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Tumor Mutation Burden and Prognosis in Patients with Colorectal Cancer Treated with Adjuvant Fluoropyrimidine and Oxaliplatin

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Abstract

Purpose: Recent sequencing studies revealed that a subset of colorectal cancer harbors a significantly higher number of somatic mutations. These hypermutated tumors show distinct clinicopathologic features. However, the prognostic impact of the hypermutated tumors is not clearly established.

Experimental Design: We analyzed tumor mutation burden (TMB) from targeted next-generation sequencing data of 40 major genes in 516 patients with colorectal cancer. TMB was defined as total number of nonsynonymous mutations per tumor. Cutoff value for TMB-high was chosen by which best discriminated relapse-free survival (RFS) using the Contal and O'Quigley method.

Results: In the TCGA data, mutation count of the selected 40 genes reflected the whole exome mutation burden (Pearson correlation = 0.873, $P < 0.001$). In our patient cohort, 8 or more mutations in the 40 genes was defined as TMB-high, which best discriminated RFS. A total of 55 patients (10.7%) had TMB-high. TMB-high tumors were more frequently found in a proximal location (63.6%) and had a higher proportion of N0 disease (30.9%) and MSI-H (49.1%) compared with TMB-low. Most importantly, TMB-high was associated with better 5-year RFS compared with TMB-low (96.3% vs. 79.8%, $P = 0.005$). Although there was significant overlap between TMB-high and MSI-H, MSI-H status was not significantly associated with RFS. Multivariate analysis revealed TMB-high as an independent positive prognostic factor for RFS [adjusted HR, 0.16 (95% confidence interval, 0.04–0.66), $P = 0.011$].

Conclusions: TMB-high is associated with better prognosis in patients with colorectal cancer treated with curative surgery followed by adjuvant fluoropyrimidine and oxaliplatin chemotherapy.

Introduction

Colorectal cancer is a heterogeneous disease resulting from complex genetic and epigenetic alterations (1). The Cancer Genome Atlas (TCGA) performed comprehensive molecular analysis of colorectal cancer using exome sequence, DNA copy number, promoter methylation, and messenger RNA and microRNA expression (1). TCGA revealed that 16% of colorectal cancer is categorized as hypermutated tumors (mutation rates of >12 per 10^6 base pair). Hypermutated tumors were more frequently found in proximal tumor location and 77% had microsatellite instability-high (MSI-H) feature. Microsatellite stable (MSS) hypermutated tumor usually had somatic mutations in mismatch-repair genes or POLE aberrations. They also identified that hypermutated tumor and nonhypermutated tumor have different genetic characteristics. *APC*, *TP53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, *TCF7L2*, and *NRAS* were the 8 most frequently mutated genes in nonhypermutated tumors. In the hypermutated tumors, *ACVR2A*, *APC*, *TGFBR2*, *MSH3*, *MSH6*, *SLC9A9*, and *TCF7L2* were frequently mutated. Although hypermutated tumors had higher mutation rates compared with nonhypermutated tumors, *TP53* (20% vs. 60%, $P < 0.0001$) and *APC* (51% vs. 81%, $P = 0.0023$; both Fisher exact test) were less frequently mutated in hypermutated tumor. Although TCGA group speculated that hypermutated tumor may have favorable prognostic role, the prognostic role of hypermutated tumors could not be evaluated due to heterogeneous population and limited number of patients. The prognostic association of tumor mutation burden (TMB) in stage II or III colorectal cancer was recently reported in patients enrolled in the QUASAR 2 clinical trial which compared the efficacy of adjuvant capecitabine plus bevacizumab with adjuvant capecitabine alone (2). In the study, TMB-high was associated with longer relapse-free survival (RFS) in patients with colorectal cancer treated with adjuvant capecitabine ± bevacizumab whereas MSI-H was not associated with prognosis (2).

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Translational Relevance

A subset of colorectal cancer is characterized as having a significantly higher number of somatic mutations. These hypermutated tumors are frequently found in proximal tumor location and have a high incidence of microsatellite instability-high feature. Recently, tumor mutation burden (TMB) has been highlighted by the fact that it may be a positive predictive factor for immune checkpoint inhibitors. However, data on the prognostic role of TMB are limited, especially in the adjuvant setting. In this study, we show that TMB-high is associated with better treatment outcome in stage III or high-risk stage II colorectal cancer treated with adjuvant fluoropyrimidine and oxaliplatin chemotherapy. In addition to validation of this finding, the mechanism underlying the better survival in TMB-high patients needs to be elucidated in future studies.

More than 80% of colorectal cancer arise in a stepwise transformation of normal colorectal epithelium to an adenoma and ultimately to an invasive carcinoma, traditionally known as the adenoma-carcinoma sequence (3). In addition, approximately 15% of colorectal cancer displays MSI-high tumor which results from deficient in mismatch repair (MMR) system (4). MSI-H colorectal cancer may have favorable outcome in stage II/III patients, whereas the prognostic role in stage IV disease is less concrete (5, 6). More importantly, it has been identified that MSI-H tumors have high response to PD-1 blockade regardless of tumor subtype (7–9). In a study performed in 86 MMR-deficient patients including 40 (47%) colorectal cancer, the objective response rate of anti–PD-1 antibody pembrolizumab was 53% (8). There was no difference in objective response rate between colorectal cancer and noncolorectal cancer (52% vs. 54%; ref. 8). Evidences show that TMB is a potential biomarker to predict efficacy of immunotherapy (10, 11). Tumors with high TMB may present more neoantigens, which can be recognized by a host immune system (10). It is suspected that the high mutation burden of MSI-H tumors may have affected its response to immunotherapy (9). However, it is not known whether TMB or MSI-H is more important in responses to immunotherapy or determining patient prognosis. Moreover, it is not clear whether the favorable prognostic role of MSI-H is due to TMB or vice versa.

The purpose of this study was to evaluate the prognostic role of TMB in patients with stage III or high-risk stage II colorectal cancer treated with adjuvant fluoropyrimidine and oxaliplatin chemotherapy, which is the current standard of care in patients with stage III colorectal cancer after surgical resection (12). TMB was calculated using a targeted sequencing data of 40 genes included in 5 critical pathways of colorectal cancer. The impact of MSI-H on survival was also analyzed.

Materials and Methods

Patients and treatment

In this study, TMB was analyzed in a previously reported colorectal cancer cohort which identified the prognostic role of 5 critical pathways (13). The study population is a retrospective consecutive series identified by searching electronic medical record database of Seoul National University Hospital (SNUH). We searched for patients who had received fluoropyrimidine and oxaliplatin after curative surgery and patients were included if they met the inclusion criteria (13). In brief, 516 patients with stage III or high-risk stage II colorectal who received at least 6 cycle of adjuvant FOLFOX or 4 cycle of adjuvant XELOX chemotherapy at Seoul National University Hospital (SNUH, Seoul, South Korea) were included. High-risk stage II was defined if the patient had any of the following: T4 lesion, obstruction or perforation, lymphovascular invasion, perineural invasion, poorly differentiated histology. All patients received curative surgery prior to adjuvant chemotherapy and upper rectal cancer were included if the patient did not receive pre- or postoperative radiation. None of the patients received anti-*EGFR* or anti-*VEGF* treatment for adjuvant chemotherapy. Patient received adjuvant chemotherapy as either FOLFOX-4 (229 patients), modified FOLFOX-6 (166 patients), or XELOX (121 patients). Adjuvant FOLFOX and XELOX chemotherapy was planned for a total of 12 and 8 cycles, respectively. The study protocol was reviewed and approved by the institutional review board of SNUH [H-1210-016-430]. This study was carried out in accordance with the recommendations of the Declaration of Helsinki for biomedical research involving human subjects.

TMB using targeted sequencing of 40 genes associated with 5 critical pathways

Every exon of the 40 genes associated with the 5 critical pathways of colorectal cancer was sequenced (13). Fourteen genes were selected from WNT pathway (*ARID1A*, *AMER1*, *APC*, *AXIN2*, *CTNNB1*, *DKK1*, *DKK2*, *DKK3*, *DKK4*, *FBXW7*, *FZD10*, *LRP5*, *SOX9*, *TCF7L2*), 2 genes from P53 pathway (*ATM*, *TP53*), 8 genes from RTK-RAS pathway (*BRAF*, *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *HRAS*, *KRAS*, *NRAS*), 7 genes from TGFβ pathway (*ACVR1B*, *ACVR2A*, *SMAD2*, *SMAD3*, *SMAD4*, *TGFBR1*, *TGFBR2*), and 9 genes from PI3K pathway (*IGF1R*, *IGF2*, *IRS2*, *MTOR*, *PDGFRA*, *PIK3CA*, *PIK3R1*, *PTEN*, *SRC*). As a result, a total of 109,161 base pair was sequenced. Detailed methods for targeted sequencing can be found in our previous article (13). In brief, genomic DNA (>200 ng) samples were sheared and prepared according to routine library preparation. The captured library was amplified and sequenced using Hiseq 2500 (Illumina). Sequencing data were filtered with a mean quality Q20 (Phred score) per read, and these filtered data were aligned to GRCh37 using bwa 0.7.5a. The aligned reads were processed with Picard Mark Duplicates and GATK base recalibration. After a series of processes, the aligned bases were piled up with SAM tools. Variant call and somatic analysis processes were performed by Varscan and were annotated with ANNOVAR. TMB were calculated using the total number of nonsynonymous somatic mutations. We used Contal and O'Quigley statistical method to define cutoff value for TMB-high tumors using RFS. Contal and O'Quigley method categorize a continuous variable into a binary variable with a cutoff point which have the maximum HR based on a log rank test (14).

Microsatellite analysis

The microsatellite status of each tumor was determined by evaluating 5 microsatellite markers (D2S123, D5S346, D17S250, BAT25, and BAT26; ref. 13). Either the forward or reverse primer for each marker was labeled with fluorescence and PCR products were electrophoresed and analyzed. We classified the MSI status as follows: MSI-high (MSI-H; instability at 2 or more microsatellite

markers), MSI-low (MSI-L; instability at 1 marker), or MSS (no instability).

Analysis of TMB using TCGA database

TCGA data was downloaded from the Genomic Data Commons Data Portal (GDC portal, https://portal.gdc.cancer.gov/). The TCGA database included 433 COAD (colon adenocarcinoma) case and 158 READ (rectal adenocarcinoma) case. In total, 591 colorectal cancer patients from the TCGA database were analyzed. Somatic mutations were identified using the MuTect algorithm as analyzed in the TCGA article (1). The correlation analysis between mutation burden of selected 40 genes and whole exome mutation burden was identified using Pearson correlation coefficient.

Statistical analysis

The primary objective of this study was to investigate the effect of TMB on RFS. Secondary objectives were to evaluate the effect of TMB on overall survival (OS), and to compare the clinical characteristics and prognostic role of TMB and MSI-H. The clinical database was last updated in February 2017. RFS was calculated from the date of operation to the first date of documented relapse. Data from patients who were free of relapse or who died in cancer-free state were censored at the date of the last follow-up visit for RFS. OS was defined as the time from date of operation to death from any cause. Categorical variables were compared by chi-square test or Fisher exact test, and continuous variables were compared using the independent-samples *T* test. RFS and OS were calculated using the Kaplan–Meier method, and comparisons were made using log-rank tests. HR were calculated using the Cox proportional hazard model, and baseline characteristics were adjusted using the forward stepwise model, including the following covariates: age (<65 vs. ≥65), sex, histology [mucinous adenocarcinoma (MAC) vs. non-MAC], tumor location (proximal vs. distal), tumor (T) stage (continuous variable), lymph node (N) stage (continuous variable), microsatellite status (MSS and MSI-low vs. MSI-high), 5 critical pathway mutations, and TMB (TMB-low vs. TMB-high). Two-sided *P* values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS software for Windows, version 18.0 (SPSS) and survMisc package of R statistical software.

Results

Patient characteristics according to TMB

The baseline characteristics are summarized in Table 1. Among 516 patients, 430 (83.3%) patients were stage III and 86 (16.7%) were stage II. In brief, the primary tumor location was proximal (from the cecum to the transverse colon) in 176 patients (34.1%) and distal (from the descending colon to the rectum) in 340 patients (65.9%). MAC histology was shown in 24 patients (4.7%) and 39 patients (7.6%) showed MSI-H feature. All patients received at least 6 cycles of FOLFOX chemotherapy or 4 cycles of XELOX chemotherapy.

We first investigated whether the mutation number of the selected 40 genes was a good surrogate of total TMB. Using the TCGA colorectal cancer data (533 patients with whole exome sequencing data), we compared the nonsynonymous mutation number in the selected 40 genes and whole exome mutation burden of each patient (Fig. 1A). There is a significant positive correlation between mutation burden of the 40 genes and whole exome ($N = 533$; Pearson correlation coefficient 0.873, $P < 0.001$). The significant correlation was maintained in a subgroup analysis of MSS/pMMR tumors ($N = 391$; Pearson correlation coefficient 0.877, $P < 0.001$).

Table 1. Baseline characteristics

	Total ($N = 516$)	TMB-Low ($N = 461$)	TMB-High ($N = 55$)	*P* value
Age				
<65 years	346 (67.1%)	307 (66.6%)	39 (70.9%)	0.52
≥65 years	170 (32.9%)	154 (33.5%)	16 (29.1%)	
Sex				
Male	304 (58.9%)	269 (58.4%)	35 (63.6%)	0.45
Female	212 (41.1%)	192 (41.6%)	20 (36.4%)	
Location				
Proximal	176 (34.1%)	141 (30.6%)	35 (63.6%)	<0.001
Distal	340 (65.9%)	320 (69.4%)	20 (36.4%)	
T stage				
T1	3 (0.6%)	2 (0.4%)	1 (1.8%)	0.20
T2	24 (4.7%)	24 (5.2%)	0 (0.0%)	
T3	412 (79.8%)	367 (79.6%)	45 (81.8%)	
T4	77 (14.9%)	68 (14.8%)	9 (16.4%)	
N stage				
N0	86 (16.7%)	69 (15.0%)	17 (30.9%)	0.004
N1	288 (55.8%)	258 (56.0%)	30 (54.5%)	
N2	142 (27.5%)	134 (29.1%)	8 (14.5%)	
Tumor stage				
II, high-risk	86 (16.7)	69 (15.0%)	17 (30.9%)	0.003
III	430 (83.3%)	392 (85.0%)	38 (69.1%)	
Histology				
Non-MAC	492 (95.3%)	438 (95.0%)	54 (98.2%)	0.29
MAC	24 (4.7%)	23 (5.0%)	1 (1.8%)	
Microsatellite status ($N = 512$)				
MSS/MSI-L	473 (92.4%)	446 (97.6%)	27 (49.1%)	<0.001
MSI-H	39 (7.6%)	11 (2.4%)	28 (50.9%)	

The distribution of nonsynonymous mutation number per patients in this study is shown in Fig. 1B. Using the Contal and O'Quigley method, TMB-high tumors was defined if the patient had 8 or more mutations (≥8). Patients with 7 or less mutation (≤7) were regarded as TMB-low. Among 516 patients, 55 (10.7%) patients had TMB-high tumor (Fig. 1C). TMB-high tumor tended to occur in proximal location (63.6% vs. 30.6% in TMB-low, $P < 0.001$), had higher incidence of N0 disease (stage II disease; 30.9% vs. 15.0% in TMB-low tumor, $P = 0.004$), and were MSI-H (50.9% vs. 2.4% in TMB-low tumor, $P < 0.001$; Table 1). However, 28.9% of MSI-H were categorized as TMB-low tumor. The detailed mutation count of patients with MSI-H is shown in Supplementary Table S1. The incidence of MAC was 12.8% in MSI-H patients but it was different according to TMB status (36.4% in MSI-H/TMB-low vs. 3.7% in MSI-H/TMB-high, $P = 0.017$). Only 1 patient with TMB-high tumor had MAC histology. Baseline characteristics including age, sex, and T stage were similar between TMB-high and TMB-low.

Mutational characteristics according to TMB is shown in Table 2. In accordance with the result from the TCGA data, *TP53* was less frequently mutated in TMB-high tumors compared with TMB-low tumors (38.2% vs. 66.8% in TMB-low tumor, $P < 0.001$; ref. 1). Although most genes were more frequently mutated in TMB-high tumors, the mutation rate of *APC* gene were similar between TMB-high and TMB-low tumors (78.2% vs. 71.6%, $P = 0.30$). Likewise, pathways were more frequently altered in TMB-high tumor, except for P53 pathway (as a result of *TP53* mutation rate).

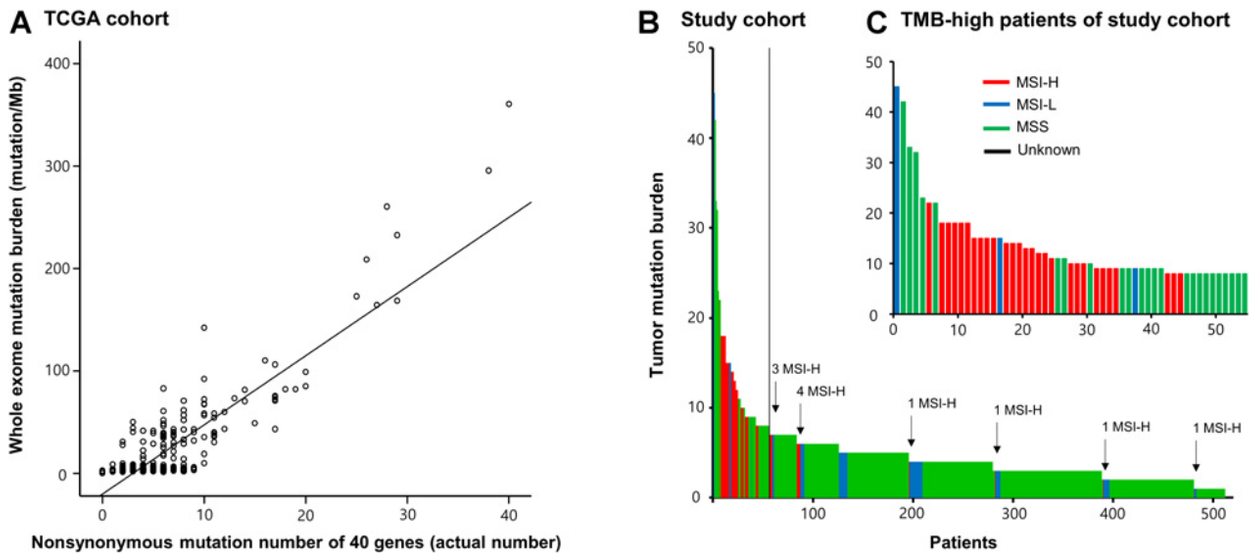

Figure 1.

TMB in the TCGA cohort and the present study cohort. **A,** TMB in the TCGA cohort. Each dot represents a single patient with X-value indicating actual mutation number in the selected 40 genes from the whole exome data and Y-value indicating whole exome mutation burden of the patient. **B,** TMB in the present study cohort including all patients. Each bar indicates actual number of nonsynonymous mutation of 40 genes of single patient. MSI-H patients with TMB-low are additionally marked with arrows, and the numbers above the arrows indicate the number of patients with MSI-H. **C,** Magnified image of TMB-high patients in **B**.

Prognostic role of TMB-high tumor and MSI-H

After a median follow up duration of 62.4 months, the 5-year RFS of the entire cohort was 81.5% [95% confidence interval (CI), 78.0%–85.0%] and the 5-year OS was 90.2% (95% CI, 87.5%–92.9%). Five-year RFS, which is the primary objective, was significantly better in TMB-high tumor compared with TMB-low tumor (5-year RFS 96.3% vs. 79.8%, respectively; $P = 0.005$; Fig. 2A). As MSI-H is historically known as a positive prognostic factor and is closely related to TMB-high, we performed additional analysis to see whether MSI-H have a prognostic role. In contrast to TMB, MSI status was not associated RFS (5-year RFS of 89.7% in MSI-H vs. 81.0% in MSS/MSI-L, $P = 0.23$; Fig. 2C). Tumor location also did not impact RFS ($P = 0.92$). Multivariate analysis using the Cox proportional hazard model revealed TMB-high tumor [adj HR for RFS 0.16 (95% CI, 0.04–0.66), $P = 0.011$] as an independent positive prognostic factor for RFS (Table 3).

Table 2. Mutation frequency of most commonly mutated genes and pathways

Gene	Total ($N = 516$)	TMB-Low ($N = 461$)	TMB-High ($N = 55$)	*P* value
APC	373 (72.3%)	330 (71.6%)	43 (78.2%)	0.30
TP53	329 (63.8%)	308 (66.8%)	21 (38.2%)	<0.001
KRAS	223 (43.2%)	184 (39.9%)	39 (70.9%)	<0.001
FBXW7	98 (19.0%)	69 (15.0%)	29 (52.7%)	<0.001
PIK3CA	91 (17.6%)	58 (12.6%)	33 (60.0%)	<0.001
SMAD4	74 (14.3%)	57 (12.4%)	17 (30.9%)	<0.001
TCF7L2	66 (12.8%)	46 (10.0%)	20 (36.4%)	<0.001
ARID1A	56 (10.9%)	32 (6.9%)	24 (43.6%)	<0.001
Pathway				
WNT	436 (84.5%)	381 (82.6%)	55 (100.0%)	0.001
P53	356 (69.0%)	323 (70.1%)	33 (60.0%)	0.13
RTK-RAS	313 (60.7%)	98 (21.3%)	47 (85.5%)	<0.001
PI3K	155 (30.0%)	258 (56.0%)	54 (98.2%)	<0.001
TGFβ	149 (28.9%)	100 (21.7%)	49 (89.1%)	<0.001

In an exploratory subgroup analysis, TMB-high tumor had a positive prognostic role in both MSI-H and MSS/MSI-L patients (Supplementary Fig. S1). TMB-L/MSI-H patients tended to have the worst prognosis. However, this result should be interpreted with caution given the small number of patients in the majority of the subgroups. Although TMB-high tumor had high incidence of stage II disease, TMB-high was a positive prognostic factor in a subgroup of patients with stage III disease (5-year RFS 97.3% vs. 79.8%, respectively; $P = 0.014$).

In the analysis of OS, which is the secondary objective, TMB-high tumor had a tendency of favorable survival compared with TMB-low tumor (5-year OS 96.2% *vs.* 89.6%, respectively; $P = 0.060$; Fig. 2B). However, TMB-high tumor was not an independent prognostic factor for OS in the multivariate analysis. MSI-H was not associated with OS (Fig. 2D).

Discussion

This study was conducted to investigate whether TMB have prognostic role in patients with stage III or high risk II colorectal cancer treated with adjuvant fluoropyrimidine and oxaliplatin chemotherapy. TMB was calculated using the targeted sequencing data of 40 genes which represent 5 critical pathways of colorectal cancer. Using the TCGA database, we confirmed that the mutation number of selected 40 genes were able to represent whole exome mutation burden. We were able to show that TMB-high is associated with favorable RFS in patients with stage III or high risk II colorectal cancer using a small set of genes important in colorectal cancer.

Development of colorectal cancer arise in a stepwise order through chromosomal instability known as the adenoma–carcinoma sequence (3). However, approximately 15% of colorectal cancer is characterized by MSI-H (4). MSI-H colorectal cancer tends to have favorable prognosis in early-stage tumors

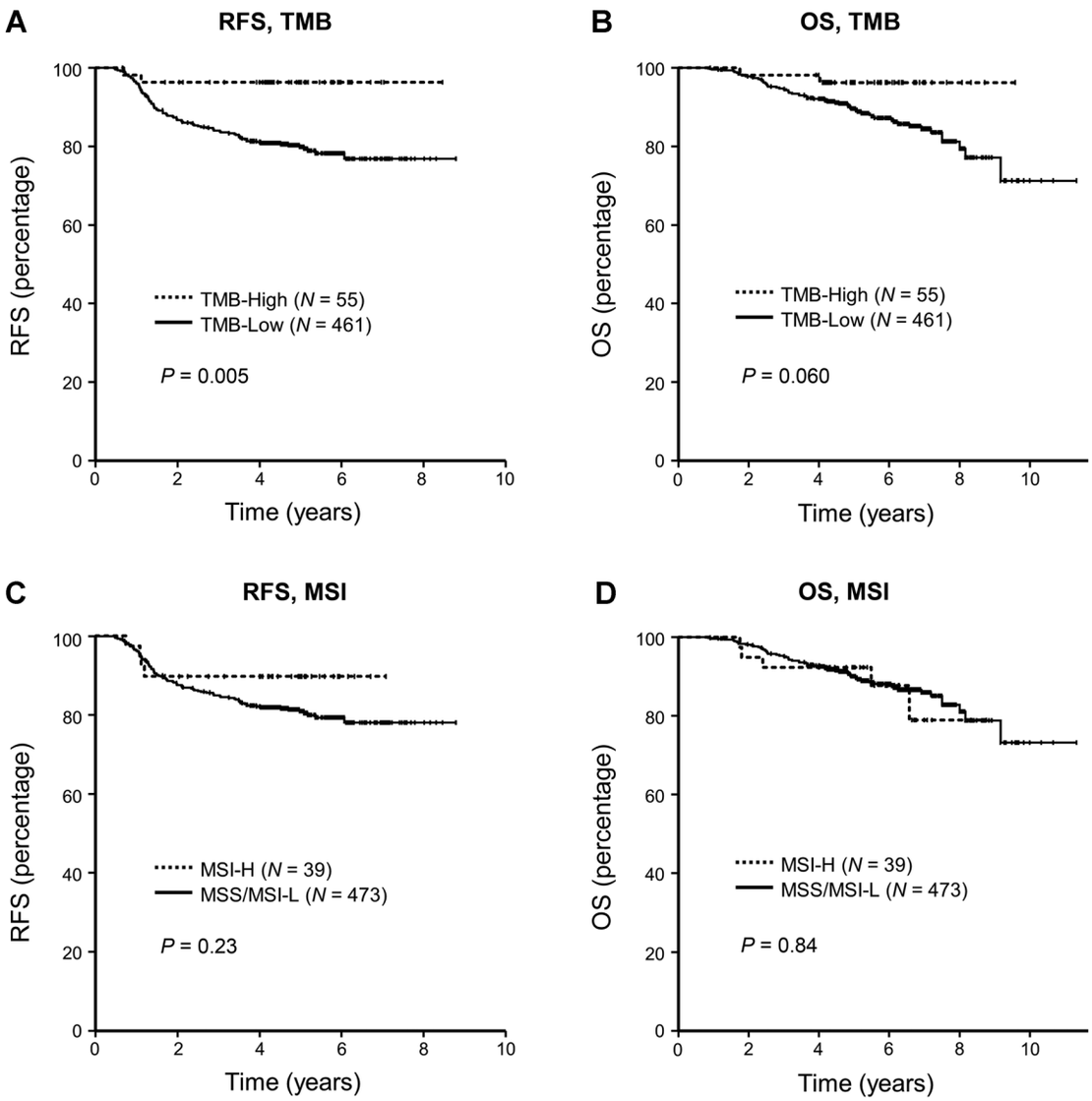

Figure 2.
Survival outcome according to TMB (**A** and **B**) and MSI (**C** and **D**). **A,** RFS according to TMB status. Kaplan–Meier curve and the corresponding *P* value of log-rank test is shown. **B,** OS according to TMB status. **C,** RFS according to MSI status. **D,** OS according to MSI status.

and have distinct clinical and pathologic features. Previous studies suggest that there is an ethnic difference in the incidence of MSI-H, CIMP, and *BRAF* mutation, which are lower in Asian compared with Western (15). Recently, the clinical relevance of MSI-H has been highlighted by the fact that it is a strong predictive biomarker for immune checkpoint inhibitors (7, 8). Because of defect in MMR system, MSI-H tumors have high TMB. As a result, MSI-H tumors create higher tumor-specific neoantigens and have more tumor infiltrating lymphocytes compared with MSS tumors (16–18). It is speculated that the high mutation burden and its resulting tumor-specific neoantigens of MSI-H tumors may have affected its response to immunotherapy (9).

Table 3. Multivariate analysis of RFS

Variable	Status	Adjusted HR (95% CI)	*P* value
Tumor stage (T)	T1–4[a]	2.91 (1.90–4.43)	<0.001
Lymph node stage (N)	N0–2[a]	1.89 (1.38–2.59)	<0.001
TMB	TMB-Low	1	0.011
	TMB-High	0.16 (0.04–0.66)	
RTK–RAS pathway	Wild type	1	0.013
	Mutation	1.73 (1.12–2.66)	

[a]Continuous variable.

Although MSI-H tumors usually have high TMB, not every colorectal cancer with high TMB show MSI-H feature (1). In this study, TMB-high tumor was shown in 55 (10.7%) among 516 patients. MSI-H was found in 39 (7.6%) patients and half of TMB-high tumors were MSI-H. In the TCGA database, 16% of colorectal cancers were found to be hypermutated and 14% had MSI-H feature. Among hypermutated tumor, 77% had MSI-H feature (1). The proportion of TMB-high tumor and the proportion of MSI-H

in high TMB tumor was lower in our cohort compared with TCGA data. The lower incidence of MSI-H in Asian patients with colorectal cancer may have affected such finding (15). Recent evidences revealed that proofreading domain mutation of *POLE* could result in hypermutated tumors (19). As we do not have explanation for the high mutation burden in MSS patients, it would be important to analyze genes including mismatch repair genes and *POLE* in the future studies to identify underlying cause of the high mutation burden. Although many studies showed favorable prognosis of MSI-H colorectal cancer, there are conflicting results especially in metastatic disease (5, 6). In our study, MSI-H was not associated with RFS and OS. By contrast, TMB-high was associated with lower recurrence and had tendency of favorable OS. Interesting finding was that MAC histology, which is frequently found in MSI-H colorectal cancer and is a potent poor prognostic factor (20, 21), was rarely shown in TMB-high tumor. In addition, TMB was variable among MSI-H tumors, and TMB-high patients had better RFS although the finding is based on a small number of patients in this study. Previous studies have shown that number of frameshift mutations and neoantigen load are associated with tumor infiltrating lymphocytes (22, 23). Immune cell infiltration is associated with better prognosis in colorectal cancer (24, 25). Taken together, different immune response among MSI-H according to TMB status may have influenced RFS. TMB and MAC histology may have partially affected contradictory role of MSI-H in previous studies. TMB maybe a more reliable prognostic factor compared with MSI-H. The favorable prognostic role of high TMB in stage II and III colorectal cancer was previously reported by Domingo and colleagues (2). However, none of the patients in the discovery cohort received standard fluoropyrimidine plus oxaliplatin chemotherapy, and only 47 patients (under 10%) received fluoropyrimidine plus oxaliplatin chemotherapy in the validation cohort (2). The novelty of our study is that all patients in our cohort were treated with curative surgery followed by adjuvant fluoropyrimidine plus oxaliplatin chemotherapy, which is the current standard treatment for patients with stage III colorectal cancer (12). Recently, the positive prognostic role of high TMB was also noticed in MSS metastatic colorectal cancer (26). From the database of CALGB/SWOG 80405 study, the authors revealed that high TMB tumor had longer OS compared with low TMB tumor (HR = 0.73; $P = 0.02$) in patients with MSS metastatic colorectal cancer. This study result once again confirms that TMB is a positive prognostic factor in patients with colorectal cancer. Future study investigating the predictive role of TMB on immune check point inhibitor would be helpful in patients with colorectal cancer.

There are several limitations in this study. As TMB was measured with targeted sequencing of 40 genes, only 109,161 base pair was sequenced in each patient. In addition, TMB-high tumor was defined using the cutoff point which have the best HR for RFS. However, our gene panel is specific to colorectal cancer and all 40 genes were included in the 5 critical pathways of colorectal cancer. As a result, 511 patients (99.0%) had at least 1 nonsynonymous mutation and the median number of nonsynonymous mutation was 4. Moreover, the mutation burdens of selected 40 genes were associated whole exome mutation burden in the TCGA database. Our study results suggest that even gene panel with limited number of genes can be used as a useful tool to identify TMB, when selected properly to a tumor subtype. Another major limitation of this study is that we could not validate the association of TMB-high with prognosis in the TCGA data set because of the limited number of stage III patients ($N = 155$) with survival data. The finding needs to be validated with an independent cohort in the future studies.

In conclusion, high TMB is associated with favorable outcome in patients with stage III or high risk II colorectal cancer treated with fluoropyrimidine and oxaliplatin chemotherapy. However, MSI-H was not associated with survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Availability of Data and Materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the institutional review board of SNUH (H-1210-016-430). This study was carried out in accordance with the recommendations of the Declaration of Helsinki for biomedical research involving human subjects. In accordance with South Korea regulation, retrospective studies without any additional therapy or monitoring procedure, do not need formal written consent from patients. Because the study was retrospectively designed without any investigational intervention, the study-specific informed consent was not obtained from each patient (https://cris.snuh.org, http://bri.snuh.org).

Authors' Contributions

Conception and design: D.-W. Lee, S.-W. Han, S.-Y. Jeong, T.-Y. Kim
Development of methodology: S.-W. Han
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.-W. Lee, S.-W. Han, J.M. Bae, S.-Y. Jeong, K.J. Park, G.H. Kang
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