

CpG Island Methylator Phenotype(CIMP) is An Independent Prognostic Marker with Tumor-Promoting Functions in Colorectal Cancer

Jiante Li

Wenzhou Medical University Second Affiliated Hospital

Kepeng Hu

Wenzhou Medical University

Chenchen Wu

Wenzhou Medical University Second Affiliated Hospital

Mao Cai

Wenzhou Medical University Second Affiliated Hospital

Chongjie Huang

Wenzhou Medical University Second Affiliated Hospital

Dansi Qi

Wenzhou Medical University Second Affiliated Hospital

Changjia Li

Weihai Municipal Hospital

Long Li

Qingdao Municipal Hospital Group

Wanle Hu (✉ wanlehu@126.com)

Wenzhou Medical University Second Affiliated Hospital

Yaojun Yu

Wenzhou Medical University Second Affiliated Hospital

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Abstract

Background

The CpG island methylator phenotype (CIMP) with extensive promoter methylation is a distinct epigenotype in colorectal cancer (CRC). Changes in microbiota and epigenetic dysregulation might be the key underlying mechanism.

Methods

Tissues with stages I–III cancer were collected after proctocolectomy. The 16S rRNA gene sequencing was carried to determine the differences in microbiota. Subsequently, BRAF mutation, the status of microsatellite instability (MSI, also known as mismatch repair deficiency) and CIMP were also tested. The Chi-square test was carried out to analyze the relationship between molecular changes (MSI and CIMP) and the development of CRC.

Results

Patients in the three groups differed in the tumor location ($P=0.034$) and the carcinoembryonic antigen (CEA) level ($P=0.036$). The positive CIMP and MSI-LOW/MSS were more common in the worse prognosis groups. The Kaplan–Meier and Cox proportional regression analyses indicated that CIMP and MSI were the independent indicators of poor survival since the positive rates of which were significantly higher in the non-survival group. Besides, there were differences in microbiota among tumor tissues of different prognoses, with the *Fusobacterium nucleatum* and *Bacteroides fragilis* more abundant in the worse prognosis groups.

Conclusion

The persistent epigenetic changes influence the prognosis of patients with CRC and the composition of the gut microbiota might be the cause.

Background

Colorectal cancer (CRC) is one of the most common malignancies worldwide, with over half a million deaths per year [1, 2]. It represents a heterogeneous process with a different set of somatic molecular alterations [3]. It has been well demonstrated that the accumulation of both genetic and epigenetic alterations can contribute to malignant transformation of normal colonic mucosa, leading to the development of CRC. Two molecular pathways have been reported: the microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP) [4–7]. MSI is caused by a defective mismatch repair system, resulting in an alteration in the number of repeated nucleotide(s), which causes frame-shift mutations of the MMR genes (MLH1, MSH2, MSH6, and PMS2) or the germ line deletion of the EPCAM gene [8, 9].

Genetic alterations and epigenetic changes, such as global and regional hypermethylation of CpG islands are the hallmarks of human cancer, which may result in the activation of oncogenes and the inactivation of tumor-suppressor genes [10–12]. A subgroup of CRC with frequent aberrant DNA methylations of the CpG island has been reported, referred to as the CIMP. Currently, 2 panels of CIMP marker genes were used: the classic panel (MINT1, MINT2, MINT31, CDKN2A, and hMLH1) defined by Toyota et al. [13] and the new panel (CACNA1G, IGF2, NEUROG1, RUNX3, and SOCS1) defined by Weisenberger et al. [14]. According to the number of methylated promoters, tumors were classified as CIMP positive or CIMP negative. It has been well demonstrated that CIMP induced CRCs have distinct clinical and histological features, such as female predominance, proximal location, frequent BRAF mutations, and MSI [15–18].

In the past few years, with the prevailing of the 16S rRNA gene sequencing approach, we could analyze the microbial community more effectively. So far, Microbiota has been a subject in intense scholarly debate. Accumulating evidence indicates that the *Fusobacterium* species (a group of non-spore-forming, anaerobic gram-negative bacteria) has a positive association with the status of CIMP and MSI [19].

Based on the above information, we aim to determine whether the CIMP status provides further insight into clinical behavior and survival in patients with CRC. Since the CIMP status associates with MSI and mutation of BRAF, we have quantified the expression levels in tumor tissues. Besides, we have also checked the structure of the microbiota community and the status of MSI and CIMP, and the mutation of the BRAF. It is expected that this study can reveal the pathological process of how the above molecular phenotypes could affect the prognosis of CRC patients.

Method

Study population and design

A total of 180 individuals scheduled for colorectal resection at Wenzhou Medical University, Wenzhou, China, were recruited to the study. All patients with stages I–III cancer underwent a standard curative surgery, while stage IV CRC tissues were collected from patients who received palliative surgery to relieve serious cancer-related contradiction. The patients were not treated with antibiotics in the month before surgery but were administered antibiotics intravenously in a few hours after resection. Patients who had received or regularly used non-steroidal anti-inflammatory drugs, statins, or probiotics were excluded. Besides, those who had chronic bowel disease, infections, food allergies, and dietary restrictions were also excluded. Surgeries were performed at the Affiliated Hospital of Wenzhou Medical University between 2014 and 2017, and the treatments were given according to the National Comprehensive Cancer Network Guidelines.

Sample preparation

Tumor samples were obtained from the 180 patients who had undergone proctocolectomy. These CRC tissue samples and adjacent normal tissue samples (at least 5cm from the tumor site) of these 180 patients were obtained from the gastrointestinal cancer specimen bank of the Affiliated Hospital of Wenzhou University. To be specific, surgically resected specimens were collected immediately after tumor removal and stored at -80°C. The TNM stages were determined according to the American Joint Committee on Cancer system and all specimens were graded histologically according to the World Health Organization classification criteria. Written informed consents of joining the specimen bank were obtained from all the patients before surgery, and the protocols used in the study were approved by the Ethics Committee of Affiliated Hospital of Wenzhou Medical University. Clinical and pathologic data were reviewed from the gastrointestinal cancer database of Affiliated Hospital of Wenzhou Medical University.

DNA extraction

CTAB method was used to extract DNA from all tumor samples with minimal modification. The concentration of DNA was measured by fluorometer or microplate reader and sample integrity was tested by agarose gel electrophoresis (1% concentration of agarose Gel; 150 V; 40 min electrophoresis time). All DNA samples were stored at -20°C.

PCR and sequencing analysis

The V4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers 319F and 806R. The reaction mix consisted of Phusion High-Fidelity PCR Master Mix (NEB, Ipswich, MA, USA) and appropriate primer/probe pairs. The PCR program was as follows: Denaturation at 98°C followed by 30 cycles of 45 s at 95°C (denaturation) in 3 min, then annealing at 55°C and 45 s at 72°C (extension) in 45 s, with a final extension at 72°C for 7 min. The PCR products were purified with AMPure XP beads (Agencourt Bioscience) to remove the unspecific products before library construction. The library was quantitated in two ways. The average molecule length was measured using the Agilent 2100 bioanalyzer instrument (Agilent DNA 1000 Reagents) and then quantified by real-time quantitative PCR (qPCR; EvaGreen TM). The sequencing of qualified libraries was performed at the BGI-Huada Genomics institute in Shenzhen using MiSeq System, with the sequencing strategy PE250 (PE251+8+8+251) or PE300 (PE301+8+8+301) (MiSeq Reagent Kit).

Bioinformatics analysis

The sequences were clustered into operational taxonomic units (OTUs) with a 97% threshold using USEARCH (v7.0.1090) [20], thus the OTU unique representative sequences were obtained. Chimeras were filtered out using UCHIME (v4.2.40) [21]. Representative OTUs were aligned to the optimized sequences and the abundance of OTUs per sample was obtained for further analysis. Ribosomal Database Project (RDP) Classifier v.2.2 was used to taxonomically classify OTU representative sequences in the following databases: Greengene V201305 [21] and RDP (Release9 201203) [22].

Promoter Methylation Analysis

The Definition of CpG is the land promoter hypermethylation of at least 2 out of 5 methylation markers (CACNA1G, CDKN2A, NEUROG1, CRABP1 and MLH1), as proposed by Weisenberger et al [23], to detect CIMP in tumor tissue of CRC. Methylation of these markers was determined by bisulfite modification of 500 ng genomic DNA using a commercially available kit (Zymo Research), to create a methylation index with the CIMP markers and subsequent methylation-specific PCR (MSP) [24, 25]. Since MSP is effective, specific, and simple, it was used to detect CIMP. Also, the research shows that there are no differences between MSP and other technologies such as MethyLight [26].

MSI Analysis

As a second-generation genetic marker, MSI has been widely applied in tumor gene diagnosis and genetic analysis because of its high polymorphism, stability, and Mendelian co-dominant inheritance. It is determined through the multiplex fluorescent PCR combined with capillary electrophoresis (CE) using the MSI markers NR-21, BAT-26, NR-27, BAT-25, NR-24, and MONO-27, as reported by Suraweera et al [27]. This method has high efficiency, stability, high sensitivity, thus it provides reliable analysis results. After amplified by Multiplex fluorescent PCR, the CE method using dual internal standards (molecular weight markers shorter and longer than the PCR fragment of interest) was implemented to measure microsatellite length. To validate the CE method in detecting MSI, a human tumor sample and a control DNA sample collected from the patients were also tested.

Statistical analysis

Statistical analyses were conducted using the SPSS 20 statistical software. The two-sided χ^2 test was used to determine the associations between CIMP status and different clinicopathological features. The significance was determined using the log-rank test. Cox proportional hazard models were used to carry out multivariate survival analyses. Metastases (<http://metastats.cbcb.umd.edu/>) and R (v3.0.3) were used to determine which taxonomic groups were significantly different. The obtained P values were adjusted by a Benjamini-Hochberg false discovery rate (FDR) correction (function 'P.adjust' in the stats package of R (v3.0.3)). $P \leq 0.05$ was considered significant [28].

Result

Associations between Molecular phenotype and clinicopathologic characteristics

The clinical characteristics of the patients with different molecular phenotypes were compared, as shown in Table 1. We could see that the patients belonging to different groups might be correlated with the patients' outcomes in CRC. Based on the stages of the tumor samples, we found that tumor location ($P = 0.022$), differentiation ($P = 0.047$), and tumor size ($P = 0.031$) were significantly associated with CIMP status. Besides, correlations between age ($P = 0.026$), gender ($P = 0.045$), remote Metastasis ($P = 0.005$) and MSI status were also significant. In addition, gender ($P = 0.046$) and differentiation ($P = 0.043$) were found associated with BRAF mutation (Table 1).

Table 1
Correlation between clinicopathological features and CIMP combined with MSI status

Variable		N (180)	CIMP		P value	MSI	P value		BRAF		P value
			(-) (145)	(+) (35)			MSS/MSI- L(138)	MSI-H(42)	(-) (129)	(+) (51)	
Age	< 60	72	60	12	0.442	49	23	0.026*	55	17	0.251
	>=60	108	85	23		89	19		74	34	
Gender	Male	109	87	22	0.756	78	31	0.045*	84	25	0.046*
	Famale	71	58	13		60	11		45	26	
Location	L Colon	37	24	13	0.022*	24	13	0.218	22	15	0.077
	R Colon	32	26	6		25	7		21	11	
	Rectal	111	95	16		89	22		86	25	
Lymphovascular invasion	No	122	101	21	0.148	98	24	0.092	92	30	0.106
	Yes	58	44	14		40	18		37	21	
Differentiation	Poor	48	34	14	0.047*	32	16	0.056	29	19	0.043*
	Well	132	111	21		106	26		100	32	
Tumor size	≥ 5 cm	69	50	19	0.031*	52	17	0.744	55	14	0.059
	≠5 cm	111	95	16		86	25		74	37	
TNM stage	I and II	44	35	9	0.846	31	13	0.165	27	17	0.081
	III and IV	136	110	26		107	29		102	34	
Remote Metastasis	Nagetive	171	140	31	0.073	135	34	0.005*	121	50	0.239
	Positive	9	5	4		3	6		8	1	

*: statistically significant $P < 0.05$

Cpg Island Methylator Phenotype (cimp) And Microsatellite Instability (msi)

After detecting the BRAF mutation and the status of MSI and CIMP, we compared these molecular features in the four groups. The results show that We could observe the relationship between different patients and CIMP status. Also, the difference could observe in MSI status. And those patients who have the statistically significant had the more positive rate in the non-survival group(Table 2)

Table 2

Variable		N (180)	survival group (N = 89)	non-survival group (N = 28)	recurrence group (N = 34)	unclear group (N = 29)	P value
B-raf	Negative	128	66	20	24	18	0.668
	Positive	52	23	8	10	11	
CIMP	Negative	132	69	18	19	26	0.011*
	Positive	48	20	10	15	3	
MSI	MSS/MSI-low	139	70	17	25	27	0.031
	MSI-high	41	19	11	9	2	
Bonferroni-correction: $\alpha = 0.017$							
*: statistically significant $P < 0.017$							

Prognostic value of CIMP,MSI, and BRAF

The univariate Cox regression analyses revealed that positive CIMP (HR 1.914; $P = 0.042$), MSI (HR 2.549; $P = 0.002$), TNM stage (HR 3.655; $P = 0.006$), and Lymph node metastasis (HR 1.940; $P = 0.025$) were associated with worse OS in CRC patients after radical surgery (FIGURE 1). However, only positive CIMP (HR 2.166; 95% CI 1.119,4.191; $P = 0.022$) and TNM stage (HR 3.007; 95% CI 1.127,8.025; $P = 0.028$) were independent predictors of the 3-year OS (Table 3). Furthermore, positive CIMP, MSI status, and differentiation were associated with poor 3-year DFS both in the univariate Cox regression analyses and the multivariate analysis (Table 4).

Table 3
Univariate and multivariable Cox regression analyses for overall survival

Factor	Univariate analysis			Multivariate analysis		
	HR	95%CI	p	HR	95%CI	p
Age(< 60/>=60)	0.554	(0.304,1.009)	0.053			
Gender(male/female)	1.073	(0.596,1.929)	0.814			
Location(colon)	0.818	(0.343,1.951)	0.651			
Location(rectum)	1.063	(0.524,2.158)	0.865			
Tumor size (< 5 cm/>=5 cm)	0.713	(0.393,1.293)	0.265			
lymph node metastasis (Yes/No)	1.94	(1.086,3.467)	0.025*	1.587	(0.868,2.900)	0.134
Grade of differentiation (well/poor)	0.749	(0.415,1.351)	0.336			
TNM stage (I-II/III-IV)	3.655	(1.441,9.273)	0.006*	3.007	(1.127,8.025)	0.028*
CIMP(+)	1.914	(1.024,3.577)	0.042*	2.166	(1.119,4.191)	0.022*
BRAF(+)	0.907	(0.471,1.745)	0.77			
MSI(+)	2.549	(1.425,4.559)	0.002*	1.845	(0.996,3.420)	0.052
HR relative risk, 95% CI 95% confidence interval.						

Table 4
Univariate and multivariable Cox regression analyses for disease free survival

Factor	Univariate analysis			Multivariate analysis		
	HR	95%CI	p	HR	95%CI	p
Age(< 60/>=60)	0.527	(0.232,1.199)	0.127			
Gender(male/female)	0.87	(0.374,2.021)	0.746			
Location(colon)	1.316	(0.264,6.552)	0.737			
Location(rectum)	2.314	(0.676,7.917)	0.181			
Tumor size (< 5 cm/>=5 cm)	0.637	(0.262,1.553)	0.322			
lymph node metastasis (Yes/No)	0.242	(0.057,1.035)	0.056			
Grade of differentiation (well/poor)	4.596	(1.073,19.688)	0.040*	4.403	(1.021,18.994)	0.047*
TNM stage (I-II/III-IV)	0.452	(0.198,1.031)	0.059			
CIMP(+)	3.263	(1.373,7.752)	0.007*	3.130	(1.305,7.503)	0.011*
BRAF(+)	0.843	(0.331,2.144)	0.719			
MSI(+)	3.637	(1.495,8.846)	0.004*	2.985	(1.198,7.434)	0.019*

Diversity and structural changes of the tumor microbiota in CRC patients with different prognosis outcomes

The overall microbiota at the phylum level is shown in Fig. 2A. The dominant phyla in all groups were *Proteobacteria* (33.8%-49.4%), *Firmicutes* (16.9%-22.7%), *Bacteroidetes* (21.1%-27.9%), and *Fusobacterium* (3.38%-10.8%). When comparing the relative abundance, we found that the abundance of *Proteobacteria* was higher in the survival group than in non-survival group (48.2% vs. 33.8%, FDR = 0.063), while *Fusobacterium* was lower in survival group (3.38% vs. 9.71%, FDR = 0.089), although the differences were not statistically significant.

The microbial composition was different at the genus level among groups. *Shewanella* (9.05% vs. 5.76%, FDR = 0.091), *Methylobacterium* (2.54% vs. 1.56%, FDR = 0.039), *Faecalibacterium* (2.99% vs. 0.93%, FDR = 0.016), and *Sphingomonas* (1.38% vs. 0.79%, FDR = 0.031) together account over 1% of the total bacteria in the survival group, exhibiting a relatively higher abundance than that in non-survival group. While *Fusobacterium* (9.23% vs. 2.70%, FDR = 0.079) was relatively more abundant in the non-survival group and the difference was borderline significant. *Methylobacterium* (2.54% vs. 1.51%, FDR = 0.09) and *Mycoplasma* (0.64% vs. 0%, FDR = 0.01) showed higher abundance in survival group (Fig. 2B).

In specie level, we found a higher level of *B. fragilis* (9.75% vs. 2.62%, FDR = 0.017) in non-survival group than in survival group, while *F. prausnitzii* (2.96% vs. 0.92%, FDR = 0.028) and *Methylobacterium suomiense* (1.91% vs. 0.78%, FDR = 0.098) were more abundant in the survival group. Moreover, borderline statistic difference was found in *F. nucleatum* between non-survival group and survival group (5.66% vs. 1.08%, FDR = 0.076) and *F. nucleatum* (5.10% vs. 1.08%, FDR = 0.08) exhibited a greater abundance in the recurrence group than in the survival group (Fig. 2C).

Discussion

CIMP status is an important indicator to show the development of CRC. Recent studies have shown that through changing epigenetic, namely changes in gene expression patterns, CIMP can cause the occurrence of CRC mediated by DNA methylation transferase and S-adenosine methionine [29–31]. Acid provides a methyl group, and a methyl group is added to the fifth carbon atom of cytosine, making a chemical modification reaction of 5-methylcytosine. This process mainly occurs on CpG dinucleotides, thus epigenetic silencing is caused by DNA methylation. CIMP, as an epigenetic phenotype of CRC, has different clinical, pathological, and biological characteristics, such as being related to proximal colon, female, poor differentiation, MSI, BRAF high-frequency mutations, tp53 low-frequency mutations [32–34], and CIMP-H, MSI-H and TGFBR2 single nucleotide mutation. During the development of CRC, CIMP that progresses to dentate adenomas shows frequent promoter methylation, while flat adenomas not [35].

To our best knowledge, this is the first study to compare the CIMP status, MSI, and BRAF among groups of cancer tissues divided by different post-operation prognoses. CIMP has been extensively studied in CRC because it reflects epigenetic aberration in tumor cells [36, 37]. CIMP, as an epigenetic phenotype of CRC, has different clinical, pathological, and biological characteristics, such as related to proximal colon, female, poor differentiation, this was also demonstrated in our study. Divergent findings of the prognostic values of CIMP have been reported. Some studies suggest an adverse effect of CIMP on the survival of CRC patients [38–42], whereas other studies reported no relationship between CIMP status and prognosis in CRC [43–46]. These contradictory results can be explained at least in part by the absence of a consensus CIMP panel since some studies used the classical panel defined by Toyota et al [37], whereas others used the new CIMP panel [13]. Overall, MSI often manifests as a Pathological type with poor prognoses such as poor differentiation or mucinous adenocarcinoma [47], and remote metastasis was also found in our study. Besides, we also examined the BRAF mutation on patients, CIMP-high status was independently associated with a bad differential, whereas BRAF mutation was associated with a significantly increasing numbers. It might explain why these two molecular phenotypes were associated with bad outcomes in many studies [48, 49].

The specific mechanisms by which gut microbiota affects the development of CRC are still not well understood. One of the most promising theories is the microbe-driven intestinal mechanism. Interestingly, *F. nucleatum*, *B. fragilis*, and *F. prausnitzii* are all key players in modifying intestinal inflammation levels. To our knowledge, CIMP is believed to promote carcinogenesis through methylation mediated transcriptional silencing in tumor suppressor genes[32]. However, the role of microbiota in the progress of CIMP and the mechanism underlying its sustained influence remains unknown. In fact, the relationships between CIMP, MSI, BRAF mutations, and the gut microbiota in colorectal cancer are complex. To decipher the complex association of CIMP, MSI, and BRAF mutation on patients' microbiota, we collect the patients' CRC tissues in four groups. The results showed the relationship in CIMP status between different groups, which was consistent with previous studies. Besides, the higher incidence was observed in the current group, with a greater abundance of *F. nucleatum*. Some studies have shown that *F. nucleatum* is associated with epigenetic changes such as the status of MSI and CIMP[50], which also corresponds with the results in this study. In addition, another study has also shown that patients with a high level of *F. nucleatum* have a significantly shorter survival time, which was similarly obtained in this study[51]. But their sample size was relatively small. A more recent study has reported a similar result using a larger database of CRC cases in the USA, revealing a correlation between a high amount of tissue *F. nucleatum* DNA and higher CRC-specific mortality. Evidence has also shown that overexpression of *BRAF* were markers of the poor prognosis[52, 53], Moreover, MSI, the primary causes of which is hypermethylation of the *MLH1* promoter, was also associated with the clinical outcomes of CRC[4, 6]. A relevant study has shown that microbiota has a relation with MMR, which is the most important mechanism for the appearance of MSI[54]. Furthermore, overexpression of *BRAF* was also considered the marker of poor prognosis which was consistent with our findings of prognostic values of *F. nucleatum*, *B. fragilis*, and *F. prausnitzii*^[55]. This might explain why *BRAF* mutation could accompany MSI as reported in the previous studies.

Herein, we classified tumors according to the CIMP status combined with *BRAF* methylation or MSI status to investigate whether these molecular characteristics affect clinical behavior and prognosis. Our data showed that clinicopathologic features and OS differed among the 4 groups of patients according to their CIMP status combined with *BRAF* methylation or MSI status. In fact, CIMP and *BRAF* tests are used to exclude HNPCC among patients who exhibit MSI-high, since HNPCC seldom exhibits CIMP or *BRAF* mutation. Therefore, studies on epigenetic and/or genetic alterations are increasingly important in cancer research. As we all know, CIMP-H is associated with poor OS, but some reports suggested that MSI-H could decrease this phenomenon, which explains why we could see better outcomes when the CIMP-H was accompanied with the MSI-H. The limited sample size might also be an important reason why we couldn't see the statistical difference when we were analyzing the data. Studies have shown that 70–80% of MSI induced CRC can be attributed to CIMP related *MLH1* methylation. The mechanism is that CIMP hypermethylation leads to methylation of the *MLH1* promoter, which results in the silencing of the *MLH1* mismatch repair gene and the final performance MSI status. Some previous studies on the correlation between microsatellite status, CIMP status, and OS have shown that CIMP positive is negatively correlated with OS. In MSI patients, CIMP and OS also show a certain correlation. Ward et al. found that CIMP positive was associated with the total OS but not survival time, and Rhee et al. also proved this. In this study, a similar conclusion was confirmed.

Finally, it is important to mention that our study had some limitations. The major one is the small size after dividing the cohort into 4 subgroups. Although we have studied the related risk factors and survival rate, we have not explored the correlation between these phenomena and cancer medication. Besides, some mechanisms between them and the intestinal micro-ecology are just a taste.

Conclusions

Our results underline the interest that CIMP can be useful to predict prognosis in CRC patients, and at present, it is believed that MSI is closely related to the occurrence and development of CRC. So far, MSI has been a tumor-related factor recognized worldwide. Microbiota, such as *B. fragilis* and *F. prausnitzii*, participate in influencing the course/progression of CRC in patients subjected to energy restriction in their early childhood, which further validates the association of *F. nucleatum* with epigenetic changes and gene mutations. The future goal is to use precision medicine to analyze the patient's genes, select the most suitable individualized treatment plan for the patient, and make the most accurate judgment on the patient's prognosis.

Abbreviations

1. CIMP: CpG Island Methylator Phenotype
2. CRC: colorectal cancer
3. CEA:carcinoembryonic antigen
4. PCR:polymerase chain reaction
5. FDR:false discovery rate

Declarations

The authors do not have any conflicts of interest with the content of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethical committee approved the procedure.

CONSENT FOR PUBLICATION

Written informed consent for publication was obtained from all participants.

AVAILABILITY OF DATA AND MATERIALS

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

COMPETING INTERESTS

None.

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AUTHORS' CONTRIBUTIONS

JTL YJY and WLH plan the study made all coordination and was involved in the laboratory processing. KPH, CCW, MC and CJH participated in the study and performed the statistical analysis. DSQ, CJL and LL carried out handling the samples. All authors read and approved the final version of manuscript.

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Figures

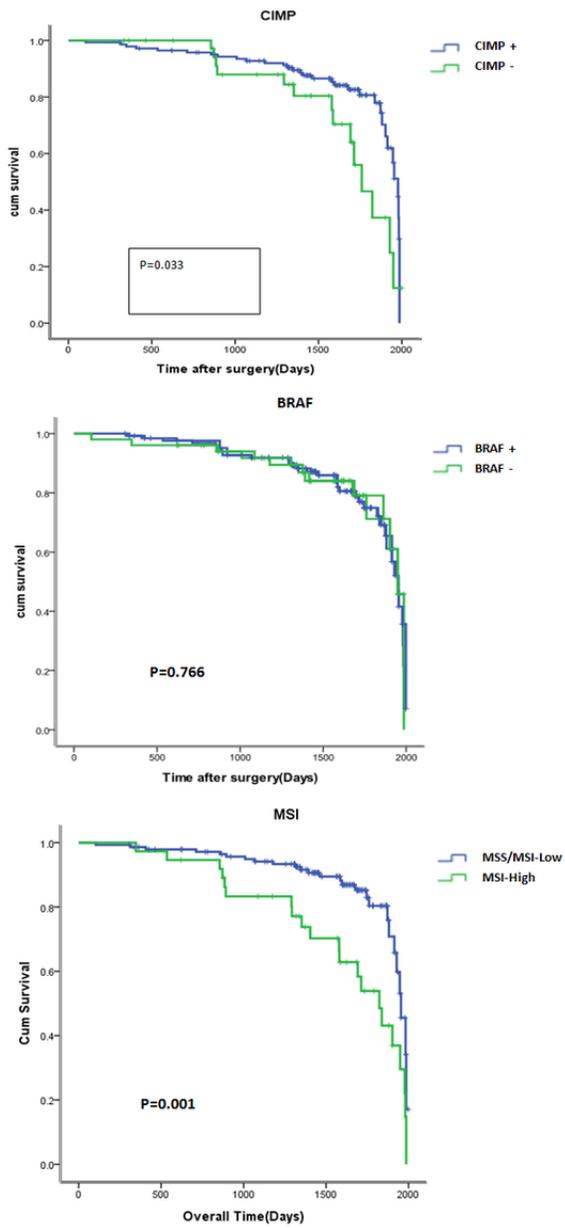


Figure 1

Kaplan–Meier survival curves for overall survival (OS) and disease free survival (DFS) in 180 CRC patients in relation to CIMP, BRAF and MSI. P values were obtained by log-rank test.

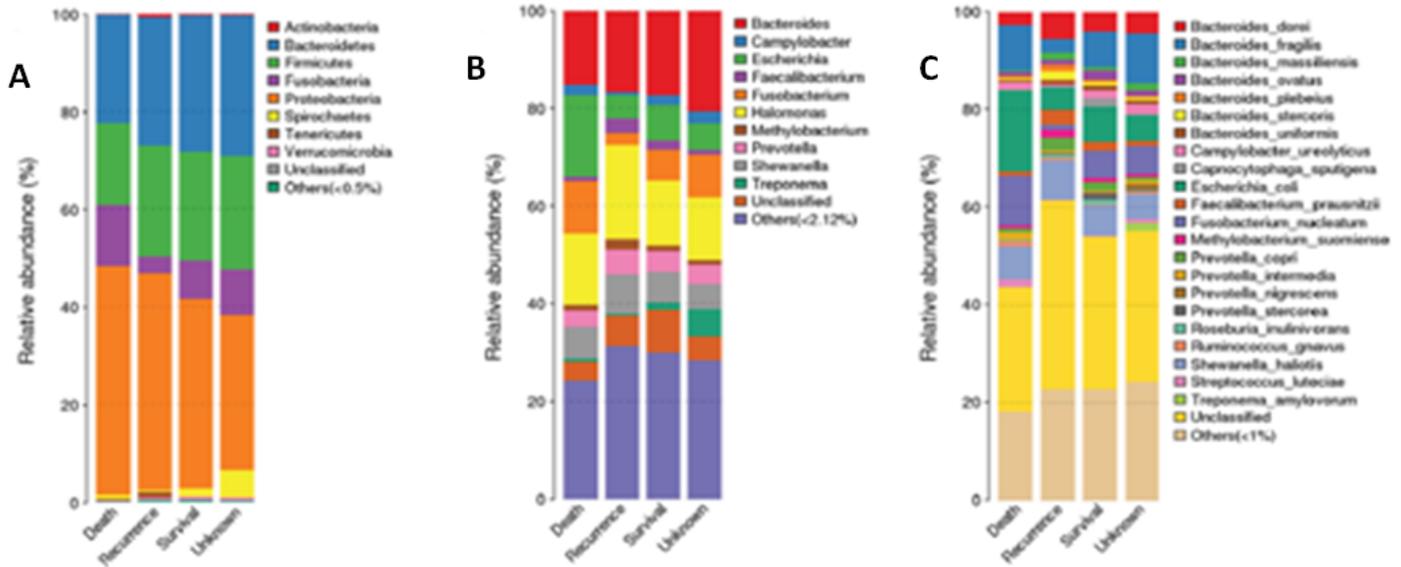


Figure 2

Diversity and structural changes of the tumor microbiota among the Non-survival group (n = 28), Recurrent group (n = 34), Survival group (n = 89) and Unclear group (n = 29). A. The dominant phyla of different groups. B. The dominant genera of different groups. C. The dominant species of different groups.