancer carcinogenic factors and future therapies for colorectal cancer.

1. Introduction

Colorectal cancer (CRC) is the third most common carcinoma worldwide. Although the treatment has made great progress, the mortality is still very high. Colorectal cancer has high heterogeneity and many gene mutations. Colorectal cancer can be divided into four molecular types according to different genomes [1-5]. Colorectal cancer involves the accumulation of a series of gene changes, leading to the occurrence of adenoma and adenocarcinoma. BRAFV600E mutation and d-MMR CRC represent unique molecular subtype [6].

Mismatch repair system defects can cause genomic instability and microsatellite instability（MSI） [7,8]. DNA mismatch repair (MMR) genes defects fail to repair DNA replication errors in repetitive sequences. This induces accumulation of frame-shift mutations in genes with microsatellites. MSI occurs at different frequencies across malignancies. MSI can be detected using immunohistochemistry (IHC) and/or PCR. In addition, next-generation sequencing (NGS) can also be used to detect microsatellite instability. Traditionally, identification of MSI has relied on immunohistochemical detection of loss of MMR protein expression (MLH1, MSH2, MHS6 and PMS2). Bethesda markers of PCR-based test often include two mononucleotide (BAT25 and BAT26) and three dinucleotide (D5S346, D2S123 and D17S250) MS loci. 44% of the Bethesda markers instability in colorectal cancer is considered to be MSI-High (MSI-H). Sporadic cases of MSI patients are due to the inactivation of MMR gene, which usually have somatic mutations. MSI can also be caused by methylation of MLH1 promoter. While Lynch syndrome is due to hereditary germline mutations.

Programmed cell death protein 1 (PD-1), a sort of inhibitory checkpoint molecule, was discovered and named in 1992 [9]. It is expressed on the surface of activated T cells, which regulate T cells' proliferation and activation. Programmed cell death 1 ligand (PD-L1) is an immune inhibitory receptor ligand that is expressed on the surface of cancer cells and immune cells in the tumor microenvironment. Interaction of PD-1 and PD-L1 inhibits T-cell activation and cytokine production. PD-L1 is induced by many cytokines, such as interferon-γ (IFN-γ), signal transducer and activator of transcription 3 (STAT3), epidermal growth factor receptor (EGFR), transforming growth factor β (TGFβ), and hypoxia inducible-factor-1α (HIF-1α) [10]. In normal immune system, PD-1/PD-L1 pathway activation will inhibit the immune function of T lymphocytes and promote the inhibitory function of regulatory T cells, which can reduce the immune response. So, it can inhibit autoimmune response. It can prevent the development of autoimmune disease. It can maintain autoimmune tolerance in healthy individuals. When cancer occurs, PD-L1 is expressed on the surface of cancer cells, which can lead to immune escape. Because cancer cells are not recognized by immune cells now. In many tumors, the PD-L1 expression is usually associated with poor prognosis [11-13]. More and more studies show that this pathway has an important relationship with the progress of cancer. Immunotherapy for this pathway is very popular now. There are many Keytruda drugs and Opdivo drugs from foreign companies, and there are many clinical experiments for different tumors, such as lung cancer, malignant melanoma, and d-MMR solid tumors [14,15].

In recent years, although the level of treatment has increased significantly, the five-year survival rate of advanced colorectal cancer is still lower than 5%, and the incidence of new colorectal cancer cases is 1.4 million per year. Colorectal cancer patients in China have also increased significantly in recent years. The occurrence of colorectal cancer involves many pathways and genes, such as the following several genes, which are related to the occurrence, development and clinical treatment of colorectal cancer. Human epidermal growth factor receptor 2 (HER-2) is a very important member of EGFR family. There are many studies on breast cancer and gastric cancer, and the conclusions are consistent [16-19]. HER-2 inhibitors have been used in the treatment of the above tumors [20,21]. At present, there are few studies on the relationship between HER-2 and colorectal cancer. We retrospectively analyzed the expression of HER-2 in

3822 primary colorectal cancers patients of Peking University Cancer Hospital. The retroviral oncogene RAF was discovered in 1983. The proto-oncogene family includes the three proteins: ARAF, CRAF and BRAF [22]. BRAF is part of the RAS-MAPK pathway. RAS-MAPK signaling controls and regulates many important cellular mechanisms, including cell division, proliferation, growth and apoptosis. Even in the absence of cytokine, hormone or growth factor stimulation, BRAF V600E mutation can also activate these signaling pathway, leading to unregulated cell proliferation and eventual cancer development [23,24]. Related research indicated that BRAF mutations have a poor prognosis in MSI-H metastatic colorectal cancer. Immunotherapy for MSI-H patients and BRAF-inhibitors combination treatment for BRAF-mutated tumors in metastatic colorectal cancer is promising [25,26]. We analyzed MMR status, gene mutation, HER-2 and PD-L1 in a large population. Our study provided an important basis for the future treatment of colorectal cancer.

2. Materials And Methods

2.1 General Information

3822 colorectal cancer patients who underwent radical surgery in our hospital were collected. All specimens were fixed in 10% neutral formaldehyde solution. The 2014 edition of the WHO Digestive System Tumor Pathology and Genetics classification of colorectal tumor TNM staging criteria was used. Of the 3822 patients, 2297 patients were man and 1525 patients were female. According to tumor differentiation, 3337 cases were well-moderately differentiated, 861 cases were poorly differentiated. 1819 cases had lymph node metastasis, and there were 2005 patients without lymph node metastasis. According to the invasion depth of tumor the number of T3+T4 stage cases was 3128, and the number of T1+T2 stage cases was 696. The number of patients who have not had preoperative neo-adjuvant therapy was 3341.The number of patients who have had preoperative neo-adjuvant therapy was 482. The research was approved Peking University Cancer Hospital ethics committees.

2.2 MMR protein expression status evaluation

The complete loss of nuclear staining in tumor cells TCs is thought to be the expression of protein deletion, including the immunohistochemical staining of MLH1, MSH2, MSH6 and PMS2. Normal epithelial cells and lymphocytes were used as an internal control. The protein absence of MLH1 and PMS2, MSH2 and MSH6, MSH6 alone and PMS2 alone are considered as d-MMR. Positive staining for all four proteins is considered to be p-MMR. Immunohistochemical staining was used to detect the expression of the four proteins. The specific clone numbers are as follows MLH1, GM002 antibody; MSH2, RED2 antibody; MSH6, EP49 antibody; PMS2, EP51 antibody (Gene Tech, Shanghai, China).

2.3 KRAS-NRAS-BRAF gene mutation analysis

A total of 1068 colorectal cancer patients had gene analysis by amplification refractory mutation system (ARMS)-PCR. We detected the point mutations of KRAS and NRAS, including exons 12, 13 and 61. We also detected V600E point mutation of BRAF gene by PCR. FFPE tissues were digested using 20 mg/mL

proteinase K in ATL buffer (Qiagen) overnight at 56 ℃. DNA isolation was performed with the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. The reaction conditions included 1 cycle at 95 ℃ for 5min; 15 cycles at 95 ℃ for 25 s, 64 ℃ for 20 s, 70 ℃ for 20 s; 31 cycles at 93 ℃ for 25 s, 60 ℃ for 35 s, 72 ℃ for 20 s. Fluorescence signals were collected at 60 ℃.

2.4 PD-L1 expression evaluation

The results of PD-L1 immunohistochemistry staining were interpreted by two experienced pathologists. PD-L1 was stained on the membrane of TCS and TILs. According to the area of staining, it was divided into four levels, including < 1%, 1% - 10%, 10% - 50% and positive staining area > 50%. The type of PD-L1 antibody was SP142 (Spring Bioscience, Pleasanton, CA, USA).

2.5 HER-2 expression in colon cancer tissues by immunohistochemistry

HER-2 was located in the cell membrane and scored for stained tissue according to the HER-2 Detection Guide for Gastric Cancer. Response or < 10% tumor cell membrane staining for HER-2 (0); ≥ 10% tumor cell membrane weak or faintly visible membrane staining, or only partial membrane staining for HER-2 (1 +); ≥ 10% tumor cells are weak to moderate basal membrane, lateral membrane or complete membrane staining for HER-2 (2 +); ≥ 10% tumor cell basal membrane, lateral membrane or complete membrane strong staining for HER-2 (3 +). The clone of HER-2 antibody was 4B5（Roche, USA）.

2.6 Statistical analysis method

We used SPSS 17.0 software for statistical analysis. $p< 0.05$ was considered to be statistically significant.

3. Results

3.1 MMR protein expression statusand clinic-pathological features

The frequency of d-MMR CRC was 8%. The number of d-MMR patients was 310, and the number of p-MMR patients was 3512. MMR status was not related to gender(p=0.397). d-MMR was more common in poorly differentiated colorectal cancer (p=0.000) (Figure 1), and d-MMR was more likely to occur in stage T3 and stage T4 (p=0.026), and lymph node metastasis often occured in p-MMR patients（p=0.000）. p-MMR patients were more likely to have distant metastasis (p=0.000). d-MMR often occured in the right colon, and p-MMR often occured in rectum and sigmoid colon (p=0.000), as shown in Table1.

3.2 KRAS, NRAS, BRAF gene mutation rate was 45%, 2.6%, 2% respectively. BRAF protein expression in 3822 colorectal cancer patients, positive expression was more often in d-MMR patients

A total of 1068 colorectal cancer patients had gene analysis by ARMS-PCR. 484 of them had KRAS gene mutation, 28 NRAS gene mutation, 20 BRAF mutation and 536 cases had no mutation. The percentage was 45%, 2.6%, 2%, and 50%, respectively. The mutation of KRAS and NRAS genes was not related to

MMR status (Table 2). When we analyzed -BRAF protein IHC data, we found BRAF positivity ration was 1.4%. d-MMR patients usually had BRAF gene mutation ($p < 0.001$) (Figure 2), as shown in Table 3. Some BRAF proteins were negative by IHC testing, but the genetic detection was positive. This indicated that gene detection was more accurate. The mutation type of BRAF gene was GTG > GAG at codon 600 (Figure 3). Of the 484 KRAS mutation CRC patients, 352 carried a KRAS mutation in the 12^{th} codon (Figure 4). The 12^{th} codon mutation included 35G>A, 35G>C, 35G>T, 34G>T. 110 patients carried a KRAS mutation in the 13^{th} codon, and the codon mutation type was 38G>A. 22 cases had 61^{th} codon mutation, and the 61^{th} codon mutation included 181C>A, 182A>T, 182A>G, 182A>C, 183A>T, 183A>C. The mutation type of NRAS gene at codon 12 was 35G > A mutation type. KRAS and NRAS gene mutations were not associated with MMR status. BRAF mutations were frequently found in d-MMR patients.

3.3 HER-2 expression in 3822 CRC patients

In our study, the ratio of HER-2 (1+ ~3+) was 47.4%, the ratio of HER-2 (3+) was 1.9%, and the ratio of HER-2 (2+ ~ 3+) was 14.7%. The number of HER2 negative case was 2017. HER-2 1+ case number was 1254. HER2 2+ case number was 490. HER-2 3+ case number was 72. HER-2 3 + was often found in p-MMR group (Table 4).

3.4 PD-L1 expression and its relation to clinicopathological factors

In our study, if 1% of TCs or TICs staining is defined as positive staining, the statistical results were as follows. PD-L1 expression was correlated with sex and differentiation, but not with T stage and lymph node metastasis. PD-L1 expression was higher in d-MMR group (p=0.000), as shown in Table 5.

If 50% of TCs or TICs staining is defined as positive staining, the statistical results are as follows. PD-L1 was highly expressed in women, poorly differentiated adenocarcinoma and d-MMR（Figure 5）, but its expression was not related to T stage and lymph node metastasis, as shown in Table 6.

4. Discussion

The World Health Organization (WHO) reported that the five years' survival rate of colorectal cancer in our country was 32%, far lower than that in developed countries. This is related to the high proportion of patients in the middle and late stages of treatment. Improving the survival of these patients can significantly improve the survival rate of colorectal cancer in China. Because of the heterogeneity and complexity of colorectal cancer genes, they play an important role in drug resistance and metastasis [27, 28]. Key genes are often used as important prognostic markers and new targets for colorectal cancer treatment. [29, 30] Mutations of some important genes can affect the treatment of colorectal cancer. In our data, 484 patients had KRAS gene mutation, and the mutation rate was 45%. 20 patients had BRAF mutation, and the mutation rate was 2%. KRAS and NRAS genes were not related to MMR status, but BRAF mutation was frequently found in d-MMR patients.

In recent years, drugs targeting immune card control have led to a new era of cancer treatment entering immunotherapy. This provided a new strategy for the treatment of colorectal cancer. MSI-H tumor is a subtype in gene molecular typing and has a good therapeutic response to immunotherapy. It is very important to select MSI-H patients from colorectal cancer patients for possible immunotherapy to avoid the toxicity and pain of chemotherapy. Immunohistochemical staining can be used as an important method to select MSI-H patients. In our research, d-MMR patients accounted for 8% of all colorectal cancer patients.

PD-L1 is one of the major members of the B7 / CD28 co-stimulatory molecule superfamily and plays a negative regulatory role. Some studies showed that PD-L1 was regulated by microRNA [31]. PD-L1 maybe a new evaluable marker for the clinical prognosis of colorectal cancer, and a possible clinical target for immunotherapy in the future. It is of great clinical significance to study its expression in colorectal cancer for its pathogenesis and future treatment. In colorectal cancer, there are a few inconsistencies in the prognosis of PD-L1 [32, 33]. Our data showed that PD-L1 expression was significantly higher in d-MMR patients. And PD-L1 expression was higher in poor differentiated patients than in well-medium differentiated patients. There were no significant differences between different genders, ages ($p > 0.05$). In our study, the frequency of d-MMR was 8%. The number of d-MMR patients is 310, and the number of p-MMR patients is 3512. d-MMR was more common in poorly differentiated colorectal cancer ($p = 0.000$), and d-MMR was more likely to occur in T3 and T4 stage ($p = 0.026$). p-MMR patients often had lymph node metastasis($p = 0.000$).d-MMR colorectal cancer was more likely to occur in right colon, and p-MMR colorectal cancer was more likely to occur in the rectum sigmoid colon ($p = 0.000$).

HER-2 overexpression can lead to anti-EGFR monoclonal antibody resistance, the mechanism is that the up-regulation can maintain MAPK (mitogen-activated protein kinase) phosphorylation, which leads to intestinal cancer cells resistance of rituximab and panitumumab, and anti-HER-2 treatment after drug resistance can slow the growth of transplanted tumors and even tumor retraction [34–36]. Therefore, it is of great clinical significance to study the expression of HER-2 in colorectal cancer. The results of our large-scale study of colorectal cancer patients in China are as follows. The positive rate of HER-2 in colorectal cancer ranged from 1.6–70.9%. In our study, the ratio of HER-2 (1 + ~ 3+) was 47.4%, and the ratio of HER-2 (2 + ~ 3+) was 14.7%. The ratio of HER-2 (3+) was 1.9%. The positive rate of HER-2 is quite different and may be related to the following: sample size, detection method and criteria, human judgment difference and HER-2 positive standard, and the HER-2 positive standard is more It is important. In gastric cancer and breast cancer, it is currently determined that HER-2 positive is IHC3 + or IHC2 + / fluorescence in situ hybridization (FISH) positive. In colon cancer, there is currently no uniform standard. It has been reported that BRAF WT MSI colon cancer is more prone to HER-2 mutation, and HER-2 signal regulation in HER-2 L755S mutant MSI colorectal cancer plays a leading role. It has also been reported that about one third of patients with metastatic colorectal cancer develop HER-2 deficient mutations, and HER-2 shortened colorectal cancer is more prone to MSI-H. At the same time, MSI-H and HER-2 are related to tumor invasion depth and differentiation degree, which indicates that there may be a certain correlation

between MSI and HER-2 expression in colorectal cancer tissues. In our study, the expression of HER-2 3 + was often found in p-MMR group.

BRAFV600E often mutates in colorectal cancer. It plays a role in the development of CRC. It is a clinical and pathological feature of CRC and a poor prognosis [37]. The effect of BRAFV600E mutation on targeted therapy still needs to be deepened. Studying the BRAF expression status of CRC improves targeted therapy. KRAS protein is downstream of the EGFR signal pathway. The mutant KRAS gene and BRAF gene can automatically activate the pathway and start the downstream signal transduction without EGFR receiving signal, so that only the patients with wild-type KRAS and BRAF gene can benefit from the treatment of anti EGFR, while the patients with mutation cannot benefit from the treatment. Therefore, before the treatment with anti EGFR monoclonal antibody, the mutation analysis of RAS must be carried out.

When analyzing all 3822 colorectal cancer patients by IHC, BRAF protein expression positivity was 1.4%. The mutation rate of BRAF gene detected by PCR was 2%. Some BRAF proteins were negative by IHC testing, but the genetic detection was positive. This indicated that gene detection was more accurate. PCR gene detection can detect some BRAF IHC negative cases. It is of great significance to select patients with BRAF mutation, which can be treated more appropriately. Sporadic MSI-H cases were often associated with BRAF gene mutation, which is often wild-type in Lynch syndrome [25]. In our study, positive BRAF protein expression was more common in d-MMR colorectal cancer ($p = 0.000$). In the future study, we will add follow-up time to discuss the prognosis.

5. Conclusion

MMR protein expression and gene mutation can affect the treatment of colorectal cancer. In our study, MMR protein status was not related to gender ($p = 0.397$). d-MMR patients were more common in poorly differentiated colorectal cancer ($p = 0.000$), and more likely to occur in T3 + T4 stage ($p = 0.026$). p-MMR patients often had lymph node metastasis and distant metastasis($p = 0.000$). Right colon often had d-MMR status, and p-MMR status was more likely to occur in the rectum sigmoid colon ($p = 0.000$). Our research indicated that PD-L1 was higher in female group ($p = 0.034$), poorly differentiated adenocarcinoma ($p = 0.026$), and d-MMR group ($p = 0.005$), but not related to T stage and lymph node metastasis. A total of 1068 colorectal cancer patients had gene analysis by ARMS-PCR. The KRAS, NRAS, and BRAF mutation rate was 45%, 2.6%, 2%. KRAS and NRAS gene mutations were not associated with MMR status. BRAF mutations were frequently found in d-MMR patients. HER-3 + cases were usually found in p-MMR group. Understanding the clinic-pathological characteristics of colorectal cancer has great significance for its treatment. In the future study, we will analyze protein expression and gene mutation with prognosis, and clarify the mechanism in colorectal cancer, so as to provide a better basis for the future treatment of colorectal cancer patients.

Abbreviations

CRC: Colorectal Cancer; MSI:Microsatellite instability; ARMS:amplification refractory mutation system; PD-L1:programmed death-ligand 1; IHC:immunohistochemistry

Declarations

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study was approved by Peking University Cancer Hospital ethics committee. People who participated in this research had complete clinical data. Signed informed consents were obtained from the patients and/or the guardians.

Consent for publication

Not applicable.

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Authors' contributions

Li Zhang and Nan Chen conceived the study and drafted the manuscript. Aiwen Wu, Zhongwu Li and Jie Chen acquired the data. All authors read and approved the manuscript.

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Competing interests

The authors declare no conflict of interest

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Tables

Table1 CRC patients' clinicopathological features *n* (%).

	MMR status		*p* value
	d-MMR	p-MMR	
Gender			
Female	131/3822(3.4%)	1394/3822(36.5%)	0.397
Male	179/3822(4.9%)	2118/3822(55.4%)	
Tumor differentiation			
Poorly	191/3822(5%)	396/3822(10.4%)	0.000*
Well-medium	119/3822(3.1%)	3116/3822(81.5%)	
Tumor stage			
T3+T4 stage	268/3822(7%)	2858/3822(75%)	0.026*
T1+T2 stage	42/3822(1.1%)	654/3822(17.1%)	
Lymph node metastasis			
Present	96/3822(2.5%)	1722/3822(45.1%)	0.000*
Absent	214/3822(5.6%)	1790/3822(47%)	
Tumor location site			
Transverse colon	15/3822(0.4%)	61/3822(2%)	0.000*
Left colon	40/3822(1%)	209/3822(5.5%)	
Right colon	170/3822(4.4%)	707/3822(18.5%)	
Rectal sigmoid colon	85/3822(2.2%)	2535/3822(66.3%)	
Metastasis			0.000*
M1	7/3480(0.2%)	314/3480(0.9%)	
M0	275/3480(8%)	2884/3480(82.9%)	

Table2 Correlation between MMR and gene mutation status in 1068 CRC patients.

Mutation status	MMR status		*p* value
	d-MMR	p-MMR	0.125
No mutation(n=536)	36/1068(3.4%)	500/1068(46.8%)	
KRAS mutation(n=484)	34/1068(3.2%)	450/1068(42.1%)	0.846
BRAF mutation(n=20)	4/1068(0.4%)	16/1068(1.5%)	0.048*
NRAS mutation(n=28)	1/1068(0.1%)	27/1068(2.5%)	0.438

Table3 d-MMR colorectal cancer patients often had BRAF gene mutation(*p*=0.000)

BRAF status	d-MMR	p-MMR	*p* value
BRAF protein IHC positive	11/2907(0.4%)	29/2907(1%)	0.000*
BRAF protein IHC negative	186/2907(6.4%)	2681/2907(92.2%)	
BRAF gene mutation-type	4/1068(0.4%)	16/1068(1.5%)	0.046*
BRAF gene wild type	71/1068(6.6%)	977/1068(91.5%)	

Table 4 HER-2 expression

	d-MMR	p-MMR	*p* value
HER-2: 0,1+,2+	304/3835(7.9%)	3459/3835(90.2%)	0.043*
HER-2: 3+	1/3835(0.3%)	71/3835(1.9%)	

HER-2	d-MMR	p-MMR
0 (n=2020)	148/3835(3.9%)	1872/3835(48.8%)
1+ (n=1253)	98/3835(2.6%)	1155/3835(30.1%)
2+ (n=490)	58/3835(1.5%)	432/3835(11.3%)
3+ (n=72)	1/3835(0.2%)	71/3835(1.9%)

Table5 Patient characteristics according to programmed death ligand 1 (PD-L1) expression (PD-L1 cut off value=1%)

	PD-L1					
	0%	1%-5%	5%-10%	10%-50%	>=50%	*p* value
Gender						
Male	43	31	7	3	3	0.022*
Female	27	7	6	1	7	
Tumor differentiation						
Poorly	18	12	1	0	6	0.028*
Well-medium	52	26	12	4	4	
Tumor stage						
T3+T4	56	31	13	3	8	0.240
T1+T2	14	7	0	1	2	
Lymph node metastasis						
Present	38	17	6	1	5	0.726
Absent	32	21	7	3	5	
MMR status						
p-MMR	40	35	6	2	2	0.000*
d-MMR	30	3	7	2	8	

Table 6 Patient characteristics according to programmed death ligand 1 (PD-L1) expression (PD-L1 cut off value=50%)

	PD-L1		
	<50%	>=50%	*p* value
Gender			
Male	84	3	0.034*
Female	41	7	
Tumor differentiation			
Poorly	31	6	0.026*
Well-medium	94	4	
Tumor stage classification			
T3+T4	103	8	1.000
T1+T2	22	2	
Lymph node metastasis			
Present	62	5	1.000
Absent	63	5	
MMR status			
p-MMR	83	2	0.005*
d-MMR	42	8	

Figures

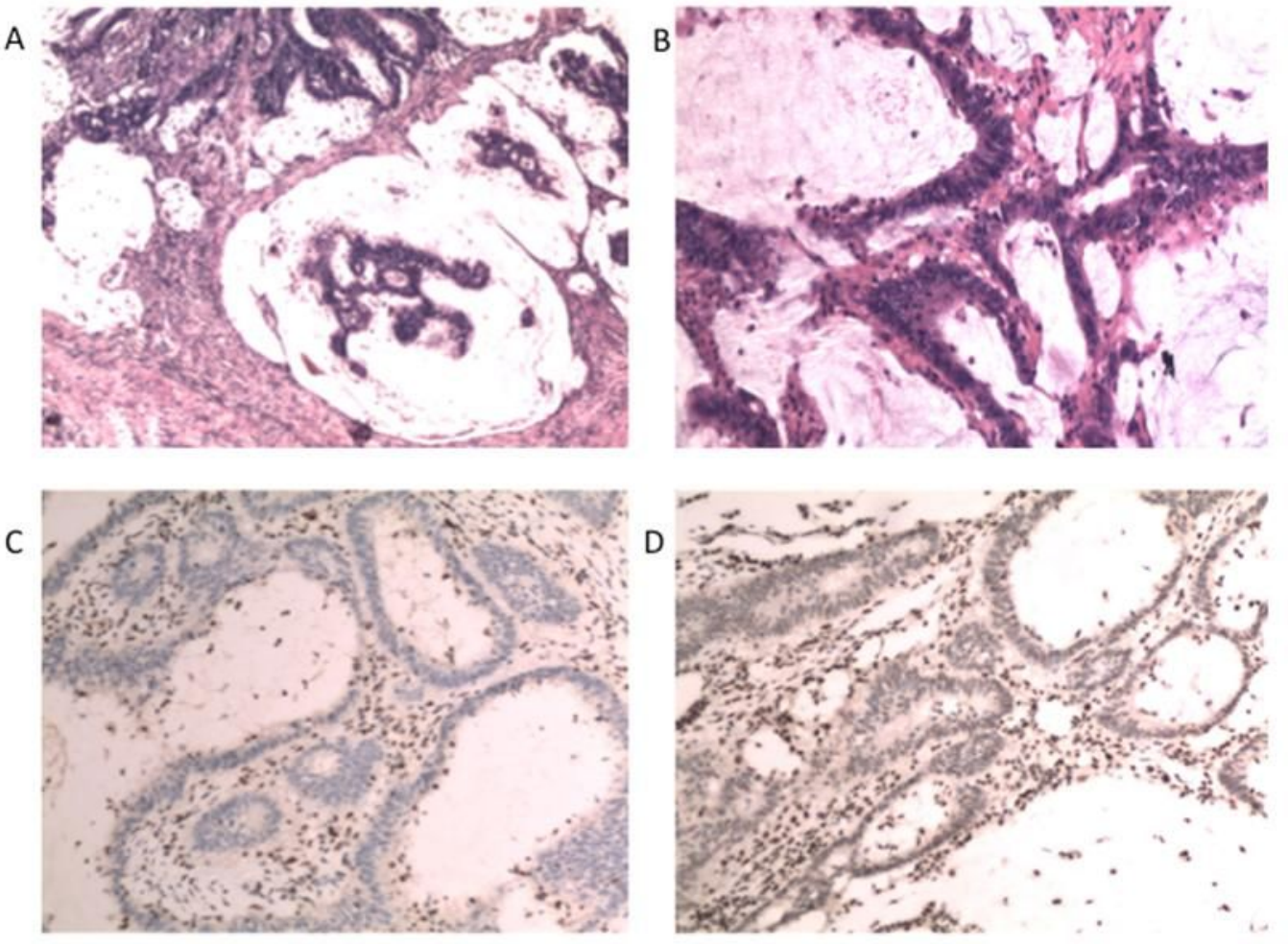

Figure 1

d-MMR was more common in poorly differentiated colorectal cancer A Mucinous adenocarcinoma of colorectal cancer (one type of poorly differentiated colorectal cancer), HE staining, 100x magnification; B Mucinous adenocarcinoma of colorectal cancer, HE staining, 200x magnification; C MLH1- IHC staining, negative tumor cell nucleus, 200x magnification D PMS2- IHC staining, negative tumor cell nucleus, 200x magnification

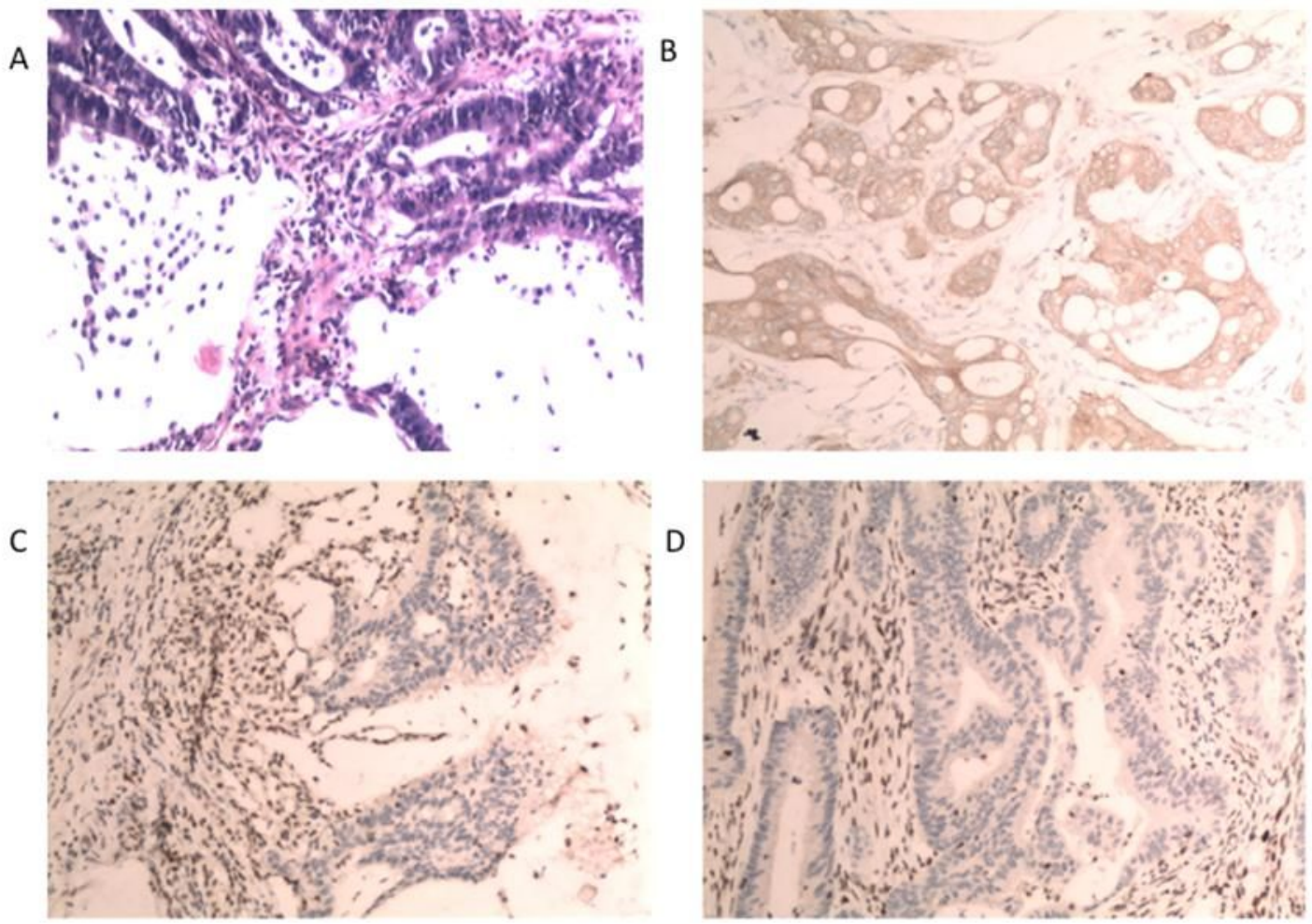

Figure 2

BRAF positive expression was more often in d-MMR patients (MLH1-, PMS2-) A Mucinous adenocarcinoma HE staining, 100x magnification B BRAF protein expression, cytoplasmic positive IHC staining, 100x magnification C-D MLH1-, PMS2- respectively tumor cell nucleus negative, 100x magnification

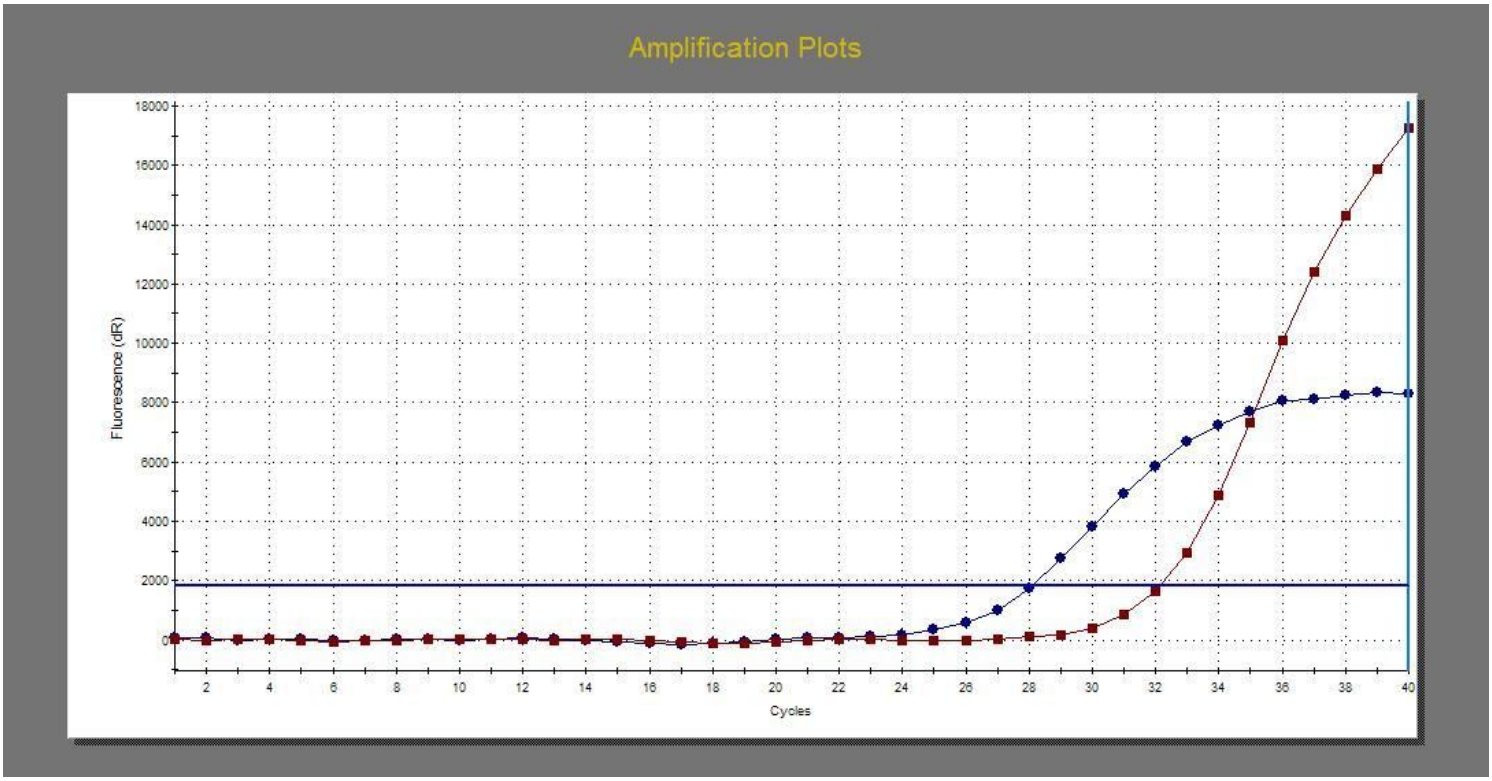

Figure 3

ARMS-PCR detection of mutated BRAF in formalin-fixed paraffin-embedded colorectal cancer tissues. The red up curve was the V600E point mutation amplification curve of BRAF gene detected in samples.

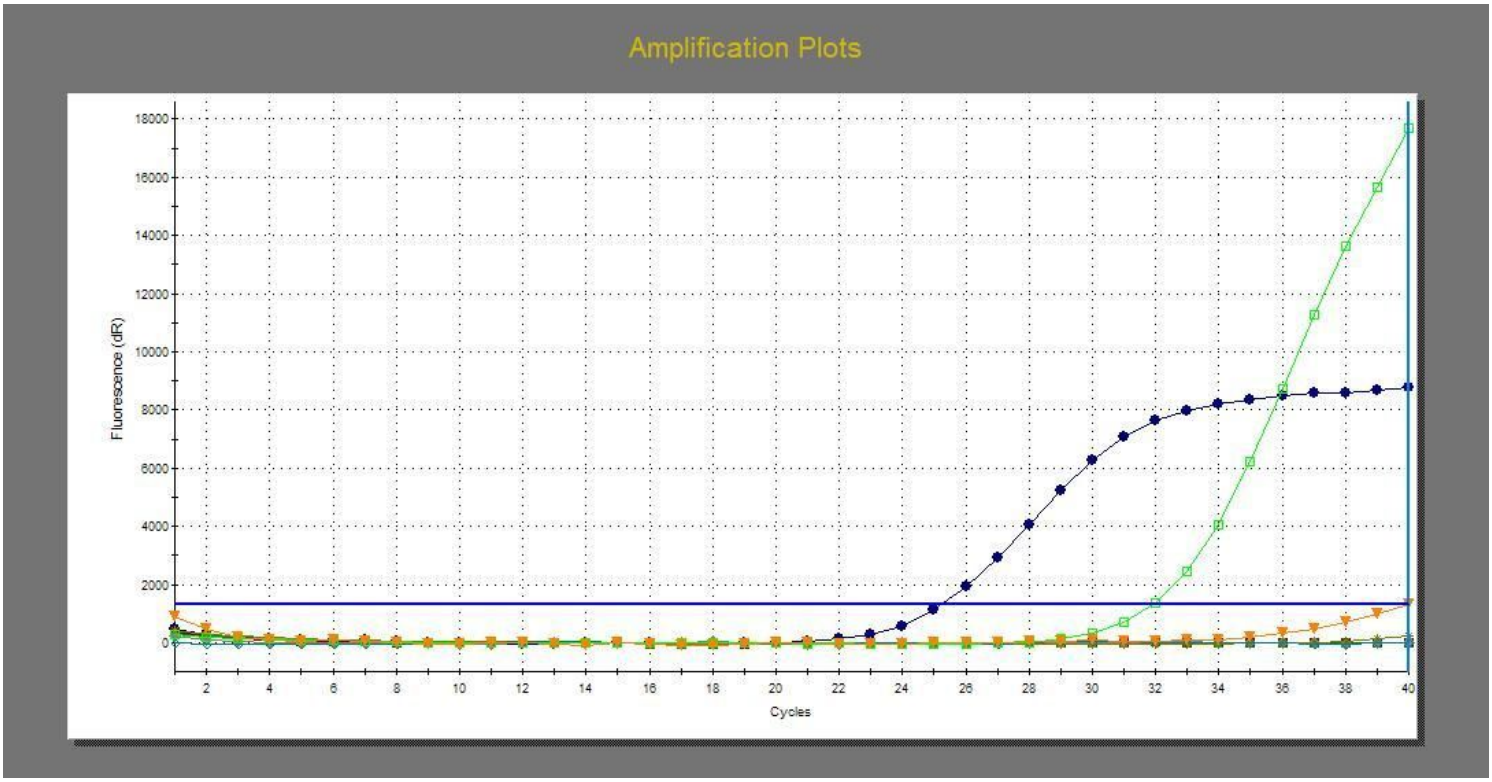

Figure 4

ARMS-PCR detection of mutated KRAS in formalin-fixed paraffin-embedded colorectal cancer tissues. The green up curve was the amplification curve of KRAS gene point mutation.

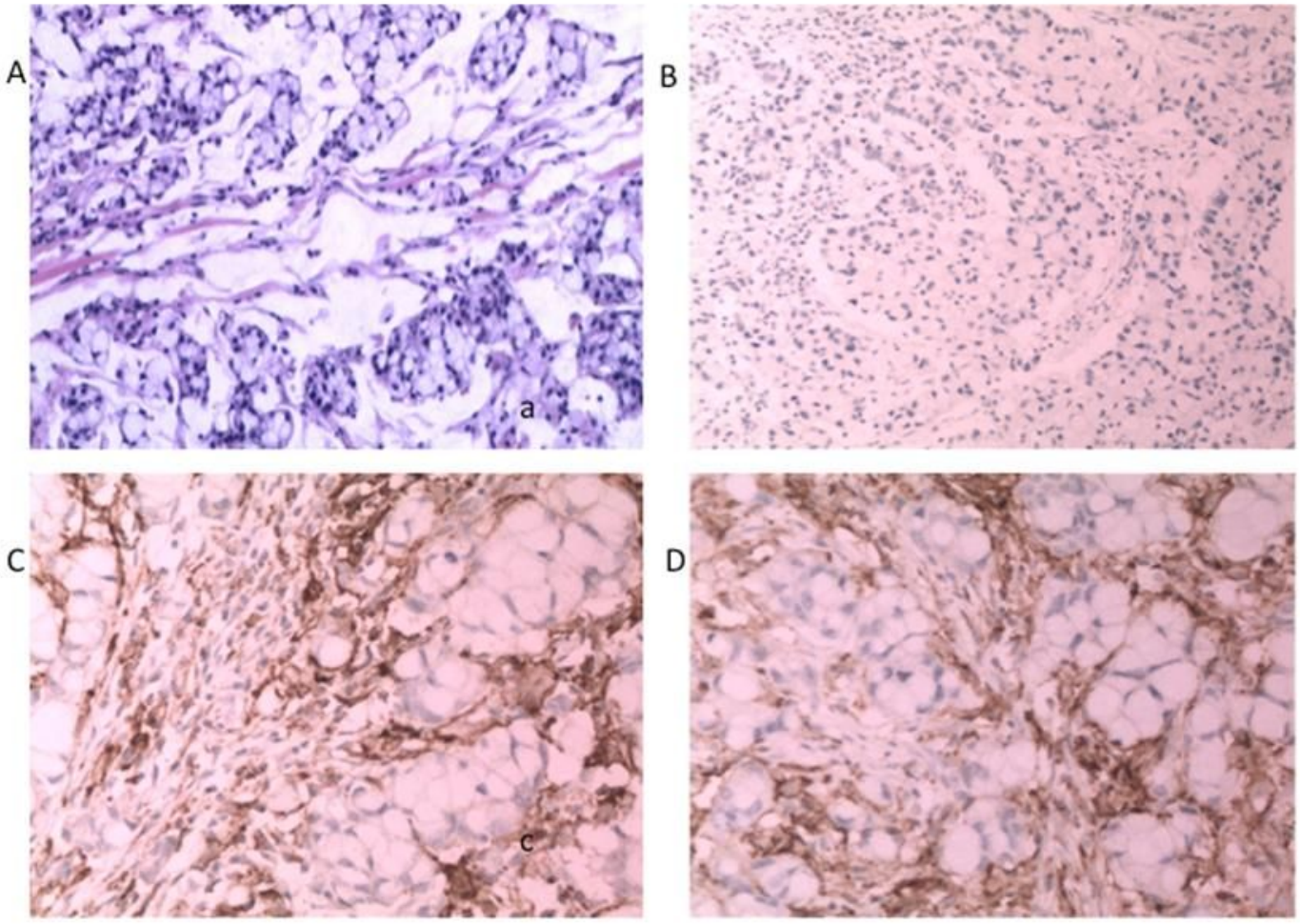

Figure 5

The staining of PD-L1 of immune cells in a d-MMR patient (MSH6-) A Colorectal cancer, the histological type is signet ring cell carcinoma, HE staining, 100x magnification; B the expression of MSH6 protein is negative, 100x magnification C-D The positive staining of PD-L1 in immune cells was more than 90%, moderately positive, 200x magnification