의학석사 학위논문

Prognostic value of microsatellite instability status in stage II/III rectal cancer patients who received upfront surgery

근치적 수술을 시행받은 2, 3 기 직장암 환자에서

현미부수체 불안정성의 예후인자로서의 역할

울산대학교대학원

의 학 과

오 충 렬

Prognostic value of microsatellite instability status in stage II/III rectal cancer patients who received upfront surgery

지도교수 김 태 원

이 논문을 의학석사 학위 논문으로 제출함

2017년 12월

울산대학교 대학원
의 학 과
오 충 렬

오충렬의 의학석사학위 논문을 인준함

심사위원　　홍 용 상　(인)

심사위원　　김 태 원　(인)

심사위원　　이 재 련　(인)

울 산 대 학 교 대 학 원

2017 년

Abstract

Background: Microsatellite instability (MSI) is one of the most important prognostic factors in patients with a resected colon cancer but its impact in rectal cancer cases has not been fully evaluated. We investigated whether the MSI status affects the survival outcomes in stage II and III rectal cancer patients who have undergone an upfront curative resection.

Methods: A total of 1,103 patients who were treated between February 2008 and August 2015 at Asan Medical Center were included in this study. The major eligibility criteria included primary adenocarcinoma of the rectum, upfront surgery of curative intent, pathologic stage II/III disease, and available polymerase chain reaction (PCR)-based MSI results. The study endpoints were disease-free survival (DFS) and overall survival (OS).

Results: Twenty-four patients (2.2%) in the total cohort were found to be MSI-high (MSI-H). MSI-H patients were significantly associated with histologically poorly differentiated tumors (P=0.001) and with a family history of colorectal cancers (P=0.008). The 5-year DFS and OS for the whole cohort was 70.1% (95% confidence interval [CI] 67.4 – 72.8) and 83.1% (95% CI 80.9 – 85.3), respectively. In univariate analysis, a high pathologic stage, poorly differentiated tumor, mid to distal located tumor (anal verge < 8 cm), positive resection margin, presence of lymphovascular or perineural invasion and high preoperative level of carcinoembryonic antigen (> 6.0 ng/mL) were significantly associated with a shorter DFS and OS. However, neither DFS nor OS were statistically significantly different according to the MSI status. The 5-year DFS rate was 78.0% in MSI-H patients and 69.9% in MSI-low (MSI-L) or microsatellite stable (MSS) patients (Hazard ratio [HR] 0.84, 95% CI 0.35 – 2.02; P=0.689). The 5-year OS rates for MSI-H and MSI-L/MSS patients were 84.0% and 83.1%, respectively (HR 0.86, 95% CI 0.27 – 2.69; P=0.790). By multivariate analysis, the MSI status did not affect either the DFS (HR 1.00, 95% CI 0.40 – 2.47; P=0.994) or OS (HR 0.85, 95% CI 0.26 – 2.73; P=0.778).

Conclusion: MSI-H tumors are rarely observed in rectal adenocarcinoma and the MSI status may not affect the survival outcome in patients with a resected rectal cancer.

Keywords: rectal cancer, microsatellite instability

차 례

표 및 그림 목차

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide with an estimated 1.7 million incident cases. Globally, CRC caused 832,000 deaths and was the second leading cause of cancer mortality in 2015 [1]. Rectal cancer accounts for about 28% of all newly diagnosed CRC cases and the majority of cases present with loco-regional disease [2]. The treatment of choice for locally advanced rectal cancer is a combination of surgical resection and chemotherapy and/or radiotherapy [3]. These patients can potentially be cured but the clinical outcome depends on the tumor biology.

Microsatellites are stretches of DNA in which a short sequence of nucleotides is repeated several times. Microsatellite instability (MSI) refers to the gain or loss of the repeat units from a microsatellite and a resulting change in its length [4]. This type of genetic destabilization occurs in approximately 15% of CRCs and is typically associated with defective function of the DNA mismatch repair (MMR) system [5]. The MSI-high (MSI-H) prevalence in rectal cancers has been known to be less than 10% with a gradual decrease in its distribution from the proximal colon to the rectum [6].

There has been considerable evidence to date on the prognostic role of the MMR or MSI status in patients with CRC. Overall, MSI-H or deficient MMR (dMMR) tumors have shown a significantly better prognosis compared with microsatellite stable (MSS) or proficient MMR (pMMR) tumors [7-11]. However, several other reports have suggested that the prognostic impact of the MMR or MSI status might vary according to the location of the CRC [12,13].

Although some previous studies have already reported an association between survival outcome and MSI status in rectal cancer patients, the prognostic impact of MSI in patients with locally advanced rectal cancer has remained unclear because of contradictory results and the heterogeneous nature of the previous study populations [14-16]. In this regard, the purpose of our current study was to evaluate the prognostic significance of MSI in patients with stage II, III rectal cancer who had undergone an upfront curative surgical resection.

Materials and Methods

Patients and Data Collection

A cohort of 1,264 patients with primary rectal cancer who underwent upfront surgical therapy in Asan Medical Center between February 2008 and August 2015 were included in the current study. Clinical data was collected from the electronic medical record review. The main eligibility criteria included a pathologically confirmed rectal cancer, upfront surgery of curative intent, pathologic stage II and III disease, and available MSI results. We excluded patients with a pathology other than adenocarcinoma (i.e., not a primary adenocarcinoma of the rectum), pathologic stage I cancer and carcinoma in situ as classified by the American Joint Committee on Cancer (AJCC) 7th edition, and those who were lost to follow-up. Patients who received palliative or R2 resection (macroscopically visible residual tumor) were also excluded. A final total of 1,103 rectal cancer patients were thus included in the final analysis.

Detection of MSI

Genomic DNA was extracted from tissue samples obtained from the surgical resections in all of the rectal cancer patients and MSI analysis was performed using fluorescence-based PCR. We analyzed the five markers of the Bethesda panel (*BAT25, BAT26, D17S250, D2S123,* and *D5S346*) to define the MSI status and the tumor was defined as MSI-H if two or more of these five markers were instable. If only one of the microsatellite sequences was found to have been mutated, the tumor was classified as MSI-low (MSI-L). Microsatellite stable (MSS) tumors was characterized by the absence of MSI in all 5 markers [17].

Statistical Analysis

The χ-square and Fisher's extract test were used for categorical variable analysis. We evaluated any differences between the continuous variables for the two groups using the Mann-Whitney *U*-test. Overall survival (OS) was calculated as the time between the date of surgery and death from any cause or last follow-up for living patients. Disease-free survival (DFS) was defined as the time from date of surgery to date of cancer recurrence, death from

any cause, or last follow-up for patients with no evidence of recurrence. Survival curves were displayed by the method of Kaplan-Meier and compared by the log-rank test. Multivariate analyses were performed with the Cox proportional hazards model. *P*-values of < 0.05 was considered statistically significant and all reported *P*-values were two-sided. All analyses were performed using SPSS version 21.0 for Microsoft Windows (IBM SPSS Inc., Chicago, IL).

Results

Patient Characteristics According to MSI Status

A total of 1,103 rectal cancer patients were finally included in the current analysis (Table 1). Of this population, MSI-H was found in 24 (2.2%), MSI-L in 32 (2.9%) and MSS in 1,047 (94.9%) patients. Among the total cohort, 708 (64.2%) patients were male and the median age was 61 years (interquartile range [IQR] 54 – 70). The median distance of the tumors from the anal verge (AV) was 9 cm (IQR 7.0 – 11.5) and approximately 90% of patients received low anterior resection. Stage II cancers were found in 446 of the study patients (40.4%) and stage III disease was present in 657 cases in this series (59.6%). Most of the patients in our cohort (90.8%) underwent postoperative treatments. Two hundred and ninety-nine patients (27.1%) received postoperative radiotherapy and 1,001 (90.8%) had postoperative chemotherapy of which 703 (70.2%) underwent chemotherapy without radiation therapy. In the stage II disease cases, 380 of 446 patients (85.2%) received adjuvant chemo- and/or radiotherapy as did 622 of 657 patients (94.7%) with stage III disease.

MSI-H was evident in 3.1% (14/446) of the stage II and 1.5% (10/657) of the stage III cases. Although the frequency of MSI-H tumors was higher in our stage II patients, no statistical significance was observed for the association between MSI status and pathologic tumor stage (P=0.071). There was also no difference found between patients with MSI-H and MSI-L/MSS tumor phenotypes with respect to the location of the tumors (P=0.351). Clinicopathological factors of the rectal cancer cases in our current series, such as presence of lymphovascular invasion, perineural invasion and the preoperative carcinoembryonic antigen (CEA) level, were also not significantly different by MSI status. One hundred and thirteen (10.2%) patients in the total cohort had a family history of CRC. Of the MSI-H tumor patients in this series, including 2 proven Lynch syndrome cases, 29.2% had a family history of CRC. However, only 9.8% of MSI-L/MSS patients had this family history. A significant association was evident between the family history of CRC and MSI status (P=0.008). The histologic tumor differentiation distribution was also found to be highly associated with the MSI status. Notably, poorly differentiated cancers were more frequently seen in MSI-H than in MSI-L/MSS rectal cancer patients (P=0.001).

Analysis of Disease-free Survival (DFS) and Overall Survival (OS)

The 5-year DFS and OS outcomes in the whole study cohort were 70.1% (95% CI 67.4 – 72.8) and 83.1% (95% CI 80.9 – 85.3), respectively. By univariate analysis, a high tumor stage, poorly differentiated tumor, mid to distal location of the rectal cancer (< 8 cm), positive resection margin, presence of lymphovascular or perineural invasion and high preoperative level of CEA (> 6.0 ng/mL) were significantly associated with a shorter DFS and OS (Table 2 and 3).

Neither the 5-year DFS nor OS in patients harboring MSI-H cancers were statistically different to those in patients with MSI-L and MSS cancers. The 5-year DFS rate was 78.0% in MSI-H and 69.9% in MSI-L/MSS (HR, 0.84; 95% CI, 0.35 – 2.02; *P*=0.689, Figure 1) cases. The 5-year OS rate was 84.0% in MSI-H and 83.1% in MSI-L/MSS (HR, 0.86; 95% CI, 0.27 – 2.69; *P*=0.790, Figure 2) cases.

Neither the DFS nor OS demonstrated significant differences between patients with MSI-H tumors and those with MSI-L or MSS tumors after adjusting for confounding variables by multivariate Cox regression analysis (*P*=0.994 and 0.778, respectively). Clinicopathological factors such as pathologic stage, location of tumor, involvement of resection margin, lymphovascular or perineural invasion and preoperative CEA level remained independent prognostic indicators associated with both DFS and OS (Table 4).

Discussion

Our current analysis indicates that the MSI status has no definite prognostic role in patients with surgically resected rectal cancer. The results of previous trials regarding the prognostic role of the MMR or MSI status in rectal cancer patients have been contradictory and non-conclusive. Colombino et al. suggested that patients with MSI-H rectal cancers had better survival outcomes, including both DFS and OS, compared to MSI-L/MSS cases [15]. In contrast, the study of 990 rectal cancer patients by Samowitz et al. indicated that an MSI-H status seemed to be an adverse prognostic factor [14]. Another report from a Korean group reported that the MSI status had no prognostic value in rectal cancer unlike colon cancer in which an MSI-H status was a strongly positive prognostic marker [16]. These earlier studies, however, shared a critical limitation. Since all included patients with various stages of rectal cancer, from early stage to metastatic disease, it was difficult to identify any prognostic role of the MSI status in locally advanced rectal cancer. To our knowledge, our current study is the largest sample-sized analysis to date to assess the impact of MSI status in surgically resected, stage II/III rectal cancer patients.

The conflicting results reported in multiple previous studies are likely to be the result of not only a selection bias but also ethnic effects. It has been previously suggested that there are distinct ethnic differences in the major molecular alterations associated with CRCs and that the frequencies of CIMP-high and $BRAF^{V600E}$ mutations in CRCs are markedly lower in Eastern Asians including Koreans compared with Western populations [18]. The molecular heterogeneity among ethnic groups might underlie the discrepancies reported for the prognostic effects of MSI. In addition, postoperative treatments may also influence the impact of MSI on the disease outcome. Most of the patients in our current study series with stage II or III rectal carcinomas (85.3% MSI-L/MSS and 83.3% MSI-H tumors) received postoperative fluoropyrimidine-containing chemotherapy or chemoradiotherapy. Since MSI-H tumors have been reported to respond poorly to 5-fluorouracil chemotherapy [19,20], although there has been some debate on this, it is possible that adjuvant treatment may dilute the prognostic significance of the MSI status. Additional research will be needed to better evaluate the real impact of MSI in rectal cancer.

Our findings also indicate that MSI-H rectal adenocarcinomas are rare and represented only 2.2% of the cases (24 out of 1,103 patients) in our present study cohort. This result is in accordance with most previous studies which have reported an MSI-H prevalence in rectal cancer ranging from 2% to 9.3% [6,16,21-23].

Another notable finding of our current analysis was that the MSI status was strongly associated with the level of tumor differentiation in rectal cancers. MSI-H CRCs have already been reported to frequently present with a poorly differentiated histology compared with MSS CRCs [24]. In addition, MSI- H poorly differentiated (PD) CRCs have been reported to have a lower incidence of regional lymph node metastases and better survival outcomes in contrast to MSS PD CRCs [25,26]. However, since the patient populations of previous investigations comprised mainly colon cancer cases, the association between tumor differentiation and MSI status in rectal cancer has remained inconclusive. In our current analysis, PD tumors were more frequently observed among the MSI-H than the MSI-L/MSS rectal cancers (16.7% vs. 3.2%, P=0.001) and MSI-H PD rectal cancer patients showed significantly less regional lymph node metastases than the MSI-L/MSS PD cases (25.0% vs. 88.2%, P=0.015). These findings demonstrate an association between PD and MSI-H rectal cancers that is similar to that in colon cancer.

One notable limitation of our present study is the absence of any evaluation of other potential predictive or prognostic molecular markers such as *KRAS* or *BRAF*. Secondly, the family history information for our cohort was incomplete. Because the family history data we collected was based on a review of the medical records, we could not obtain an accurate or entire family history for each patient, organize it in a pedigree and thus identify specific patients with Lynch syndrome. In this respect, although we have provided good evidence for a significant association between family history of CRC and the MSI status, this should be interpreted with some caution. In addition, since this was a single-center retrospective study, further prospective investigations will be needed to confirm whether the MSI status plays a role in the prognosis of rectal cancer patients.

Conclusions

MSI-H tumors are rarely observed in rectal cancer and the MSI status has no prognostic value in stage II/III rectal cancer patients who have undergone an upfront curative resection.

References

1. Global Burden of Disease Cancer: Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol* 2017;3:524-548.
2. Siegel RL, Miller KD, Fedewa SA, et al.: Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017;67:177-193.
3. Lutz MP, Zalcberg JR, Glynne-Jones R, et al.: Second St. Gallen European Organisation for Research and Treatment of Cancer Gastrointestinal Cancer Conference: consensus recommendations on controversial issues in the primary treatment of rectal cancer. *Eur J Cancer* 2016;63:11-24.
4. de la Chapelle A: Microsatellite instability. *New Engl J Med* 2003;349:209-210.
5. de la Chapelle A, Hampel H: Clinical relevance of microsatellite instability in colorectal cancer. *J Clin Oncol* 2010;28:3380-3387.
6. Meng W-J, Sun X-F, Tian C, et al.: Microsatellite instability did not predict individual survival in sporadic stage II and III rectal cancer patients. *Oncology* 2007;72:82-88.
7. Sinicrope FA, Foster NR, Thibodeau SN, et al.: DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 2011;103:863-875.
8. Hutchins G, Southward K, Handley K, et al.: Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011;29:1261-1270.
9. Klingbiel D, Saridaki Z, Roth A, et al.: Prognosis of stage II and III colon cancer treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: results of the PETACC-3 trial. *Ann Oncol* 2014;26:126-132.
10. Popat S, Hubner R, Houlston RS: Systematic Review of Microsatellite Instability and Colorectal Cancer Prognosis. *J Clin Oncol* 2005;23:609-618.

11. Lin CC, Lin JK, Lin TC, et al.: The prognostic role of microsatellite instability, codon-specific KRAS, and BRAF mutations in colon cancer. *J Surg Oncol* 2014;110:451-457.
12. Missiaglia E, Jacobs B, D'Ario G, et al.: Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. *Ann Oncol* 2014;25:1995-2001.
13. Sinicrope FA, Mahoney MR, Smyrk TC, et al.: Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy. *J Clin Oncol* 2013;31:3664-3672.
14. Samowitz WS, Curtin K, Wolff RK, et al.: Microsatellite instability and survival in rectal cancer. *Cancer Causes Control* 2009;20:1763-1768.
15. Colombino M, Cossu A, Manca A, et al.: Prevalence and prognostic role of microsatellite instability in patients with rectal carcinoma. *Ann Oncol* 2002;13:1447-1453.
16. Hong SP, Min BS, Kim TI, et al.: The differential impact of microsatellite instability as a marker of prognosis and tumour response between colon cancer and rectal cancer. *Eur J Cancer* 2012;48:1235-1243.
17. Boland CR, Thibodeau SN, Hamilton SR, et al.: A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;58:5248-57.
18. Kim JH, Kang GH: Molecular and prognostic heterogeneity of microsatellite-unstable colorectal cancer. *World J Gastroenterol* 2014;20:4230.
19. Sargent D, Marsoni S, Thibodeau S, et al.: Confirmation of deficient mismatch repair (dMMR) as a predictive marker for lack of benefit from 5-FU based chemotherapy in stage II and III colon cancer (CC): a pooled molecular reanalysis of randomized chemotherapy trials [abstract]. *J Clin Oncol* 2008;26:4008-4008.
20. Sargent DJ, Marsoni S, Monges G, et al.: Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* 2010;28:3219-3226.

21. Nilbert M, Planck M, Fernebro E, et al.: Microsatellite instability is rare in rectal carcinomas and signifies hereditary cancer. *Eur J Cancer* 1999;35:942-945.

22. Ishikubo T, Nishimura Y, Yamaguchi K, et al.: The clinical features of rectal cancers with high-frequency microsatellite instability (MSI-H) in Japanese males. *Cancer Lett* 2004;216:55-62.

23. Morán A, Iniesta P, de Juan C, et al.: Stromelysin-1 promoter mutations impair gelatinase B activation in high microsatellite instability sporadic colorectal tumors. *Cancer Res* 2002;62:3855-3860.

24. Gryfe R, Kim H, Hsieh ET, et al.: Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *New Engl J Med* 2000;342:69-77.

25. Kazama Y, Watanabe T, Kanazawa T, et al.: Microsatellite instability in poorly differentiated adenocarcinomas of the colon and rectum: relationship to clinicopathological features. *J Clin Pathol* 2007;60:701-704.

26. Xiao H, Yoon YS, Hong S-M, et al.: Poorly differentiated colorectal cancers: correlation of microsatellite instability with clinicopathologic features and survival. *Am J Clin Pathol* 2013;140:341-347.

국문요약

배경: 현미부수체 불안정성은 절제술을 받은 결장암 환자에게서 중요한 예후 인자 중 하나로 알려져 있지만, 직장암 환자에서의 역할은 아직 충분히 평가되지 않았다. 본 연구에서는 현미부수체 불안정성이 수술을 시행 받은 2, 3 기 직장암 환자의 생존율에 어떠한 영향을 미치는 지 조사하였다.

방법: 2008 년 2 월부터 2015 년 8 월까지의 기간 동안 수술을 시행 받은 총 1,103 명의 환자가 최종 분석에 포함되었다. 병리학적으로 확인된 원발성 직장 선암 환자이면서 완치 목적의 수술을 받은 2, 3 기 직장암 환자 중, 중합효소 연쇄반응 기반의 현미부수체 불안정성 검사 결과가 확인 가능한 환자를 연구 대상으로 하였다. 연구 목표는 현미부수체 불안정성에 따른 무병생존율과 전체생존율을 비교하는 것이었다.

결과: 고도 현미부수체 불안정성 종양은 총 1,103 명의 환자 중 24 명(2.2%)에서 확인되었다. 현미부수체 불안정성은 조직학적 분화도와 유의한 연관성이 있었으며 (*P*=0.001) 환자의 직결장암 가족력 유무와도 연관이 있었다 (*P*=0.008). 전체 환자의 5 년 무병 생존율은 70.1% (95% 신뢰구간 67.4 - 72.8)이었고, 5 년 전체 생존율은 83.1% (95% 신뢰구간 80.9 - 85.3)이었다. 단변량 생존분석에서, 높은 병기, 나쁜 분화도, 직장 하부 (항문연 8 cm 미만), 종양의 절제면 침범, 림프혈관강침윤, 신경주위침윤, 그리고 수술 전 높은 수치의 암배아항원 (> 6.0 ng/mL)과 같은 다양한 임상적 요소가 나쁜 무병 및 전체 생존율과 유의한 연관이 있었다. 그러나 현미부수체 불안정성은 생존율에 영향을 미치지 못하였다. 5 년 무진행 생존율은 고도 현미부수체 불안정성 종양에서 78.8%, 저도 현미부수체 불안정성 종양 및 현미부수체 안정성 종양에서 69.9% (위험비 0.84, 95% 신뢰구간 0.35 – 2.02; *P*=0.689)였고, 5 년 전체 생존율은 고도 현미부수체 불안정성 종양에서 84.0%, 저도 현미부수체 불안정성 및 현미부수체 안정성 종양에서 83.1% 였다 (위험비 0.86, 95% 신뢰구간 0.40 – 2.47; *P*=0.790). 다변량 분석에서도 현미부수체 불안정성은 무진행 생존율 (위험비 1.00, 95% 신뢰구간 0.40 – 2.47; *P*=0.994) 및 전체 생존율 (위험비 0.85, 95% 신뢰구간 0.26 – 2.73; *P*=0.778)에 유의한 영향을 미치지 못하였다.

결론: 고도 현미부수체 불안정성 종양은 직장암에서 상당히 드문 빈도로 관찰되었으며, 현미부수체 불안정성은 절제술을 받은 직장암 환자의 생존율에 유의한 영향을 주지 못하였다.

중심 단어: 직장암, 현미부수체 불안정성

Table 1. Clinicopathological features according to microsatellite status of 1,103 study patients with rectal adenocarcinoma

	MSI-L/MSS (N = 1,079)	MSI-H (N = 24)	*P*
Age, median (IQR)	61 (54 – 70)	57 (43.5 – 71.5)	0.221
Gender			0.545
Male	694 (64.3)	14 (58.3)	
Female	385 (35.7)	10 (41.7)	
Tumor stage			0.071
II	432 (40.0)	14 (58.3)	
III	647 (60.0)	10 (41.7)	
Anal verge, median (IQR)	9 (7 – 12)	9 (7.5 – 10)	0.351
Surgery			1.000
Sphincter saving surgery	1,060 (98.2)	24 (100)	
APR or pelvic exenteration	19 (1.8)	0	
Family history of CRC			0.008
No	973 (90.2)	17 (70.8)	
Yes	106 (9.8)	7 (29.2)	
Tumor differentiation			0.001
Well	68 (6.3)	3 (12.5)	
Moderately	957 (88.7)	16 (66.7)	
Poorly	34 (3.2)	4 (16.6)	
Mucinous	20 (1.8)	1 (4.2)	
Lymphovascular invasion			0.156
No	655 (60.7)	18 (75.0)	
Yes	424 (39.3)	6 (25.0)	
Perineural invasion			0.321
No	803 (74.4)	20 (83.3)	
Yes	276 (25.6)	4 (16.7)	
Preoperative CEA level (IQR)	2.5 (1.5 – 4.8)	2.2 (1.45 – 6.6)	0.762
Resection margin status			0.625
Negative	1,026 (95.1)	24 (100)	
Positive	53 (4.9)	0	

Postoperative treatment			0.268
No	97 (9.0)	4 (16.7)	
Radiation therapy	296 (27.4)	3 (12.5)	
Chemotherapy	981 (90.9)	20 (83.3)	
Fluoropyrimidine alone	713	12	
Oxaliplatin-based	268	8	

MSI-L, microsatellite-low; MSS, microsatellite stable; MSI-H, microsatellite-high; IQR, interquartile range; APR, abdominoperineal resection; CRC, colorectal cancer; CEA, carcinoembryonic antigen

Table 2. Univariate analysis of disease-free survival according to clinical prognostic factors

	No.	HR (95% CI)	*P* (Log-rank)	5-year DFS
Gender				
Male	708	1		69.1%
Female	395	0.87 (0.68 – 1.12)	0.283	72.1%
Age (years)				
≤ 61 (median)	559	1		71.9%
> 61 (median)	544	1.08 (0.86 – 1.37)	0.507	68.1%
Tumor stage				
II	446	1		80.6%
III	657	2.24 (1.71 – 2.94)	< 0.001	63.0%
Tumor location				
Mid to lower (< 8 cm)	351	1		60.7%
Upper (≥ 8 cm)	752	0.65 (0.51 – 0.82)	< 0.001	74.3%
Family history of CRC				
No	990	1		69.4%
Yes	113	0.70 (0.45 – 1.09)	0.112	76.4%
Tumor differentiation				
Well	71	1		72.7%
Moderately	973	1.20 (0.71 – 2.02)	0.498	71.1%
Poorly	38	2.84 (1.40 – 5.75)	0.004	56.6%
Mucinous	21	3.34 (1.50 – 7.43)	0.003	42.7%
Resection margin status				
Negative	1,050	1		71.7%
Positive	53	3.32 (2.27 – 4.86)	< 0.001	41.0%
Lymphovascular invasion				
No	673	1		76.2%
Yes	430	1.95 (1.54 – 2.48)	< 0.001	59.6%
Perineural invasion				
No	823	1		76.9%
Yes	280	2.65 (2.09 – 3.37)	< 0.001	49.4%

Preoperative CEA level				
Normal (≤ 6 ng/mL)	872	1		73.5%
Increased (> 6 ng/mL)	231	1.87 (1.44 – 2.42)	< 0.001	57.1%
MSI status				
MSI-L/MSS	1,079	1		69.9%
MSI-H	24	0.84 (0.35 – 2.02)	0.689	78.0%

HR, hazard ratio; CI, confidence interval; DFS, disease-free survival; CRC, colorectal cancer; CEA, carcinoembryonic antigen; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable

Table 3. Univariate analysis of overall survival according to clinical prognostic factors

	No.	HR (95% CI)	*P* (Log-rank)	5-year OS
Gender				
Male	708	1		82.0%
Female	395	0.82 (0.57 – 1.16)	0.260	85.1%
Age (years)				
≤ 61 (median)	559	1		85.7%
> 61 (median)	544	1.77 (1.26 – 2.49)	0.001	80.4%
Tumor stage				
II	446	1		90.7%
III	657	2.38 (1.62 – 3.50)	< 0.001	77.8%
Tumor location				
Mid to lower (< 8 cm)	351	1		73.7%
Upper (≥ 8 cm)	752	0.55 (0.39 – 0.77)	< 0.001	87.1%
Family history of CRC				
No	990	1		82.2%
Yes	113	0.55 (0.28 – 1.07)	0.074	91.1%
Tumor differentiation				
Well	71	1		76.9%
Moderately	973	0.69 (0.37 – 1.28)	0.235	84.7%
Poorly	38	3.90 (1.77 – 8.62)	0.001	63.7%
Mucinous	21	2.40 (0.83 – 6.92)	0.105	63.2%
Resection margin status				
Negative	1,050	1		84.4%
Positive	53	3.56 (2.17 – 5.86)	< 0.001	58.3%
Lymphovascular invasion				
No	673	1		87.5%
Yes	430	2.52 (1.80 – 3.52)	< 0.001	74.5%
Perineural invasion				
No	823	1		87.6%
Yes	280	2.53 (1.81 – 3.54)	< 0.001	69.0%

Preoperative CEA level				
Normal (≤ 6 ng/mL)	872	1		86.2%
Increased (> 6 ng/mL)	231	1.81 (1.27 – 2.60)	0.001	71.3%
MSI status				
MSI-L/MSS	1,079	1		83.1%
MSI-H	24	0.86 (0.27 – 2.69)	0.790	84.0%

HR, hazard ratio; CI, confidence interval; OS, overall survival; CRC, colorectal cancer; CEA, carcinoembryonic antigen; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable

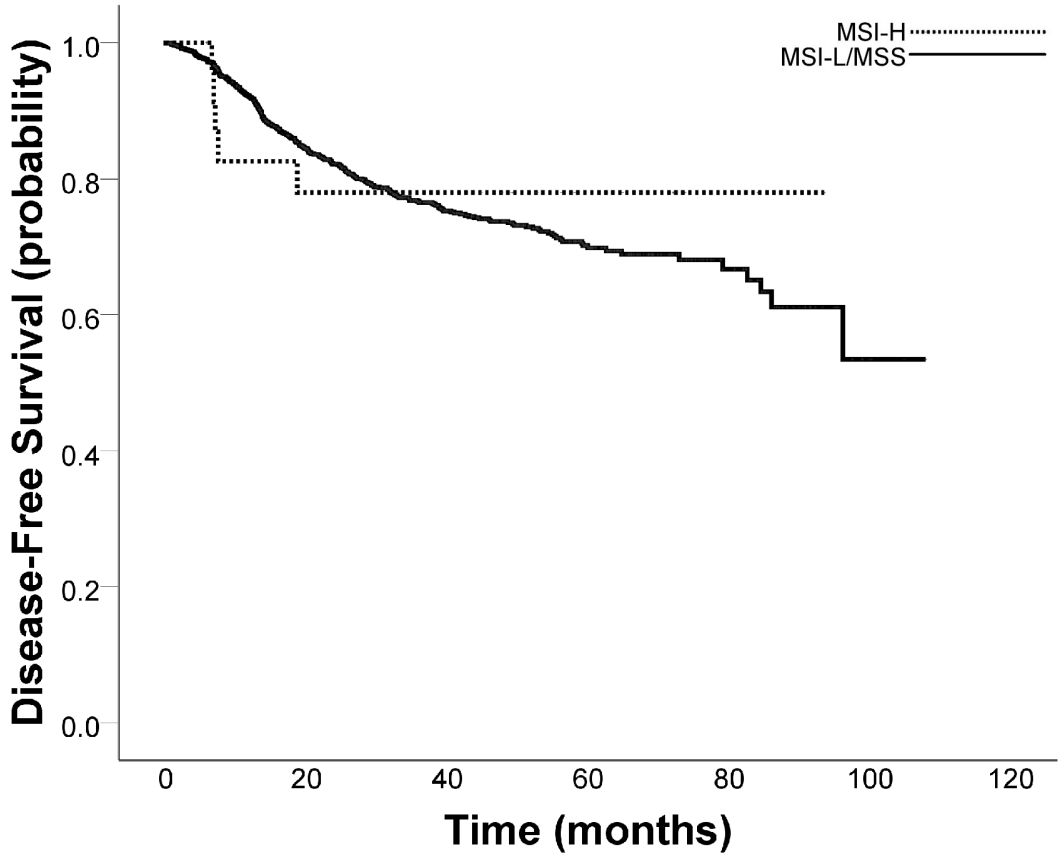
Table 4. Multivariate Cox regression analysis of disease-free survival and overall survival

	DFS		OS	
Clinicopathologic factors	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**
Age				
> 61 vs. ≤ 61	0.287	1.14 (0.90 – 1.45)	< 0.001	1.96 (1.39 – 2.78)
Gender				
female vs. male	0.068	0.79 (0.61 – 1.02)	0.121	0.75 (0.52 – 1.08)
Tumor stage				
III vs. II	< 0.001	1.75 (1.31 – 2.33)	0.012	1.70 (1.12 – 2.56)
Tumor differentiation				
PD vs. WD	0.237	1.55 (0.75 – 3.19)	0.047	2.29 (1.01 – 5.17)
Tumor location				
≥ 8 cm vs. < 8 cm	0.005	0.70 (0.55 – 0.90)	0.006	0.62 (0.44 – 0.87)
Resection margin status				
positive vs. negative	< 0.001	2.58 (1.75 – 3.80)	< 0.001	2.82 (1.69 – 4.69)
Lymphovascular invasion				
yes vs. no	0.024	1.34 (1.04 – 1.73)	0.012	1.60 (1.11 – 2.30)
Perineural invasion				
yes vs. no	< 0.001	2.13 (1.66 – 2.74)	< 0.001	2.13 (1.50 – 3.03)
Preoperative CEA level				
> 6 ng/mL vs. ≤6 ng/mL	0.001	1.56 (1.20 – 2.04)	0.033	1.49 (1.03 – 2.16)

MSI status				
MSI-H vs. MSI-L/MSS	0.994	1.00 (0.40 – 2.47)	0.778	0.85 (0.26 – 2.73)

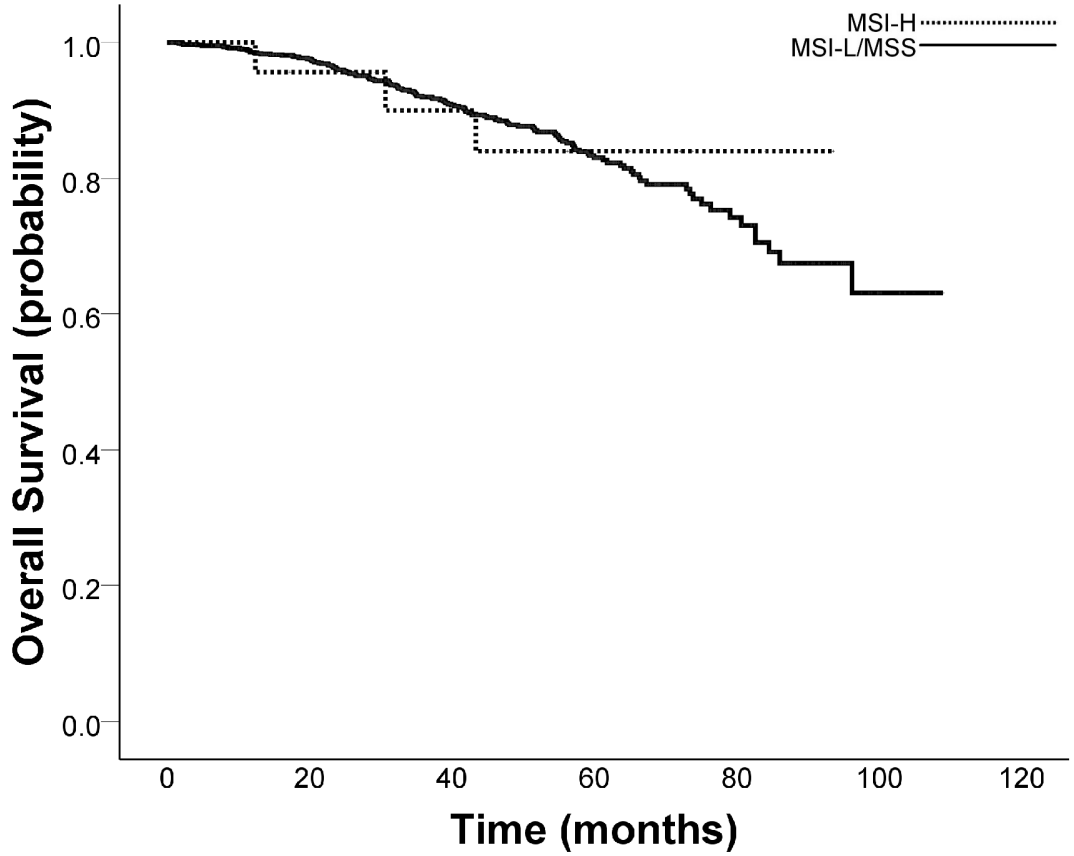
DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; PD, poorly differentiated; WD, well differentiated; CEA, carcinoembryonic antigen; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable

Figure 1. Kaplan-Meier analysis of disease-free survival according to microsatellite phenotype



MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable

Figure 2. Kaplan-Meier analysis of overall survival according to microsatellite phenotype



MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable