의학박사 학위논문

동시성 다발성 대장암의 면역유전체 발현양상 분석

Immunogenomic analysis of synchronous colorectal cancer

울산대학교대학원
의 학 과
이 현 구

동시성 다발성 대장암의 면역유전체 발현양상 분석

지도교수 박 인 자

이 논문을 의학박사 학위 논문으로 제출함

2023년 8월

울산대학교 대학원
의 학 과
이 현 구

이현구의 의학박사학위 논문을 인준함

심사위원장 홍 승 모 (인)

심사위원 박 인 자 (인)

심사위원 이 종 률 (인)

심사위원 김 덕 훈 (인)

심사위원 정 성 민 (인)

울 산 대 학 교 대 학 원

2023 년 8 월

Abstracts

Immunogenomic analysis of synchronous colorectal cancer

Background and Objectives: Synchronous colorectal cancers (SCRC) are defined as having more than one primary colorectal cancer at the time of the initial diagnosis. The molecular characteristics of SCRC are not fully understood despite their importance in determining targeted therapy such as immune checkpoint blockade. The aim of this study was to compare molecular characteristics, including microsatellite instability (MSI) and somatic mutations between synchronous tumors.

Materials and methods: From 2012 to 2014, 100 patients with SCRC treated with surgical resection were retrospectively reviewed. Clinical, pathologic, and molecular characteristics, including MSI and tumor infiltrating lymphocytes (TILs), were analyzed for all tumor lesions in SCRC patients. The Bethesda panel was used to assess the MSI status, and two or more altered microsatellite markers were classified as high MSI (MSI-H). The density of TILs was determined as a percentage of all mononuclear cells over the stromal area on hematoxylin and eosin-stained sections, based on the recommendation of the International TILs Working Group. Whole exome sequencing (WES) was performed on 18 tumors from 9 patients who had at least one MSI-H tumor to evaluate the intertumoral heterogeneity of SCRC.

Results: SCRC showed male predominance and a higher rate of MSI-H and right-sided location compared to solitary colorectal cancer. MSI-H tumors were more frequently located in the right colon (68.4% vs 33.7%, $p = 0.011$) and had a higher proportion of mucinous adenocarcinoma (15.8% vs 0%, $p < 0.001$) and a high density of TILs (57.9% vs 25.0%, $p < 0.001$) than MSS tumors. Among the 100 SCRC patients, 12 had at least one MSI-H tumor, and 5 showed discordant MSI status. Patients with discordant MSI status did not differ from those with concordant MSI status in terms of recurrence-free survival or overall survival. In WES analysis, most synchronous tumors shared only a few variants in the same patient (0.09–0.36%), except for one patient (6.5%). The concordance rates for *BRAF*, *KRAS*, *PIK3CA*, and *NRAS* of synchronous tumors in the same individual were 66.7%, 66.7%,

55.6%, and 66.7%, respectively. However, almost all synchronous tumors had different mutant subtypes among them, even when they shared the same mutated gene.

Conclusion: In this study, SCRC showed a discordance rate of 5% in MSI status and a high discordance rate in somatic variants. As intertumoral heterogeneity may affect the response to target therapy, molecular analysis of all tumor lesions in a single patient is recommended in determining the treatment strategies for SCRC.

Key words: synchronous colorectal cancer, microsatellite instability, whole exome sequencing, somatic mutation

Contents

Lists of figures and tables

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors, and the third leading cause of cancer-related mortality worldwide.[1] Synchronous colorectal cancer (SCRC) is defined as the presence of more than one primary colorectal carcinoma in the same individual at the time of the initial diagnosis. The proportion of SCRC ranged from 1.1% to 8.1% of all CRC cases.[2] SCRC is more prevalent in the proximal colon and have a higher male to female ratio and higher incidence of mucinous adenocarcinoma than solitary CRC.[2] Moreover, SCRC has clinical importance in terms of surgical treatment strategy, postoperative surveillance, and family screening compared to solitary CRC. Patients with hereditary colorectal cancer syndromes or inflammatory bowel disease are known to have an increased risk of SCRC[2-4]. However, these predisposing conditions account for only about 10% of CRC patients,[5] with the majority of cases caused by other genetic and environmental factors. SCRC have arisen from very similar or identical background factors (genetic or environmental), therefore providing a unique model to investigate multistep carcinogenesis.

CRC is a highly heterogenous disease and is caused by a variety of accumulations of genetic and epigenetic changes in colonic mucosa. Three molecular pathways are known crucial features in colorectal carcinogenesis: chromosomal instability (CIN), microsatellite instability (MSI), and CpG Island Methylator Phenotype (CIMP). CIN refers to an increased rate of gains or losses of chromosomes, which leads to cell-to-cell karyotypic heterogeneity. In addition to karyotypic changes, CIN tumors exhibit an accumulation of somatic mutations in specific oncogenes and tumor suppressor genes that activate pathways essential for the initiation and progression of CRC.[6] CIN is observed in 65-70% of sporadic CRC and is the most essential pathway in colorectal tumorigenesis.[7] MSI is a hypermutable phenotype caused by genetic or epigenetic alterations in mismatch repair (MMR) genes such as *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*. MSI was first identified in hereditary nonpolyposis colorectal cancer (HNPCC). HNPCC is an autosomal dominant genetic condition associated with a high risk of colon cancer and other cancers, including endometrial, ovarian, stomach, small intestine, and hepatobiliary tract tumors. HNPCC is caused by a germline mutation in one of the MMR genes. MSI was also observed in sporadic CRC, and sporadic MSI cancers are caused by methylation of *MLH1* promoters, which is strongly associated with the V600E mutation of the *BRAF* gene. The CIMP pathway is characterized by abnormal DNA

methylation patterns in specific regions of the genome known as CpG islands. DNA methylation is a process by which a methyl group is added to the DNA molecule. Methylation of CpG islands can affect gene expression by silencing tumor suppressor genes, such as *MLH1*, *MGMT*, and *CDKN2A*.[8,9] CIMP-positive CRC is more prevalent in the proximal colon, has a high frequency of MSI-H, and tends to have *BRAF* mutations rather than *KRAS* mutations.[10] CRC can originate from one or a combination of these three molecular pathways. Currently, molecular subtyping based on gene expression is widely accepted and provides a greater understanding of etiology and features of CRC as well as subtype-based targeted therapies.[11]

Despite advances in understanding of molecular pathways in colorectal carcinogenesis, the molecular mechanisms of SCRC remain unclear. Several hypotheses have been suggested to explain the occurrence of SCRC. According to the clonal hypothesis, multifocal tumors originate from a founder tumor via intraluminal or intraepithelial spread.[12] In contrast, another hypothesis, known as the field effect,[13,14] suggested that a large area of cells is transformed by a carcinogen, followed by neoplastic transformation through subsequent mutations. This hypothesis highlighted the importance of the tumor microenvironment in the development of synchronous tumors. Given that cancer is caused by the sequential accumulation of genetic and epigenetic mutations, each hypothesis would result in a distinct genetic signature.

SCRC has been reported to have a strong correlation with the MSI pathway compared to solitary CRC.[15] The rate of high MSI (MSI-H) in solitary CRC ranged from 12 to 17%, whereas it was 30–37% in SCRC[16-18] and 90% in HNPCC.[19] The majority of SCRC patients showed concordant MSI status; however, a few SCRC patients exhibit discordant MSI status, which was reported to be associated with a worse prognosis than concordant MSI status.[20] CRC is known to have intratumoral and intertumoral heterogeneity in molecular abnormalities, and these characteristics may have greater significance in SCRC. Previous studies showed contradictory findings concerning the concordance of molecular findings in individuals with SCRC. Some studies have reported that SCRC frequently has discordance in molecular alterations.[21-25] K. Eguchi et al. reported that all of the enrolled cases showed intertumoral heterogeneity in the mutation pattern of *TP53*, suggesting most SCRC are multicentric in origin.[23] In other studies, the concordance rate for *KRAS* mutations and *BRAF* mutations between lesions in the same SCRC patients ranged from 11 to 40% and 0 to 14%,

respectively.[16,26,27] On the other hand, some reports have demonstrated a strong concordance in epigenetic alterations among synchronous tumors in the same individual.[14,17,28]

Current trends have focused on the individualization of treatment for colorectal cancer based on the molecular characteristics of each tumor. Especially, Immunotherapy has demonstrated outstanding success in eliminating cancer cells by utilizing the innate immune mechanisms of the host.[29,30] Immune checkpoint blockade regenerates T lymphocytes and enables the adaptive immune system to prevent immune escape resulting from cascade activation of tumor-specific immune checkpoints regulated by programmed cell death protein (PD-1), programmed death-ligand 1 (PD-L1), or cytotoxic T lymphocyte-associated protein 4 (CTLA-4).[31,32] Pembrolizumab and nivolumab, PD-1 inhibitors, have shown a durable outcomes in patients with MSI-H CRC.[29,30] In addition to MSI status, tumor mutation burden (TMB) and tumor-infiltrating lymphocytes (TILs) have also been found to be correlated with the response to immune checkpoint blockade.[33,34] With a growing interest in immunotherapy as a treatment paradigm for CRC, the significance of MSI status and TILs as biomarkers for predicting response to immunotherapy has been emphasized.

In contrast to solitary CRC, for which treatment strategies can be determined based on the characteristics of a single tumor, SCRC has numerous characteristics of multiple tumors, and it is unclear how each affects the response to treatment. Therefore, this study aimed to investigate the clinical, pathologic, and molecular characteristics of SCRC, including TILs and MSI status. In addition, we performed whole exome sequencing (WES) to assess intertumoral heterogeneity in individuals with SCRC.

Materials and Methods

Study population

This analysis included 200 synchronous tumors from 100 patients with SCRC who underwent surgical resection at Asan Medical Center between 2012 and 2014. Patients with more than one primary adenocarcinoma in the colon or rectum at the time of the first diagnosis were included. All cases were surgically resected without receiving any neoadjuvant treatment. Patients with colon cancer associated with inflammatory bowel disease, familial adenomatous polyposis, or concurrent other cancer were excluded. The study was approved by the institutional review board of Asan Medical Center (approval number: 2022-0771).

Clinicopathologic evaluation and postoperative surveillance

Clinical and pathologic data were collected retrospectively from the hospital's database of medical records. The index tumor (labeled as T1) was defined for each patient as the one with the most advanced T staging. If the tumors were of the same T stage, the tumor with the longest diameter was designated T1. T2 was assigned to the second-most advanced T stage or second-largest tumor in the same patient. Tumor size was defined as the longest diameter of each tumor. Tumor locations were classified into three groups: the right colon, the left colon, and the rectum. The right colon includes the cecum, ascending colon, hepatic flexure, and transverse colon, while the left colon includes the splenic flexure, descending colon, and sigmoid colon. HNPCC was clinically diagnosed based on the Amsterdam II criteria[35]: i) $\geq$3 family members with colorectal cancer, where one is a first-degree relative of the other two members; ii) at least two successive generations affected; iii) at least one case diagnosed before the age of 50 years; and iv) familial adenomatous polyposis should be excluded.

The pathologic staging was based on the eighth edition of the AJCC classification of malignant tumors. Metastasis was clinically diagnosed through imaging, except in cases where simultaneous resection was performed. Differentiation, histologic type, lymphovascular invasion (LVI), perineural invasion (PNI), and tumor budding were also evaluated for synchronous tumors in each patient. The Bethesda panel was used to assess the MSI status; two or more altered microsatellite markers were classified as MSI-H, only one altered marker as MSI-low (MSI-L), and none as microsatellite stable (MSS).[36]

In accordance with our institution's guidelines, all patients received surveillance every 3–6 months for at least five postoperative years. Postoperative surveillance included a physical examination, laboratory test, serum carcinoembryonic antigen (CEA) level, and/or chest radiographs, abdominopelvic computed tomography (CT), chest CT, and colonoscopy. Positron emission tomography and magnetic resonance imaging were considered for patients with suspected recurrences in scheduled surveillance with serum CEA level or abdominopelvic/chest CT. Recurrences were identified by imaging modalities and/or histopathologic confirmation.

Evaluation of tumor infiltrating lymphocytes

TILs were assessed in tissue material stained with hematoxylin-eosin (H&E) and evaluated by two independent pathologists (S.M. Hong & Y. S. Kim) blinded to the clinical information. The density of TILs was determined based on the recommendation by the International TILs Working Group (ITWG).[37] TILs were determined as a percentage of all mononuclear inflammatory cells (e.g., lymphocytes and plasma cells), and other inflammatory cells (i.e., neutrophils and granulocytes) were excluded. The density of TILs was assessed within the intra-tumoral stromal area and counted in 5 areas (total magnification, x200-x400) on the average (not hotspots). Only TILs within the border of invasive tumors were assessed, so that tumor areas with dysplasia, in-situ area, crush artifacts, necrosis, or regressive hyalinization were excluded. For statistical analysis, three levels of infiltration in the stroma TILs were determined: weak (0-10% of stromal TILs), moderate (20-40% of stromal TILs) and strong (50-90% of stromal TILs). Examples of TILs assessment using the ITWG system are shown in Fig. 1.

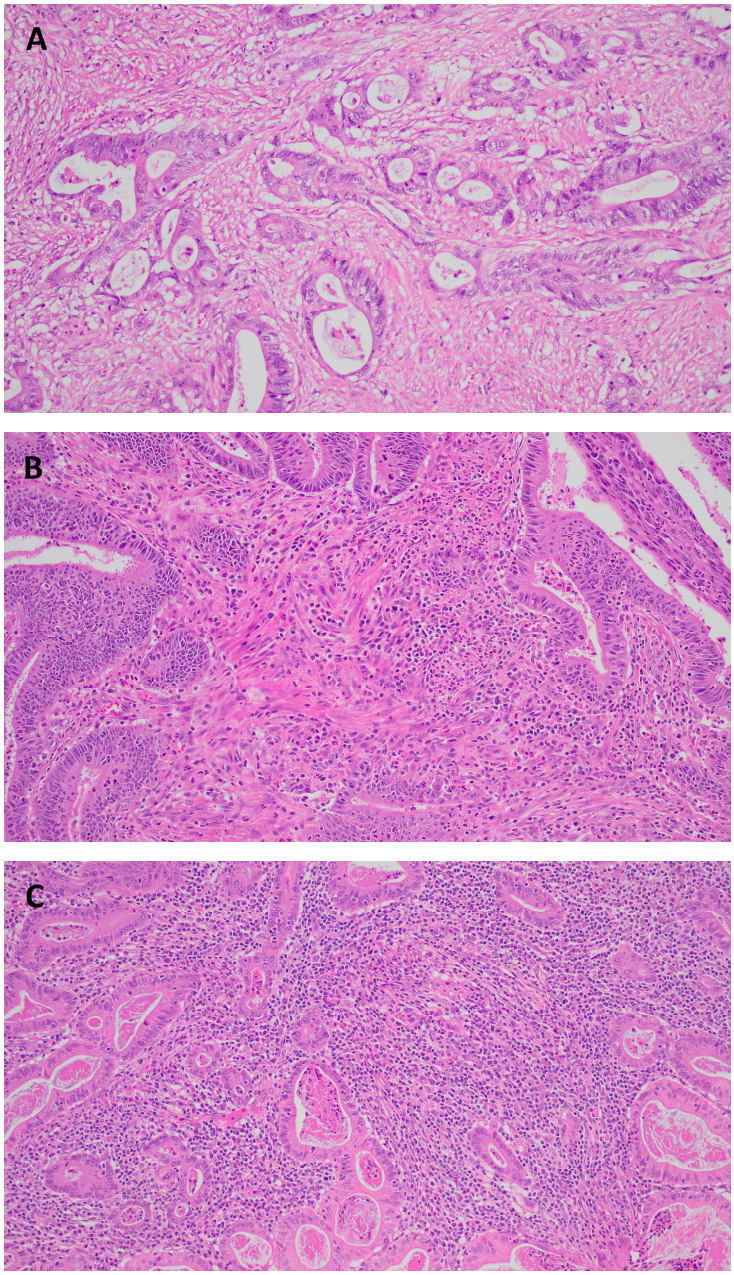


Fig. 1. Examples of TILs scored using the methodology of the ITWG (H&E, original magnifications 200x) (A) Weak (<20%): A few lymphocytes are scattered in tissue surrounding the cancer nests. (B) Moderate (20-40%): Some lymphocytes are present in stroma. (C) Strong (>40%): Numerous lymphocytes are distributed adjacent to the cancer nests.

DNA extraction

For whole exome sequencing, twelve patients who had at least one MSI-H tumor out of a total of 100 SCRC patients were selected. Patient-paired two synchronous tumor and normal samples were obtained by manual microdissection with H&E-stained guide slides. Thirty-six samples were collected by manual microdissection from formalin-fixed paraffin-embedded (FFPE) tissues obtained from 12 colon cancer patients. Genomic DNA was extracted using QIAamp DNA FFPE tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA quality was assessed by PicoGreen (Invitrogen, CA, USA) using Victor 3 fluorometry and gel electrophoresis methods. One case did not satisfy the quality criteria, and DNA libraries for whole exome sequencing were constructed using 33 samples from 11 patients.

Whole exome sequencing

Whole exome sequencing was performed using SureSelect Human all Exon V8 (FFPE) library kit (Agilent Technologies, CA, USA) and NovaSeq 6000 platform (Illumina, CA, USA), in accordance with the manufacturer's protocol. Briefly, genomic DNA samples were randomly fragmented and then ligated to the 5' and 3' molecular barcoded adapters. Adapter-ligated DNA fragments are amplified with SureSelect Human all Exon V8 primers, which anneal to the ends of each adapter. The quality and quantity of the thirty-three library templates were assessed using 2100 Bioanalyzer (Agilent Technologies, CA, USA) and Illumina qPCR Quantification (Illumina, CA, USA). Since two samples of capture libraries were not amplified, 27 samples from nine patients were hybridized and amplified. And samples were performed for paired-end sequencing using NovaSeq 6000 sequencer with a 101-bp read length. The base calling files are converted into FASTQ files using bcl2fastq, which is an Illumina-provided package. Variant calling was performed by comparing the sequencing reads of the tumor and matched normal genomes to identify somatic mutations. Tumor-specific somatic SNV and INDEL were determined if they were absent in the normal counterpart.

Statistical analysis

Statistical analysis comparing the characteristics of the patients and the tumors were done using Fisher's exact test or Pearson's chi-squared test for categorical variables and the

Student's *t*-test for continuous variables. Survival outcomes included recurrence-free survival (RFS) and overall survival (OS). RFS and OS of groups divided according to TILs and MSI status were analyzed using the Kaplan-Meier method and compared by log-rank tests. To assess the genetic heterogeneity of paired tumors evaluated by WES, the Jaccard coefficient was calculated as the proportion of shared variants over the total number of variants. The Jaccard coefficient of each pair was compared using Student's *t*-test. All statistical analyses were performed using SPSS software (version 25.0; IBM Statistics, Armonk, NY), and statistical significance was defined as p values less than 0.05.

Results

Clinicopathological characteristics of patients with synchronous colorectal carcinoma

Of the 100 enrolled patients, male patients (72.0%) were more common than female patients, and the mean age was 62.2 years (range, 22–89 years, Table 1). Forty-seven patients had synchronous tumors on the same side of the colon, or rectum. Pathologic T3 stage was the most common, and 46.0% of patients had regional lymph node metastasis. The initial staging based on the index tumor was the following: stage I in 10.0% of patients, stage II in 42.0% of patients, stage III in 32.0% of patients, and stage IV in 16.0% of patients. The mean follow-up period was 59 months.

Nineteen out of 200 tumors in 100 SCRC patients were MSI-H tumors (Table 2). MSI-H tumors were more frequently located in the right colon (68.4% vs 33.7%, $p = 0.011$) and had a higher proportion of mucinous adenocarcinoma (15.8% vs 0%, $p < 0.001$) than MSS tumors. MSI-H tumors exhibited significantly higher densities of TILs than MSS tumors ($p < 0.001$).

Table 3 shows a comparison of the index lesions and concurrent lesions in the patients with SCRC. The index lesions showed larger size (5.2 ± 2.3 cm vs 2.9 ± 1.7 cm, $p < 0.001$), deeper depth of invasion ($p < 0.001$) and poorer differentiation ($p = 0.003$) than the concurrent lesions. LVI (44.0% vs 26.0%, $p = 0.008$) and PNI (24.0% vs 13.0%, $p = 0.045$) were more frequent in the index lesions. There were no significant differences between the index lesions and the concurrent lesions in location, MSI status, and TILs. The distance between the index lesion and the concurrent lesion ranged from 0.7 to 85 cm (Fig. 2). In 52 patients, concurrent tumors were located in the more distal colon or rectum than the index tumor.

Table 1. Demographic, clinical, and pathological characteristics

Characteristics	Value
Sex	
Male	72 (72.0)
Female	28 (28.0)
Age, years	62.2 ± 13.3
Location	
Both right colon	21 (21.0)
Both left colon	23 (23.0)
Different colon	25 (25.0)
Colon & rectum	28 (28.0)
Both rectum	3 (3.0)
pT category	
T1	3 (3.0)
T2	19 (19.0)
T3	68 (68.0)
T4	10 (10.0)
pN category	
N0	54 (54.0)
N1	32 (32.0)
N2	14 (14.0)
cM/pM category	
M0	84 (84.0)
M1	16 (16.0)
AJCC Stage	
I	10 (10.0)
II	42 (42.0)
III	32 (32.0)
IV	16 (16.0)

Values are presented as number (%) or mean ± standard deviation unless otherwise indicated.

AJCC Stage, clinical or pathologic staging according to the American Joint Committee on Cancer (8^{th} ed)

Table 2. Comparison of clinicopathologic features of tumors according to MSI status

Characteristics	MSS tumor (n = 181)	MSI-H tumor (n = 19)	*p*-value
Location			0.011
Right colon	61 (33.7)	13 (68.4)	
Left colon	88 (48.6)	4 (21.1)	
Rectum	32 (17.7)	2 (10.5)	
Tumor size, cm	4.0 ± 2.3	4.5 ± 2.5	0.359
Depth of invasion			0.498
Tis	2 (1.1)	0 (0)	
T1	34 (18.8)	1 (5.3)	
T2	37 (20.4)	6 (31.6)	
T3	96 (53.0)	10 (52.6)	
T4	12 (6.6)	2 (10.5)	
Histologic type			< 0.001
Adenocarcinoma	180 (99.4)	16 (84.2)	
Mucinous	0 (0)	3 (15.8)	
Signet ring cell	1 (0.6)	0 (0)	
Differentiation[a]			0.074
Well	40 (22.2)	2 (12.5)	
Moderate	131 (72.8)	11 (68.8)	
Poor	9 (5.0)	3 (18.8)	
LVI			0.806
Absent	117 (64.6)	13 (68.4)	
Present	64 (35.4)	6 (31.6)	
PNI			0.749
Absent	147 (81.2)	16 (84.2)	
Present	34 (18.8)	3 (15.8)	
Tumor budding			0.078
Low	128 (70.7)	18 (94.7)	
Intermediate	41 (22.7)	1 (5.3)	
High	12 (6.6)	0 (0)	
TILs			< 0.001
Weak (0–10%)	154 (85.1)	8 (42.1)	
Moderate (20–40%)	24 (13.3)	4 (21.1)	
Strong (50–90%)	3 (1.7)	7 (36.8)	

[a] Only adenocarcinomas were included, except for mucinous and signet ring cell carcinomas.

Table 3. Comparison of clinicopathologic features between the index lesions and concurrent lesions

Characteristics	Index lesion (n = 100)	Concurrent lesion (n = 100)	*p*-value
Location			0.952
Right colon	38 (38.0)	36 (36.0)	
Left colon	45 (45.0)	47 (47.0)	
Rectum	17 (17.0)	17 (17.0)	
Tumor size, cm	5.2 ± 2.3	2.9 ± 1.7	< 0.001
Depth of invasion			< 0.001
Tis	0 (0)	2 (2.0)	
T1	3 (3.0)	32 (32.0)	
T2	19 (19.0)	24 (24.0)	
T3	68 (68.0)	38 (38.0)	
T4	10 (10.0)	4 (4.0)	
Histologic type			0.508
Adenocarcinoma	97 (97.0)	99 (99.0)	
Mucinous	2 (2.0)	1 (1.0)	
Signet ring cell	1 (1.0)	0 (0)	
Differentiation			0.003
Well	11 (11.3)	31 (31.3)	
Moderate	80 (82.5)	62 (62.6)	
Poor	6 (6.2)	6 (6.1)	
LVI			0.008
Absent	56 (56.0)	74 (74.0)	
Present	44 (44.0)	26 (26.0)	
PNI			0.045
Absent	76 (76.0)	87 (87.0)	
Present	24 (24.0)	13 (13.0)	
Tumor budding			0.237
Low	68 (68.0)	78 (78.0)	
Intermediate	24 (24.0)	18 (18.0)	
High	8 (8.0)	4 (4.0)	
MSI status			0.809
MSS	90 (90.0)	91 (91.0)	
MSI-H	10 (10.0)	9 (9.0)	
TILs			0.726
Weak (0–10%)	83 (83.0)	79 (79.0)	
Moderate (20–40%)	13 (13.0)	15 (15.0)	
Strong (50–90%)	4 (4.0)	6 (6.0)	

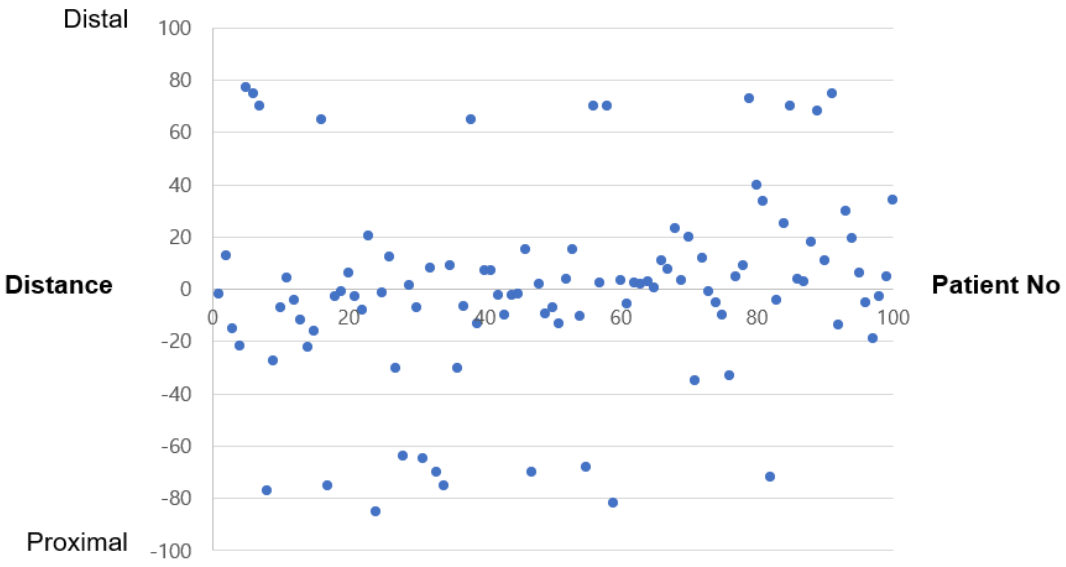


Fig. 2. Relationship between the locations of synchronous tumors in each patient. The horizontal axis represents each patient, and the vertical axis represents the distance between two lesions. Positive values indicate that the concurrent tumor is more distal than the index tumor, whereas negative values indicate that the concurrent tumor is more proximal than the index tumor.

Relationship between tumor location and clinicopathologic characteristics

To evaluate the relationship between tumor location and clinicopathologic characteristics, patients were divided into three subgroups: Both right, all tumors are located in the right colon; Both left, all tumors are located in the left colon or rectum; and Right/Left, each tumor is located in the right and left sides, respectively (Table 4). The Both right group showed a significantly higher proportion of MSI-H tumors (21.4% vs 3.2%, $p = 0.001$) and moderate or strong densities of TILs (35.7% vs 13.0%, $p = 0.005$) than the Both left group. The Both left group showed a significantly higher grade of tumor budding compared to the Both right (Both left vs Both right 36.5% vs 19.0%, $p = 0.026$) and Right/Left groups (Both left vs Right/Left 36.5% vs 18.4%, $p = 0.001$).

Table 5 compares 47 patients who had synchronous tumors on the same side of the colon, or rectum, with 53 patients who had tumors on different sites. There were no significant differences in clinicopathologic characteristics between the two groups.

Table 4. Clinicopathologic characteristics according to tumor location in SCRC

Characteristics	Both right (n= 21 patients, 42 tumors)	Both left (n = 47 patients, 94 tumors)	*p*-value	Both right (n = 21 patients, 42 tumors)	Right/Left (n = 32 patients, 64 tumors)		Right/Left (n = 32 patients, 64 tumors)	Both left (n = 47 patients, 94 tumors)	*p*-value
Sex			0.117			0.978			0.064
Male	17 (81.0)	29 (61.7)		17 (81.0)	26 (81.3)		26 (81.3)	29 (61.7)	
Female	4 (19.0)	18 (38.3)		4 (19.0)	6 (18.8)		6 (18.8)	18 (38.3)	
Age (yr)	59.4 ± 15.0	63.0 ± 10.2	0.254	59.4 ± 15.0	62.7 ± 15.9	0.455	62.7 ± 15.9	63.0 ± 10.2	0.918
pT category			0.227			0.577			0.474
T1	0 (0)	0 (0)		0 (0)	1 (3.1)		1 (3.1)	0 (0)	
T2	5 (23.8)	4 (8.5)		5 (23.8)	4 (12.5)		4 (12.5)	4 (8.5)	
T3	14 (66.7)	38 (80.9)		14 (66.7)	22 (68.8)		22 (68.8)	38 (80.9)	
T4	2 (9.5)	5 (10.6)		2 (9.5)	5 (15.6)		5 (15.6)	5 (10.6)	
pN category			0.597			0.561			0.612
N0	12 (57.1)	23 (48.9)		12 (57.1)	19 (59.4)		19 (59.4)	23 (48.9)	
N1	5 (23.8)	17 (36.2)		5 (23.8)	10 (31.3)		10 (31.3)	17 (36.2)	
N2	4 (19.0)	7 (14.9)		4 (19.0)	3 (9.4)		3 (9.4)	7 (14.9)	
cM/pM category			0.546			0.241			0.425
M0	19 (90.5)	40 (85.1)		19 (90.5)	25 (78.1)		25 (78.1)	40 (85.1)	
M1	2 (9.5)	7 (14.9)		2 (9.5)	7 (21.9)		7 (21.9)	7 (14.9)	
Histologic type			0.689			0.369			0.319
Adenocarcinoma	42 (100.0)	92 (98.9)		42 (100.0)	62 (95.4)		62 (95.4)	92 (98.9)	
Mucinous	0 (0)	1 (1.1)		0 (0)	2 (3.1)		2 (3.1)	1 (1.1)	
Signet ring cell	0 (0)	0 (0)		0 (0)	1 (1.5)		1 (1.5)	0 (0)	
Differentiation			0.122			0.087			0.188

Well	13 (31.0)	15 (16.3)		13 (31.0)	14 (22.6)		14 (22.6)	15 (16.3)	
Moderate	25 (59.5)	70 (76.1)		25 (59.5)	47 (75.8)		47 (75.8)	70 (76.1)	
Poor	4 (9.5)	7 (7.6)		4 (9.5)	1 (1.6)		1 (1.6)	7 (7.6)	
LVI			0.21			0.683			0.337
Absent	30 (71.4)	56 (60.2)		30 (71.4)	44 (67.7)		44 (67.7)	56 (60.2)	
Present	12 (28.6)	37 (39.8)		12 (28.6)	21 (32.3)		21 (32.3)	37 (39.8)	
PNI			0.784			0.197			0.186
Absent	36 (85.7)	78 (83.9)		36 (85.7)	49 (75.4)		49 (75.4)	78 (83.9)	
Present	6 (14.3)	15 (16.1)		6 (14.3)	16 (24.6)		16 (24.6)	15 (16.1)	
Tumor budding			0.026			0.856			0.001
Low	34 (81.0)	59 (63.4)		34 (81.0)	53 (81.5)		53 (81.5)	59 (63.4)	
Intermediate	5 (11.9)	31 (33.3)		5 (11.9)	6 (9.2)		6 (9.2)	31 (33.3)	
High	3 (7.1)	3 (3.2)		3 (7.1)	6 (9.2)		6 (9.2)	3 (3.2)	
MSI status			0.001			0.131			0.055
MSS	33 (78.6)	90 (96.8)		33 (78.6)	58 (89.2)		58 (89.2)	90 (96.8)	
MSI-H	9 (21.4)	3 (3.2)		9 (21.4)	7 (10.8)		7 (10.8)	3 (3.2)	
TILs			0.005			0.082			0.641
Weak (0–10%)	27 (64.3)	81 (87.1)		27 (64.3)	54 (83.1)		54 (83.1)	81 (87.1)	
Moderate (20–40%)	10 (23.8)	10 (10.8)		10 (23.8)	8 (12.3)		8 (12.3)	10 (10.8)	
Strong (50–90%)	5 (11.9)	2 (2.2)		5 (11.9)	3 (4.6)		3 (4.6)	2 (2.2)	

Table 5. Comparison of clinicopathologic characteristics between patients with synchronous tumors located at the same site and at different sites

Characteristics	Same location (n = 47 patients, 94 tumors)	Different location (n = 53 patients, 106 tumors)	*p*-value
Sex			0.173
Male	72 (76.6)	72 (67.9)	
Female	22 (23.4)	34 (32.1)	
Age (yr)	60.9 ± 12.4	63.3 ± 13.9	0.207
pT category			0.409
T1	0 (0)	1 (1.9)	
T2	8 (17.0)	5 (9.4)	
T3	35 (74.5)	39 (73.6)	
T4	4 (8.5)	8 (15.1)	
pN category			0.907
N0	73 (77.7)	81 (76.4)	
N1	14 (14.9)	18 (17.0)	
N2	7 (7.4)	7 (6.6)	
cM/pM category			0.188
M0	89 (94.7)	95 (89.6)	
M1	5 (5.3)	11 (10.4)	
Histologic type			0.569
Adenocarcinoma	93 (98.9)	103 (97.2)	
Mucinous	1 (1.1)	2 (1.9)	
Signet ring cell	0 (0)	1 (0.9)	
Differentiation			0.917
Well	20 (21.5)	22 (21.4)	
Moderate	68 (73.1)	74 (71.8)	
Poor	5 (5.4)	7 (6.8)	
LVI			0.976
Absent	61 (64.9)	69 (65.1)	
Present	33 (35.1)	37 (34.9)	
PNI			0.216
Absent	80 (85.1)	83 (78.3)	
Present	14 (14.9)	23 (21.7)	
Tumor budding			0.698
Low	66 (70.2)	80 (75.5)	
Intermediate	22 (23.4)	20 (18.9)	
High	6 (6.4)	6 (5.7)	
MSI status			0.605
MSS	84 (89.4)	97 (91.5)	

MSI-H	10 (10.6)	9 (8.5)	
TILs			0.323
Weak (0–10%)	72 (76.6)	90 (84.9)	
Moderate (20–40%)	16 (17.0)	12 (11.3)	
Strong (50–90%)	6 (6.4)	4 (3.8)	

Comparison of clinicopathologic characteristics and oncologic outcomes in the groups divided according to TILs

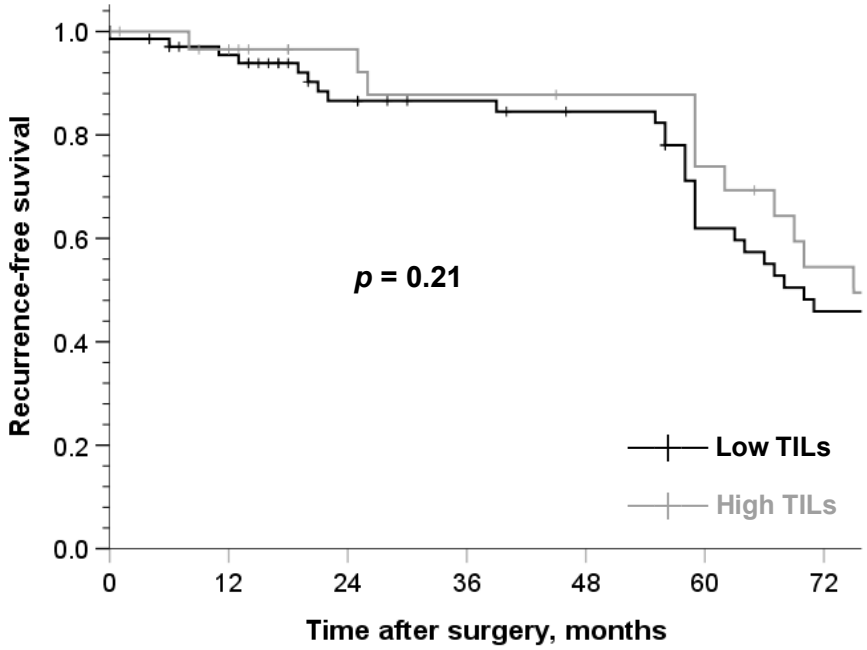
Of the 200 tumors in 100 patients with SCRC, 38 tumors exhibited moderate or strong densities of TILs (Table 6). Those tumors were more prevalent in the right colon (52.6% vs 33.3%, $p = 0.029$) and had a higher proportion of mucinous adenocarcinoma (7.9% vs 0%, $p = 0.001$) and poorly differentiated adenocarcinoma (17.2% vs 3.7%, $p = 0.006$) than tumors with weak densities of TILs. The proportion of MSI-H tumors was significantly higher in tumors with moderate or strong densities of TILs compared to tumors with weak densities of TILs (28.9% vs 4.9%, $p < 0.001$)

To investigate the impact of TILs on recurrence and survival, patients were divided into two groups. Seventy patients who had only tumors with low density of TILs were assigned to Low TILs group, while thirty patients who had at least one tumor with a moderate or strong density of TILs were assigned to the High TILs group. The median RFS of Low TILs group and High TILs group were 70 months and 75 months, respectively. The 5-year RFS did not differ between the two groups (Low TILs 58.8% vs High TILs 69.3%, $p = 0.21$, Fig. 3A). There were no significant differences in the median OS (Low TILs 76 months vs High TILs 86 months) and 5-year OS (Low TILs 62.4% vs High TILs 75.2%, $p = 0.367$, Fig. 3B) between the two groups.

Table 6. Comparison of clinicopathologic features of tumors according to TILs

Characteristics	Week (n = 162)	Moderate or strong (n = 38)	*p*-value
Location			0.029
Right colon	54 (33.3)	20 (52.6)	
Left colon	76 (46.9)	16 (42.1)	
Rectum	32 (19.8)	2 (5.3)	
Tumor size	4.1 ± 2.2	3.9 ± 2.6	0.558
Depth of invasion			0.082
Tis	1 (0.6)	1 (2.6)	
T1	29 (17.9)	6 (15.8)	
T2	29 (17.9)	14 (36.8)	
T3	91 (56.2)	15 (39.5)	
T4	12 (7.4)	2 (5.3)	
Histologic type			0.001
Adenocarcinoma	161 (99.4)	35 (92.1)	
Mucinous	0 (0)	3 (7.9)	
Signet ring cell	1 (0.6)	0 (0)	
Differentiation			0.006
Well	33 (20.5)	9 (25.7)	
Moderate	122 (75.8)	20 (57.1)	
Poor	6 (3.7)	6 (17.2)	
LVI			0.385
Absent	103 (63.6)	27 (71.1)	
Present	59 (36.4)	11 (28.9)	
PNI			0.989
Absent	132 (81.5)	31 (81.6)	
Present	30 (18.5)	7 (18.4)	
Tumor budding			0.201
Low	115 (71.0)	31 (81.6)	
Intermediate	38 (23.5)	4 (10.5)	
High	9 (5.6)	3 (7.9)	
MSI status			< 0.001
MSS	154 (95.1)	27 (71.1)	
MSI-H	8 (4.9)	11 (28.9)	

(A)



(B)

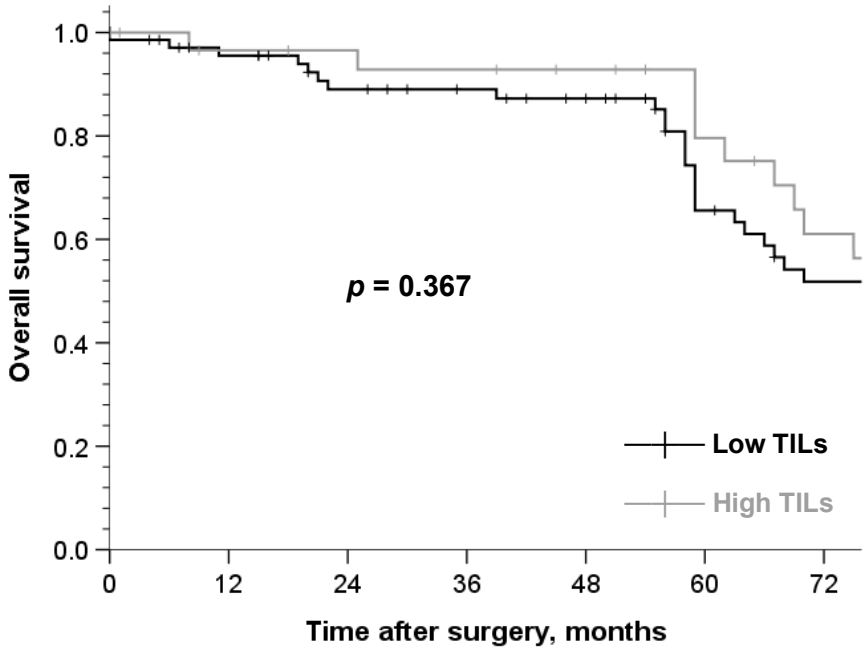


Fig. 3. Survival analysis of subgroups divided according to TILs. (A) Recurrence-free survival (B) Overall survival

Comparison of clinicopathologic characteristics and oncologic outcomes in the groups divided according to MSI status

Among the 100 enrolled patients, 12 had at least one MSI-H tumor, and 5 showed discordant MSI status. Patients were divided into three groups according to the MSI status of synchronous tumors: All MSS group, All MSI-H group, and MSI-H/MSS group. Five patients were diagnosed with HNPCC. Three of these patients had both MSI-H tumors and two patients had a discordant MSI status (Fig. 4).

All seven patients in the All MSI-H group were male, and the All MSI-H group was significantly younger than the All MSS group (49.3 ± 9.0 years vs 63.3 ± 12.8 years, p = 0.006. Table 7). In the All MSI-H group, four patients (57.1%) had synchronous tumors in the right colon. On the other hand, the MSI-H/MSS group showed a similar tumor location pattern to the All MSS group. Sixteen patients (18.2%) in the All MSS group had stage IV disease, whereas there was no patient with stage IV disease in the All MSI-H and MSI-H/MSS groups. The All MSI-H group had a higher proportion of mucinous adenocarcinoma (14.3% vs 0%, $p < 0.001$) and a stronger density of TILs (67.2% vs 14.7%, $p < 0.001$) than the All MSS group. Similarly, the proportion of mucinous adenocarcinoma (10.0% vs 0%, $p < 0.001$) and moderate or strong density of TILs (40.0% vs 14.7%, $p < 0.001$) were significantly higher in the MSI-H/MSS group compared to the MSS group.

The median RFS of three groups was the following: 58 months in the All MSS group, 68 months in the All MSI-H group, and 55 months in the MSI-H/MSS group (Fig. 5A). The median OS for the three groups was 59 months in the All MSS group, 68 months in the All MSI-H group, and 55 months in the MSI-H/MSS group, with no significant between-group differences (Fig. 5B). Since only the All MSS group included stage IV CRC patients, the survival analysis excluding stage IV patients is shown in Fig. 5C and 5D. RFS and OS did not differ significantly between any two MSI groups, even after excluding stage IV patients.

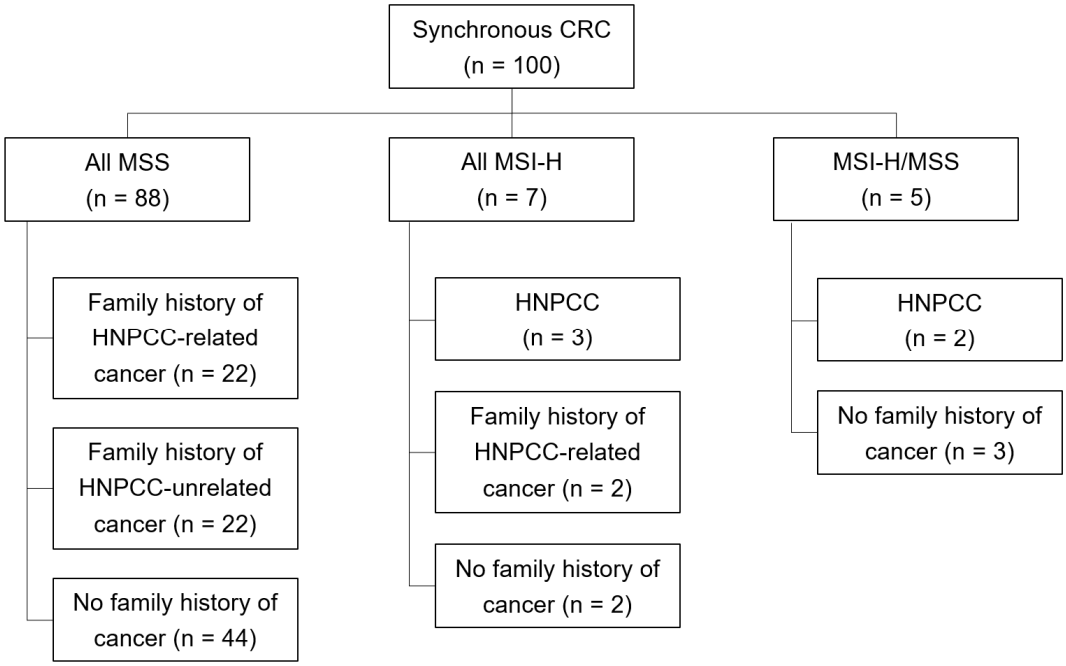


Fig. 4. Associated family history of 100 patients with synchronous colorectal cancer

Table 7. Comparison of clinicopathologic characteristics between the groups divided according to MSI status

Characteristics	All MSI-H (n = 7 patients, 14 tumors)	All MSS (n = 88 patients, 176 tumors)	*p*-value	MSI-H/MSS (n = 5 patients, 10 tumors)	All MSS (n = 88 patients, 176 tumors)	*p*-value	MSI-H/MSS (n = 5 patients, 10 tumors)	All MSI-H (n = 7 patients, 14 tumors)	*p*-value
Sex			0.083			0.613			0.417
Male	7 (100.0)	61 (69.3)		4 (80.0)	61 (69.3)		4 (80.0)	7 (100.0)	
Female	0 (0)	27 (30.7)		1 (20.0)	27 (30.7)		1 (20.0)	0 (0)	
Age (yr)	49.3 ± 9.0	63.3 ± 12.8	0.006	59.6 ± 20.0	63.3 ± 12.8	0.536	59.6 ± 20.0	49.3 ± 9.0	0.216
Location type			0.127			0.974			0.359
Both right colon	4 (57.1)	16 (18.2)		1 (20.0)	16 (18.2)		1 (20.0)	4 (57.1)	
Both left colon	0 (0)	22 (25.0)		1 (20.0)	22 (25.0)		1 (20.0)	0 (0)	
Different colon	2 (28.6)	22 (25.0)		1 (20.0)	22 (25.0)		1 (20.0)	2 (28.6)	
Colon & rectum	1 (14.3)	25 (28.4)		2 (40.0)	25 (28.4)		2 (40.0)	1 (14.3)	
Both rectum	0 (0)	3 (3.4)		0 (0)	3 (3.4)		0 (0)	0 (0)	
pT category			0.72			0.215			0.523
T1	0 (0)	1 (1.1)		0 (0)	1 (1.1)		0 (0)	0 (0)	
T2	0 (0)	13 (14.8)		0 (0)	13 (14.8)		0 (0)	0 (0)	
T3	6 (85.7)	65 (73.9)		3 (60.0)	65 (73.9)		3 (60.0)	6 (85.7)	
T4	1 (14.3)	9 (10.2)		2 (40.0)	9 (10.2)		2 (40.0)	1 (14.3)	
pN category			0.966			0.436			0.598
N0	4 (57.1)	46 (52.3)		4 (80.0)	46 (52.3)		4 (80.0)	4 (57.1)	
N1	2 (28.6)	29 (33.0)		1 (20.0)	29 (33.0)		1 (20.0)	2 (28.6)	
N2	1 (14.3)	13 (14.8)		0 (0)	13 (14.8)		0 (0)	1 (14.3)	
cM/pM category			0.216			0.583			1
M0	7 (100.0)	72 (81.8)		5 (100.0)	72 (81.8)		5 (100.0)	7 (100.0)	

M1	0 (0)	16 (18.2)		0 (0)	16 (18.2)		0 (0)	0 (0)	
AJCC Stage			0.413			0.299			0.576
I	0 (0)	10 (11.4)		0 (0)	10 (11.4)		0 (0)	0 (0)	
II	4 (57.1)	34 (38.6)		4 (80.0)	34 (38.6)		4 (80.0)	4 (57.1)	
III	3 (42.9)	28 (31.8)		1 (20.0)	28 (31.8)		1 (20.0)	3 (42.9)	
IV	0 (0)	16 (18.2)		0 (0)	16 (18.2)		0 (0)	0 (0)	
Tumor location			0.018			0.477			0.504
Right colon	10 (71.4)	59 (33.5)		5 (50.0)	59 (33.5)		5 (50.0)	10 (71.4)	
Left colon	3 (21.4)	86 (48.9)		3 (30.0)	86 (48.9)		3 (30.0)	3 (21.4)	
Rectum	1 (7.1)	31 (17.6)		2 (20.0)	31 (17.6)		2 (20.0)	1 (7.1)	
Depth of invasion			0.676			0.357			0.234
Tis	0 (0)	2 (1.1)		0 (0)	2 (1.1)		0 (0)	0 (0)	
T1	1 (7.1)	31 (17.6)		3 (30.0)	31 (17.6)		3 (30.0)	1 (7.1)	
T2	5 (35.7)	37 (21.0)		1 (10.0)	37 (21.0)		1 (10.0)	5 (35.7)	
T3	7 (50.0)	95 (54.0)		4 (40.0)	95 (54.0)		4 (40.0)	7 (50.0)	
T4	1 (7.1)	11 (6.3)		2 (20.0)	11 (6.3)		2 (20.0)	1 (7.1)	
Histologic type			< 0.001			< 0.001			0.47
Adenocarcinoma	12 (95.7)	176 (100.0)		8 (80.0)	176 (100.0)		8 (80.0)	12 (85.7)	
Mucinous	2 (14.3)	0 (0)		1 (10.0)	0 (0)		1 (10.0)	2 (14.3)	
Signet ring cell	0 (0)	0 (0)		1 (10.0)	0 (0)		1 (10.0)	0 (0)	
Differentiation			0.024			0.617			0.267
Well	2 (16.7)	39 (22.2)		1 (12.5)	39 (22.2)		1 (12.5)	2 (16.7)	
Moderate	7 (58.3)	128 (72.7)		7 (87.5)	128 (72.7)		7 (87.5)	7 (58.3)	
Poor	3 (25.0)	9 (5.1)		0 (0)	9 (5.1)		0 (0)	3 (25.0)	
LVI			0.971			0.736			0.77
Absent	9 (64.3)	114 (64.8)		7 (70.0)	114 (64.8)		7 (70.0)	9 (64.3)	

Present	5 (35.7)	62 (35.2)		3 (30.0)	62 (35.2)		3 (30.0)	5 (35.7)	
PNI			0.848			0.124			0.239
Absent	11 (78.6)	142 (80.7)		10 (100.0)	142 (80.7)		10 (100.0)	11 (78.6)	
Present	3 (21.4)	34 (19.3)		0 (0)	34 (19.3)		0 (0)	3 (21.4)	
Tumor budding			0.19			0.39			0.803
Low	13 (92.9)	124 (70.5)		9 (90.0)	124 (70.5)		9 (90.0)	13 (92.9)	
Intermediate	1 (7.1)	40 (22.7)		1 (10.0)	40 (22.7)		1 (10.0)	1 (7.1)	
High	0 (0)	12 (6.8)		0 (0)	12 (6.8)		0 (0)	0 (0)	
TILs			< 0.001			< 0.001			0.504
Weak (0–10%)	6 (42.9)	150 (85.2)		6 (60.0)	150 (85.2)		6 (60.0)	6 (42.9)	
Moderate (20–40%)	2 (14.3)	24 (13.6)		2 (20.0)	24 (13.6)		2 (20.0)	2 (14.3)	
Strong (50–90%)	6 (42.9)	2 (1.1)		2 (20.0)	2 (1.1)		2 (20.0)	6 (42.9)	

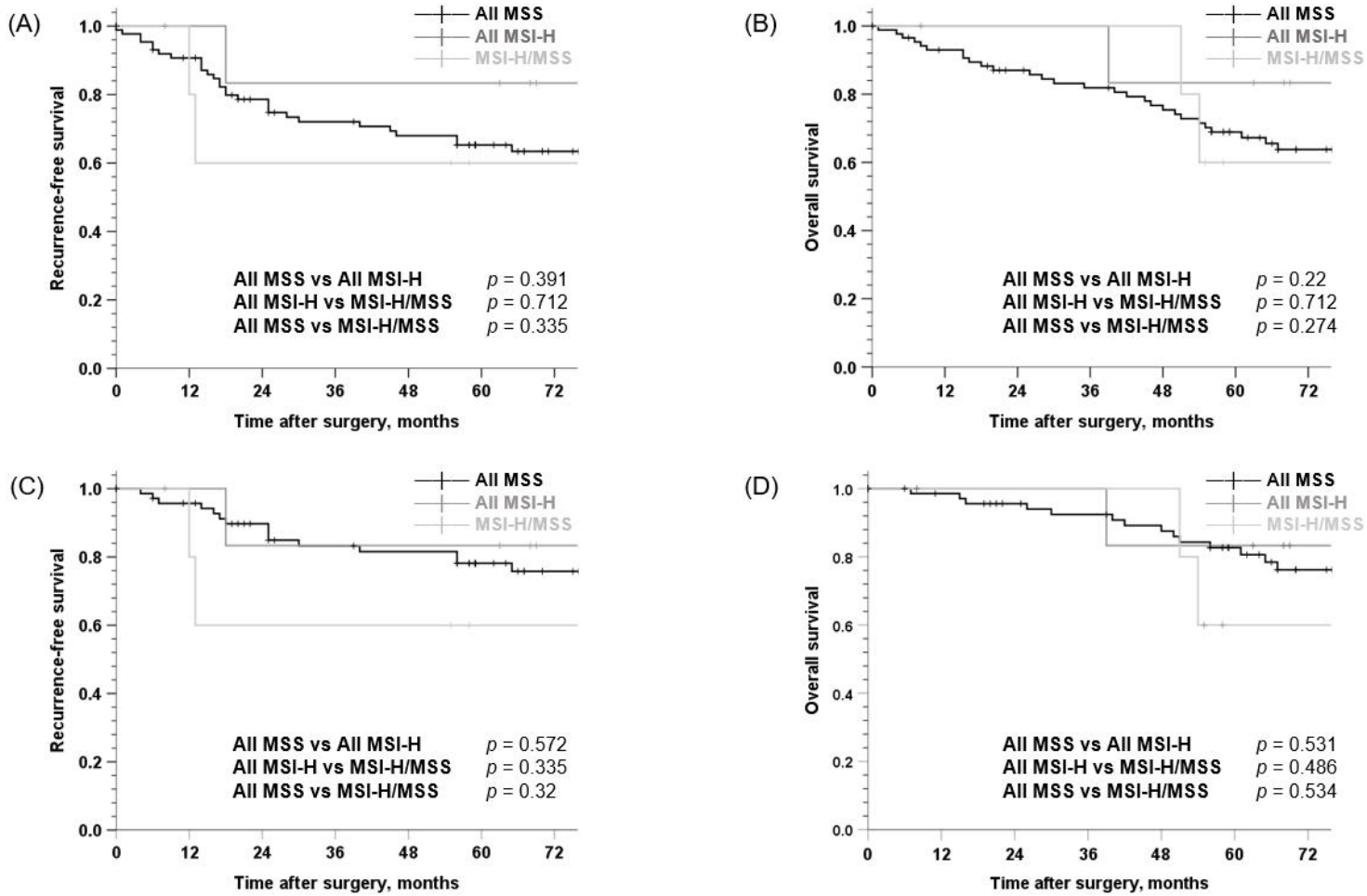


Fig. 5. Survival analysis in the All MSS, All MSI-H, and MSI-H/MSS groups. (A) Recurrence-free survival and (B) Overall survival in all patients. (C) Recurrence-free survival and (D) Overall survival in stage I-III patients

Mutation analysis of synchronous tumors using WES

Characteristics of 18 tumors analyzed with WES were shown in Table 8. Five patients had only MSI-H tumors, and four patients had both MSI-H and MSS tumors. Three of nine patients were diagnosed with HNPCC in accordance with Amsterdam II criteria.

The median number of total mutations in each tumor identified by WES was 5850 (range, 3365–14070). Fig. 6 shows the numbers of different mutation types in each tumor. To evaluate the heterogeneity of mutations between the two tumors of the same patient, variants simultaneously found in both tumors were identified. T02-1 and T02-2 shared 6.5% variants (Fig. 7). Except for T02, the other synchronous tumors in the same patient shared only a few variants, and the proportion of shared mutated genes over the total number of mutated genes was lower than 1% (range, 0.09–0.36%, Fig. 8). Nevertheless, synchronous lesions from the same patient shared a significantly greater proportion of shared variants than pairs of tumors from different patients (0.89 ± 2.1 % vs 0.13 ± 0.04 %, $p < 0.001$). This statistical significance remained unchanged after excluding the T02 case, which shared a notably high proportion of variants. (0.19 ± 0.08 % vs 0.13 ± 0.04 %, $p < 0.001$)

We mapped the driver genes reported in the literature or known to be associated with oncogenic transformation from the cancer gene census list in the Catalogue Of Somatic Mutations In Cancer (COSMIC) (Fig. 9). A mutation of APC was identified in samples from 8 patients except T09. However, 5 of these patients had different mutation types, and all 8 patients had different mutation sites between synchronous tumors, even if the synchronous tumors shared the *APC* mutation. The concordance rates for *BRAF*, *KRAS*, *PIK3CA*, and *NRAS* were 66.7%, 66.7%, 55.6%, and 66.7%, respectively. However, only T08-1 and T08-2 shared the same mutation site, *KRAS G12D*, while the other synchronous tumors had different mutation subtypes even if they shared the same mutated genes.

Three out of nine patients (T02, T05, and T09) exhibited more than 20% differences in tumor mutation burden between synchronous tumor samples. Table 9 compared the characteristics of three patients with synchronous tumors with different mutation burdens (labeled as Different group) to those of the remaining six patients (labeled as Similar group). The Different group tended to be associated with a shorter distance between tumors and a higher proportion of moderate or strong density of TILs than the Similar group. Three HNPCC patients were included in the Similar group, and the Similar group was younger than the Different group. However, all the differences were not statistically significant.

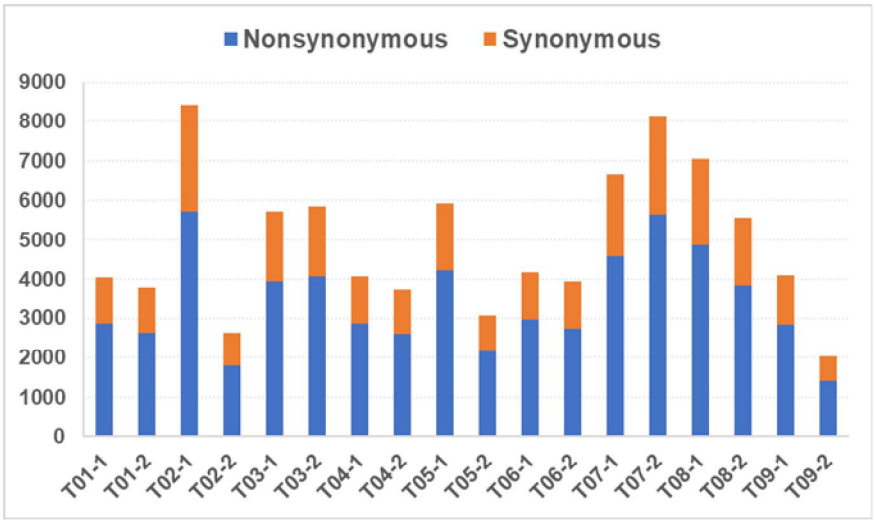
Table 8. Characteristics of tumors analyzed with whole exome sequencing

Tumor sample	MSI	Location	Distance[a]	Histology type & Differentiation	TILs	Associated history
T01-1	MSI-H	Rectum		PD	Weak	Family history of CRC
T01-2	MSI-H	Sigmoid colon	- 15 cm	MD	Weak	
T02-1	MSI-H	Transverse colon		MD	Moderate	Past history of CRC and
T02-2	MSI-H	Transverse colon	- 1 cm	MD	Weak	duodenal cancer
T03-1	MSI-H	Hepatic flexure		PD	Strong	HNPCC
T03-2	MSI-H	Transverse colon	20 cm	WD	Strong	
T04-1	MSI-H	Cecum		MD	Weak	HNPCC
T04-2	MSI-H	Hepatic flexure	12 cm	WD	Weak	
T05-1	MSI-H	Transverse colon		MUC	Strong	Past history of CRC
T05-2	MSI-H	Splenic flexure	- 5.3 cm	MUC	Moderate	
T06-1	MSI-H	Rectum		MD	Weak	Family history of other
T06-2	MSS	Ascending colon	- 77 cm	MD	Weak	cancer
T07-1	MSI-H	Rectum		MUC	Moderate	HNPCC
T07-2	MSS	Splenic flexure	- 27.2 cm	WD	Strong	
T08-1	MSI-H	Transverse colon		MD	Weak	No
T08-2	MSS	Sigmoid colon	33.5 cm	MD	Weak	
T09-1	MSS	Hepatic flexure		MD	Weak	No
T09-2	MSI-H	Ascending colon	- 3 cm	MD	Strong	

WD, well differentiated; MD, moderately differentiated; PD, poorly differentitated; MUC, mucinous adenocarcinoma

[a] Positive values indicate that the concurrent tumor is more distal than the index tumor, whereas negative values indicate that the concurrent tumor is more proximal than the index tumor.

(A)



(B)

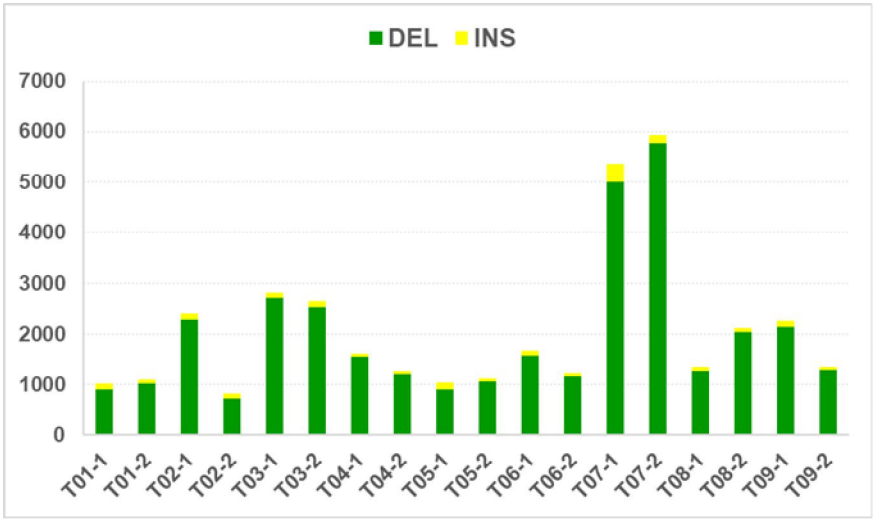


Fig. 6. Mutations identified by whole exome sequencing in 9 patients with 18 synchronous tumors. (A) Single nucleotide variants (B) Insertion or deletion of bases in the genome

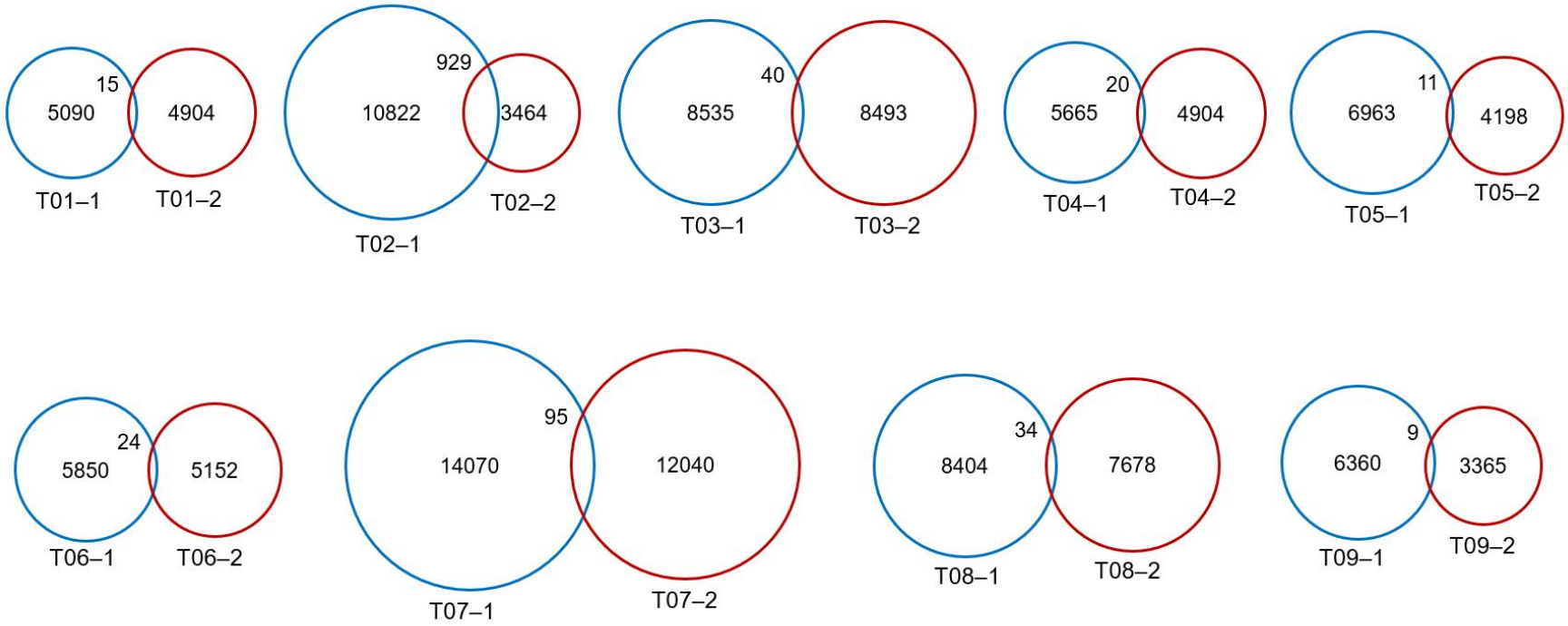
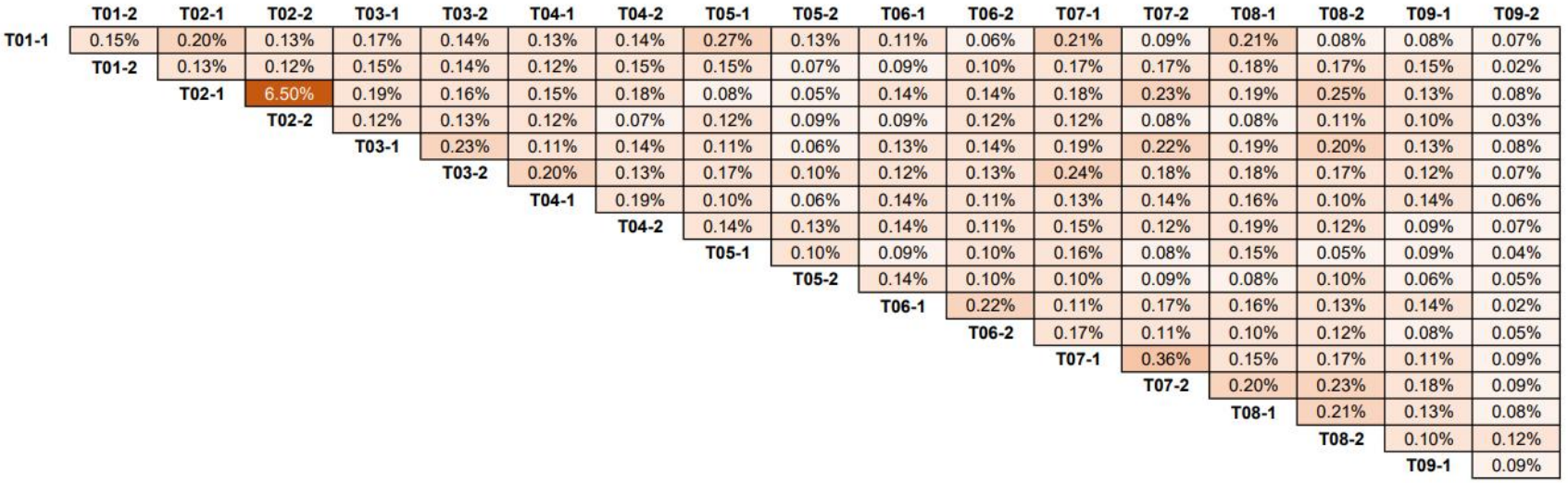


Fig. 7. The Venn plots of distribution of mutations in patients with SCRC. The number of mutations identified in the index tumor (blue circle), that in the concurrent tumor (red circle), and the number of mutations found in both tumors simultaneously are marked.



	T01-2	T02-1	T02-2	T03-1	T03-2	T04-1	T04-2	T05-1	T05-2	T06-1	T06-2	T07-1	T07-2	T08-1	T08-2	T09-1	T09-2
T01-1	0.15%	0.20%	0.13%	0.17%	0.14%	0.13%	0.14%	0.27%	0.13%	0.11%	0.06%	0.21%	0.09%	0.21%	0.08%	0.08%	0.07%
T01-2		0.13%	0.12%	0.15%	0.14%	0.12%	0.15%	0.15%	0.07%	0.09%	0.10%	0.17%	0.17%	0.18%	0.17%	0.15%	0.02%
T02-1			6.50%	0.19%	0.16%	0.15%	0.18%	0.08%	0.05%	0.14%	0.14%	0.18%	0.23%	0.19%	0.25%	0.13%	0.08%
T02-2				0.12%	0.13%	0.12%	0.07%	0.12%	0.09%	0.09%	0.12%	0.12%	0.08%	0.08%	0.11%	0.10%	0.03%
T03-1					0.23%	0.11%	0.14%	0.11%	0.06%	0.13%	0.14%	0.19%	0.22%	0.19%	0.20%	0.13%	0.08%
T03-2						0.20%	0.13%	0.17%	0.10%	0.12%	0.13%	0.24%	0.18%	0.18%	0.17%	0.12%	0.07%
T04-1							0.19%	0.10%	0.06%	0.14%	0.11%	0.13%	0.14%	0.16%	0.10%	0.14%	0.06%
T04-2								0.14%	0.13%	0.14%	0.11%	0.15%	0.12%	0.19%	0.12%	0.09%	0.07%
T05-1									0.10%	0.09%	0.10%	0.16%	0.08%	0.15%	0.05%	0.09%	0.04%
T05-2										0.14%	0.10%	0.10%	0.09%	0.08%	0.10%	0.06%	0.05%
T06-1											0.22%	0.11%	0.17%	0.16%	0.13%	0.14%	0.02%
T06-2												0.17%	0.11%	0.10%	0.12%	0.08%	0.05%
T07-1													0.36%	0.15%	0.17%	0.11%	0.09%
T07-2														0.20%	0.23%	0.18%	0.09%
T08-1															0.21%	0.13%	0.08%
T08-2																0.10%	0.12%
T09-1																	0.09%

Fig. 8. Similarity matrix of all mutated genes across the 18 synchronous tumors. The Jaccard coefficient was calculated for each combination of tumors from the same or different patients.

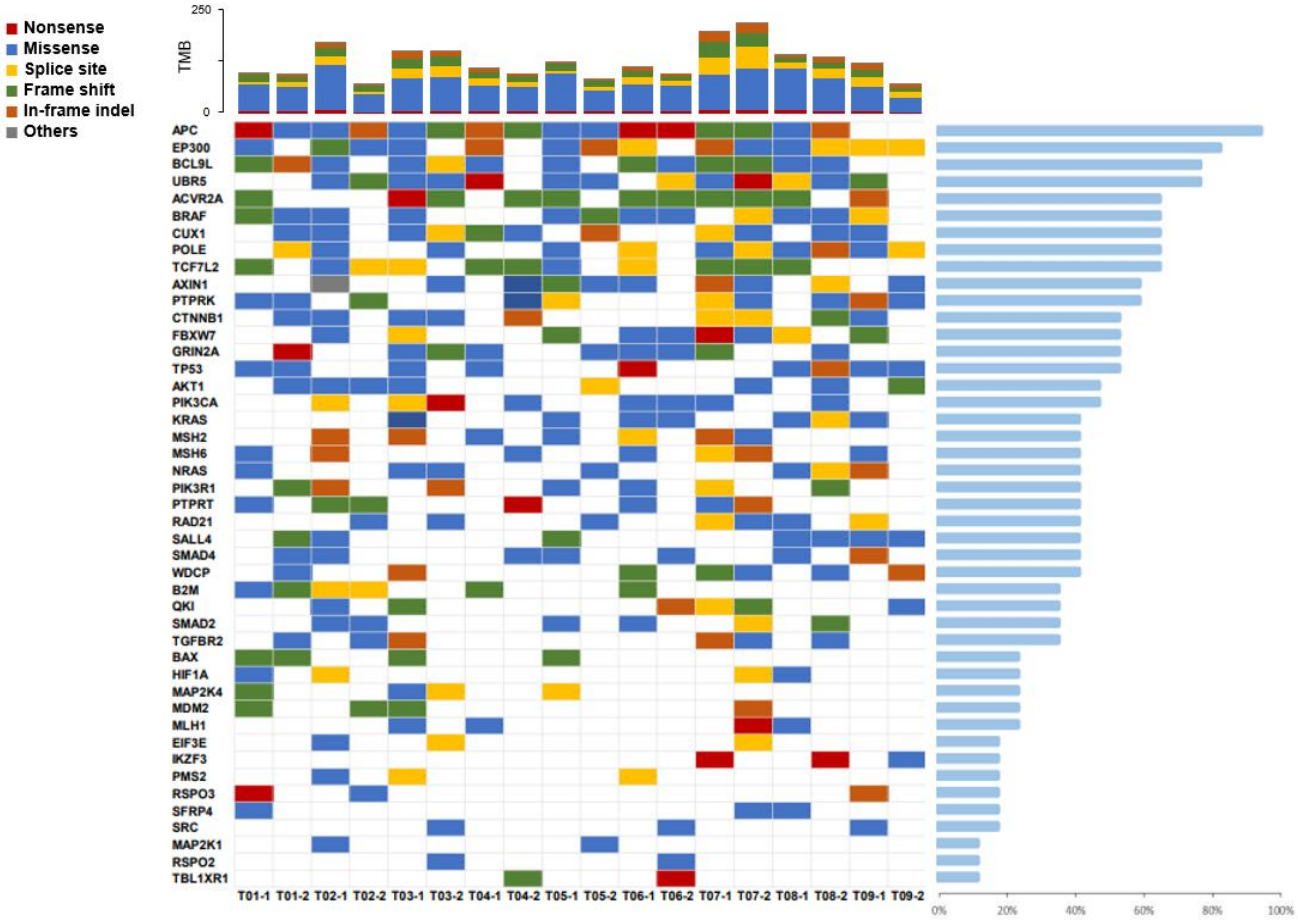


Fig. 9. Types of mutations in synchronous tumors. Each square represents a mutated cancer gene in a single tumor, with the color representing the mutation type. The upper panel indicate total mutation burden of each tumor. The blue bars on the right panel indicate the proportions of lesions with each mutated cancer genes.

Table 9. Comparison of clinicopathologic characteristics between patients who had tumors with similar mutation burden and different mutation burden

Characteristics	Similar (n = 6 patients, 12 tumors)	Different (n = 3 patients, 6 tumors)	*p*-value
Sex			0.098
Male	12 (100.0)	4 (66.7)	
Female	0 (0)	2 (33.3)	
Age (yr)	49.3 ± 11.8	61.0 ± 9.3	0.051
HNPCC	3 (50.0)	0 (0)	0.464
MSI group			1
All MSI-H	3 (50.0)	2 (66.7)	
MSI-H/MSS	3 (50.0)	1 (33.3)	
Location			0.472
Both right	2 (33.3)	2 (66.7)	
Both left	2 (33.3)	1 (33.3)	
Right/left	2 (33.3)	0 (0)	
Distance	30.8 ± 24.0	3.1 ± 2.2	0.095
pStage			1
II	4 (66.7)	2 (66.7)	
III	2 (33.3)	1 (33.3)	
Histologic type			0.245
Adenocarcinoma	11 (91.7)	4 (66.7)	
Mucinous	1 (8.3)	2 (33.3)	
Signet ring cell	0 (0)	0 (0)	
Differentiation			0.917
Well	3 (27.3)	0 (0)	
Moderate	5 (45.5)	4 (100.0)	
Poor	3 (27.3)	0 (0)	
LVI, yes	3 (25.0)	3 (50.0)	0.344
PNI, yes	3 (25.0)	0 (0)	0.515
Tumor budding			0.333
Low	12 (100.0)	5 (83.3)	
Intermediate	0 (0)	1 (16.7)	
High	0 (0)	0 (0)	
TILs			0.301
Weak (0–10%)	8 (66.7)	2 (33.3)	
Moderate (20–40%)	1 (8.3)	2 (33.3)	
Strong (50–90%)	3 (25.0)	2 (33.3)	

Discussion

On the basis of the molecular characteristics of each tumor, an individualized approach to the treatment of colorectal cancer has been emphasized. For the appropriate selection of treatment strategies for SCRC, it is essential to assess the degree of similarity in molecular characteristics between synchronous tumors. In this study, 95% of enrolled patients showed concordance in MSI status, while only 5% showed discordant MSI status. On the other hand, WES analysis of patients with SCRC revealed synchronous tumors in one individual shared only a few somatic variants, indicating substantial intertumoral heterogeneity.

In our study, SCRC was more prevalent in men than in women (2.57:1). This result was similar with previous studies that reported a predominance of male patients in SCRC compared to solitary CRC (1.3:1–1.7:1).[38-41] SCRC has been reported to occur more frequently in the right colon than in solitary CRC[42-44]. In this study, 37% of synchronous tumors were located in the right colon, which is a higher proportion than the proportion of right-sided colon cancer among solitary CRC from the Korea Central Cancer Registry Data (25%)[45] and our previous result (22%).[41] Some previous studies have shown that many SCRC arise on the same side of the large intestine.[46,47] More than half of the patients enrolled in this study had synchronous tumors on different sides of the large intestine. The distance between two synchronous lesions ranged from less than 1 cm to nearly 90 cm, and in about half of the patients, the concurrent tumors were located in more proximal colon than the index tumors. When a malignant lesion is present in the distal colon or rectum, it is occasionally difficult to evaluate the proximal colon because of obstruction. These results might emphasize the importance of a preoperative examination of the entire colon.

As described earlier, SCRC is known to have a higher proportion of MSI-H cancers than solitary CRC. In our study, 12% of SCRC patients had at least one MSI-H tumor, which is lower than the findings of other studies (30–37%).[16-18] One of the possible explanations for this result is ethnic differences in the molecular pathogenesis of CRC.[48] It has also been reported that the incidence of MSI-H tumors in solitary CRC is relatively low (4–6%) in Korea[48] and Japan[49,50] However, since this is a retrospective study, there may be a selection bias, so there may be limitations in interpreting the incidence of MSI-H.

MSI-H tumors had a higher proportion of right-sided location, mucinous adenocarcinoma, and high T cell infiltration compared to MSS tumors in our SCRC cohorts. There are several explanations for the relationship between right-sided colon cancer and MSI status. The right

and left sides of the colon have distinct embryologic origins, resulting in differences in the underlying biology. Indeed, the patterns of gene methylation differ significantly between the right and left colons.[51] Notably, the prevalence of methylation of the hMLH1 gene is significantly higher in the right colon mucosa,[52] suggesting the right colon is more vulnerable to MMR deficiency. The other explanation is the distinct environmental background between the right and left colons. Environmental factors, such as colonic microbiota, exposure to carcinogens, or bile acid levels, may differ between the right and left colons.[53,54] These factors can also affect the occurrence of genetic mutations and the probability of MSI-H tumor formation.

The significance of tumor immune infiltration, which includes lymphocytes, macrophages, neutrophils, and dendritic cells, is supported by an increasing number of studies.[55] In the past, CRC was considered immunogenic and resistant to immunotherapy; however, advances in techniques for the molecular characterization of tumor-associated antigens and the detection of antigen-specific T cell reactions have altered the perspective of the scientific community. In MSI-H tumors, the frequent frameshift mutations and high mutational burden result in the production of a greater number of neoantigens, which increases T cell infiltration.[56] $CD8^+$ cytotoxic T lymphocytes, which play an essential role in the adaptive immune system, were considered as the key promoter of anti-tumor immunity. $CD8^+$ T cells mediate tumor rejection through the recognition of tumor antigens and the production of several substances, such as granulysin, granzymes, and tumor necrosis factor α, leading to tumor cell killing.[57,58] Several studies demonstrated that elevated levels of TILs correlated with antitumor effects and a more favorable prognosis, and a lower risk of metastasis.[59,60] In our study, the High TILs group showed a more favorable survival outcomes than the Low TILs group; however, the difference was not statistically significant. It may be due to small sample sizes and other variables, such as location, stage, and histologic type, that influence survival analysis.

Regarding MSI status in SCRC patients, the reported discordance rate ranged from 6 to 13%,[17,20,61] and our study found a discordance rate of 5%. The All MSI-H group was associated with younger ages, a higher proportion of mucinous adenocarcinoma, and a moderate or strong density of TILs than the All MSS group. These results correspond to the comparison between MSI-H and MSS tumors. The discordant group also showed a higher proportion of mucinous adenocarcinoma and a moderate or strong density of TILs than the All MSS group, which is similar to the result of the All MSI-H group. Three of the five MSI-

H/MSS patients had an MSI-H tumor as the index tumor, while two had an MSS tumor as the index tumor. In other words, if the MSI test is only performed on the index tumor, it is possible for MSI-H/MSS cancers to be regarded as MSS cancers, and treatment decisions may be targeted to MSS CRC. According to current guidelines, MSI status can be used to guide adjuvant therapy for colorectal cancer and enhance the efficacy of individualized treatment. In this regard, the National Comprehensive Cancer Network® guidelines recommend that patients with stage II MSI-H CRC may benefit from fluorouracil-assisted chemotherapy.[62] Furthermore, as mentioned above, the MSI status of tumors is closely linked to the response to immunotherapy. Consequently, the MSI status of synchronous tumors should play a significant role in treatment decisions. The discordant group had similar characteristics to the All MSI-H group regarding histologic type and TILs, so it is hypothesized that patients with discordant MSI status may have a similar treatment response to those with MSI-H. However, it is still unclear how discordant MSI status affects the response to treatment in SCRC. Therefore, it should be investigated through further studies.

Five of the twelve patients with MSI-H tumors were diagnosed with HNPCC according to the Amsterdam II criteria. As mentioned above, HNPCC is an autosomal dominant disease characterized by a high risk of developing not only CRC but also other associated cancers, such as endometrial, ovarian, and gastric cancer. Consequently, it is essential to diagnose HNPCC in terms of early detection of de Novo cancers, consideration for prophylactic surgery to reduce the risk of developing other cancers, and family screening. HNPCC can be underdiagnosed due to inadequate family history assessment or small family size[63]; therefore, genetic testing is recommended to confirm HNPCC. Testing for germline mutation of the MMR genes and hypermethylation of *MLH1* promoter were not included in this analysis, and this is one of the limitations of our study. The significance of MSI status in SCRC will be elucidated through additional research that includes these analyses.

MSI status is known to be prognostic in CRC, with the impact on prognosis varying by stage. MSI-H tumors have a better prognosis than MSS tumors in stage II and III CRC.[15,64,65] On the contrary, Stage IV disease with MSI-H has been linked to poor survival, although the prevalence in stage IV CRC is only 3–4%.[66,67] There were few studies on the survival analysis of SCRC according to MSI status. Generally, MSI-H has been reported to be associated with a more favorable prognosis than MSS, which is a similar result to solitary CRC.[20,68] Interestingly, Bae et al. found that patients with discordant MSI status among their

tumors had the worst clinical prognosis compared to patients with MSI-H or MSS concordant status.[17] In our cohorts, there were no statistically significant differences in RFS or OS between groups, even when stage IV patients were excluded. Given the small sample size, it may have been difficult to demonstrate statistical differences. The prognostic impact of MSI status discordance must be investigated through additional research with a larger sample size. Furthermore, the influence of adjuvant treatment regimen should also be evaluated.

In WES analysis, synchronous tumors in a patient shared only a few somatic mutations, indicating that most synchronous tumors may have independent clonal origins. This is consistent with previous studies that demonstrated the intertumoral heterogeneity of SCRC by WES.[12,69] Cereda M, et al. showed that SCRC had independent genetic origins, somatic alterations, and clonal compositions.[69] In whole exome sequencing analysis of 32 tumor lesions from 15 patients with SCRC, very few ubiquitously mutated genes were identified, ranging from 0.34% to 4.22% in non-hypermutated tumors and 0.8% to 7.0% in hypermutated tumors, respectively.[12] These findings suggest the field effect theory of colorectal carcinogenesis.[13,14,70] The molecular basis of this theory may be a genetic susceptibility to cancer development or extensive exposure to carcinogens. Inherited mutations of immune-related genes may increase the frequency of independent events of cancer initiation, indicating that the inflammatory microenvironment promotes carcinogenesis via cytokine secretion or genomic instability.[69]

On the other hand, in only one patient, synchronous tumors showed a substantially higher proportion of shared variants. In this case, both lesions were located in the transverse colon, and the distance between them was only 1 cm. It is hypothesized that these tumors may have a common origin and may have spread to adjacent lesions via monoclonal seeding. Intraluminal spread is one of the possible explanations for multifocal colorectal carcinogenesis.[71,72] Simmer F, et al. suggested that clonally related CRC was quite common in multifocal CRC by comparing DNA copy number profiles and mutations.[71] Another study found that tumor dissemination during colonoscopy could be a potential cause of metachronous CRC by comparing the molecular profiles of primary and secondary tumors using next-generation sequencing.[72] In our analysis, only one case exhibited genetic similarity, making it difficult to determine the impact of tumor location and distance between synchronous tumors.

In general, tumor heterogeneity affects the efficiency of therapies targeting specific key genes, such as *KRAS*, *BRAF*, or *PIK3CA*.[73] In the case of SCRC, the presence of genetically different tumors complicates the situation, and there are currently no specific guidelines for managing SCRC. As described earlier, the concordance rates of *TP53*, *KRAS*, and *BRAF* mutations have been reported to be less than 40%.[16,23,26,27] The concordance rates in our study were higher than those reported previously; however, the majority of the concordant variants differed in mutation type and site. The efficacy of targeted agents and prognosis may vary depending on the specific mutation subtypes.[74-76] Therefore, it is essential to assess all synchronous tumors within a single individual in order to make appropriate treatment decisions.

This study had several limitations. It was a retrospective study, and there might be a selection bias. This study was also limited by a relatively small sample size, especially the small number of patients with MSI-H tumors. Therefore, it was difficult to demonstrate significant differences in survival analysis according to MSI status. Patients with MSS tumors only were not included in WES analysis. MSS tumors have different clinical, pathologic, and molecular characteristics compared to MSI-H tumors; they should also be analyzed by WES through further studies. This study did not include analyses of germline mutation and DNA methylation. In addition, we did not validate WES using polymerase chain reaction and Sanger sequencing to assess the accuracy of variant calling.

Conclusion

This study found discordance in MSI status between synchronous tumors were identified in 5% of SCRC patients, and the discordant nature of concurrent tumors could be missed if all tumor lesions are not analyzed. Moreover, WES analysis revealed that synchronous tumors in a patient shared only a few somatic mutations, indicating substantial intertumoral heterogeneity of SCRC. This intertumoral heterogeneity was evident in known druggable mutations, including KRAS, BRAF, or PIK3CA. Although the relationship between intertumoral heterogeneity and the response to treatment has not yet been studied in SCRC, molecular analysis of all tumors in patients with SCRC is recommended in determining the treatment strategies for SCRC.

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Cli n.* Jan 2023;73(1):17-48.

2. Lam AK, Chan SS, Leung M. Synchronous colorectal cancer: clinical, pathological a nd molecular implications. *World J Gastroenterol.* Jun 14 2014;20(22):6815-6820.

3. Greenstein AJ, Slater G, Heimann TM, Sachar DB, Aufses AH, Jr. A comparison of multiple synchronous colorectal cancer in ulcerative colitis, familial polyposis coli, a nd de novo cancer. *Ann Surg.* Feb 1986;203(2):123-128.

4. Kiran RP, Khoury W, Church JM, Lavery IC, Fazio VW, Remzi FH. Colorectal canc er complicating inflammatory bowel disease: similarities and differences between Cr ohn's and ulcerative colitis based on three decades of experience. *Ann Surg.* Aug 201 0;252(2):330-335.

5. Lam AK, Carmichael R, Gertraud Buettner P, Gopalan V, Ho YH, Siu S. Clinicopath ological significance of synchronous carcinoma in colorectal cancer. *Am J Surg.* Jul 2011;202(1):39-44.

6. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroe nterology.* Jun 2010;138(6):2059-2072.

7. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Natu re.* Dec 17 1998;396(6712):643-649.

8. Shima K, Nosho K, Baba Y, et al. Prognostic significance of CDKN2A (p16) promot er methylation and loss of expression in 902 colorectal cancers: Cohort study and lite rature review. *Int J Cancer.* Mar 1 2011;128(5):1080-1094.

9. Tariq K, Ghias K. Colorectal cancer carcinogenesis: a review of mechanisms. *Cance r Biol Med.* Mar 2016;13(1):120-135.

10. Bae JM, Kim JH, Kwak Y, et al. Distinct clinical outcomes of two CIMP-positive col orectal cancer subtypes based on a revised CIMP classification system. *Br J Cancer.* Apr 11 2017;116(8):1012-1020.

11. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of color ectal cancer. *Nature Medicine.* 2015/11/01 2015;21(11):1350-1356.

12. Di J, Yang H, Jiang B, Wang Z, Ji J, Su X. Whole exome sequencing reveals intertu mor heterogeneity and distinct genetic origins of sporadic synchronous colorectal ca ncer. *Int J Cancer.* Mar 1 2018;142(5):927-939.

13. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squa mous epithelium; clinical implications of multicentric origin. *Cancer.* Sep 1953;6(5): 963-968.

14. Ogino S, Brahmandam M, Kawasaki T, Kirkner GJ, Loda M, Fuchs CS. Epigenetic profiling of synchronous colorectal neoplasias by quantitative DNA methylation anal ysis. *Mod Pathol.* Aug 2006;19(8):1083-1090.

15. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology.* Jun 2010;138(6):2073-2087.e2073.

16. Nosho K, Kure S, Irahara N, et al. A prospective cohort study shows unique epigenet ic, genetic, and prognostic features of synchronous colorectal cancers. *Gastroenterol ogy.* Nov 2009;137(5):1609-1620.e1601-1603.

17. Dykes SL, Qui H, Rothenberger DA, García-Aguilar J. Evidence of a preferred mole cular pathway in patients with synchronous colorectal cancer. *Cancer.* Jul 1 2003;98 (1):48-54.

18. Hu H, Chang DT, Nikiforova MN, Kuan SF, Pai RK. Clinicopathologic features of s ynchronous colorectal carcinoma: A distinct subset arising from multiple sessile serr ated adenomas and associated with high levels of microsatellite instability and favor able prognosis. *Am J Surg Pathol.* Nov 2013;37(11):1660-1670.

19. Müller A, Edmonston TB, Dietmaier W, Büttner R, Fishel R, Rüschoff J. MSI-testing in hereditary non-polyposis colorectal carcinoma (HNPCC). *Dis Markers.* 2004;20(4-5):225-236.

20. Bae JM, Cho NY, Kim TY, Kang GH. Clinicopathologic and molecular characteristics of synchronous colorectal cancers: heterogeneity of clinical outcome depending on microsatellite instability status of individual tumors. *Dis Colon Rectum.* Feb 2012;55(2):181-190.

21. Aslanian HR, Burgart LJ, Harrington JJ, et al. Altered DNA mismatch repair expression in synchronous and metachronous colorectal cancers. *Clin Gastroenterol Hepatol.* Dec 2008;6(12):1385-1388.

22. Balschun K, Haag J, Wenke AK, von Schönfels W, Schwarz NT, Röcken C. KRAS, NRAS, PIK3CA exon 20, and BRAF genotypes in synchronous and metachronous primary colorectal cancers diagnostic and therapeutic implications. *J Mol Diagn.* Jul 2011;13(4):436-445.

23. Eguchi K, Yao T, Konomoto T, Hayashi K, Fujishima M, Tsuneyoshi M. Discordance of p53 mutations of synchronous colorectal carcinomas. *Mod Pathol.* Feb 2000;13(2):131-139.

24. Norrie MW, Hawkins NJ, Todd AV, Meagher AP, O'Connor TW, Ward RL. The role of hMLH1 methylation in the development of synchronous sporadic colorectal carcinomas. *Dis Colon Rectum.* May 2002;45(5):674-680.

25. Sedivy R, Wolf B, Kalipciyan M, Steger GG, Karner-Hanusch J, Mader RM. Genetic analysis of multiple synchronous lesions of the colon adenoma-carcinoma sequence. *Br J Cancer.* Apr 2000;82(7):1276-1282.

26. Jesinghaus M, Pfarr N, Kloor M, et al. Genetic heterogeneity in synchronous colorectal cancers impacts genotyping approaches and therapeutic strategies. *Genes Chromosomes Cancer.* Mar 2016;55(3):268-277.

27. Zauber P, Huang J, Sabbath-Solitare M, Marotta S. Similarities of molecular genetic changes in synchronous and metachronous colorectal cancers are limited and related to the cancers' proximities to each other. *J Mol Diagn.* Sep 2013;15(5):652-660.

28. Konishi K, Shen L, Jelinek J, et al. Concordant DNA methylation in synchronous colorectal carcinomas. *Cancer Prev Res (Phila).* Sep 2009;2(9):814-822.

29. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med.* Jun 25 2015;372(26):2509-2520.

30. Cercek A, Lumish M, Sinopoli J, et al. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. *N Engl J Med.* Jun 23 2022;386(25):2363-2376.

31. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A.* Sep 17 2002;99(19):12293-12297.

32. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science.* Mar 22 1996;271(5256):1734-1736.

33. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Cancer.* Mar 2019;19(3):133-150.

34. Lavin Y, Kobayashi S, Leader A, et al. Innate Immune Landscape in Early Lung Adenocarcinoma by Paired Single-Cell Analyses. *Cell.* May 4 2017;169(4):750-765.e717.

35. Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum.* May 1991;34(5):424-425.

36. Loukola A, Eklin K, Laiho P, et al. Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC). *Cancer Res.* Jun 1 2001;61(11):4545-4549.

37. Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lympho cytes (TILs) in breast cancer: recommendations by an International TILs Working Gr oup 2014. *Ann Oncol.* Feb 2015;26(2):259-271.

38. Choy AM, Lebwohl B, Krigel A. Impact of social determinants of health on colorect al cancer screening and surveillance in the COVID reopening phase. *Eur J Gastroent erol Hepatol.* Jul 1 2022;34(7):739-743.

39. White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A. A rev iew of sex-related differences in colorectal cancer incidence, screening uptake, route s to diagnosis, cancer stage and survival in the UK. *BMC Cancer.* Sep 20 2018;18 (1):906.

40. Latournerie M, Jooste V, Cottet V, Lepage C, Faivre J, Bouvier AM. Epidemiology a nd prognosis of synchronous colorectal cancers. *Br J Surg.* Dec 2008;95(12):1528-1 533.

41. Lee BC, Yu CS, Kim J, et al. Clinicopathological features and surgical options for sy nchronous colorectal cancer. *Medicine (Baltimore).* Mar 2017;96(9):e6224.

42. Passman MA, Pommier RF, Vetto JT. Synchronous colon primaries have the same pr ognosis as solitary colon cancers. *Dis Colon Rectum.* Mar 1996;39(3):329-334.

43. Adloff M, Arnaud JP, Bergamaschi R, Schloegel M. Synchronous carcinoma of the c olon and rectum: prognostic and therapeutic implications. *Am J Surg.* Mar 1989;157 (3):299-302.

44. Chen HS, Sheen-Chen SM. Synchronous and "early" metachronous colorectal adeno carcinoma: analysis of prognosis and current trends. *Dis Colon Rectum.* Aug 2000;4 3(8):1093-1099.

45. Hur H, Oh CM, Won YJ, Oh JH, Kim NK. Characteristics and Survival of Korean Pa tients With Colorectal Cancer Based on Data From the Korea Central Cancer Registr y Data. *Ann Coloproctol.* Aug 2018;34(4):212-221.

46. Kaibara N, Koga S, Jinnai D. Synchronous and metachronous malignancies of the co lon and rectum in Japan with special reference to a coexisting early cancer. *Cancer.* Nov 1 1984;54(9):1870-1874.

47. Eu KW, Seow-Choen F, Goh HS. Synchronous colorectal cancer in an Oriental popu lation. *Int J Colorectal Dis.* Dec 1993;8(4):193-196.

48. Oh JR, Kim DW, Lee HS, et al. Microsatellite instability testing in Korean patients with colorectal cancer. *Fam Cancer.* Sep 2012;11(3):459-466.

49. Asaka S, Arai Y, Nishimura Y, et al. Microsatellite instability-low colorectal cancer a cquires a KRAS mutation during the progression from Dukes' A to Dukes' B. *Carcin ogenesis.* Mar 2009;30(3):494-499.

50. Yamada K, Kanazawa S, Koike J, et al. Microsatellite instability at tetranucleotide re peats in sporadic colorectal cancer in Japan. *Oncol Rep.* Feb 2010;23(2):551-561.

51. Kaz AM, Wong CJ, Dzieciatkowski S, Luo Y, Schoen RE, Grady WM. Patterns of D NA methylation in the normal colon vary by anatomical location, gender, and age. *E pigenetics.* Apr 2014;9(4):492-502.

52. Menigatti M, Truninger K, Gebbers JO, Marbet U, Marra G, Schär P. Normal colorec tal mucosa exhibits sex- and segment-specific susceptibility to DNA methylation at t he hMLH1 and MGMT promoters. *Oncogene.* Feb 12 2009;28(6):899-909.

53. Lee MS, Menter DG, Kopetz S. Right Versus Left Colon Cancer Biology: Integrating the Consensus Molecular Subtypes. *J Natl Compr Canc Netw.* Mar 2017;15(3):411-419.

54. Flemer B, Lynch DB, Brown JM, et al. Tumour-associated and non-tumour-associate d microbiota in colorectal cancer. *Gut.* Apr 2017;66(4):633-643.

55. Senovilla L, Vacchelli E, Galon J, et al. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. *Oncoimmunology.* Nov 1 2012;1(8):1323-1343.

56. Baran B, Mert Ozupek N, Yerli Tetik N, Acar E, Bekcioglu O, Baskin Y. Difference Between Left-Sided and Right-Sided Colorectal Cancer: A Focused Review of Litera ture. *Gastroenterology Res.* Aug 2018;11(4):264-273.
57. Russell JH, Ley TJ. Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol.* 2002;2 0:323-370.
58. Golstein P, Griffiths GM. An early history of T cell-mediated cytotoxicity. *Nat Rev I mmunol.* Aug 2018;18(8):527-535.
59. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cell s within human colorectal tumors predict clinical outcome. *Science.* Sep 29 2006;31 3(5795):1960-1964.
60. Ogino S, Nosho K, Irahara N, et al. Lymphocytic reaction to colorectal cancer is ass ociated with longer survival, independent of lymph node count, microsatellite instabi lity, and CpG island methylator phenotype. *Clin Cancer Res.* Oct 15 2009;15(20):64 12-6420.
61. Arriba M, Sánchez R, Rueda D, et al. Toward a Molecular Classification of Synchro nous Colorectal Cancer: Clinical and Molecular Characterization. *Clin Colorectal C ancer.* Mar 2017;16(1):31-37.
62. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive m arker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Cl in Oncol.* Jul 10 2010;28(20):3219-3226.
63. Singh H, Schiesser R, Anand G, Richardson PA, El-Serag HB. Underdiagnosis of Ly nch syndrome involves more than family history criteria. *Clin Gastroenterol Hepatol.* Jun 2010;8(6):523-529.
64. Samowitz WS, Curtin K, Ma KN, et al. Microsatellite instability in sporadic colon ca ncer is associated with an improved prognosis at the population level. *Cancer Epide miol Biomarkers Prev.* Sep 2001;10(9):917-923.
65. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol.* Jan 20 2005;23(3):609-618.
66. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instabilit y on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *C ancer.* Oct 15 2011;117(20):4623-4632.
67. Uhlig J, Cecchini M, Sheth A, Stein S, Lacy J, Kim HS. Microsatellite Instability an d KRAS Mutation in Stage IV Colorectal Cancer: Prevalence, Geographic Discrepan cies, and Outcomes From the National Cancer Database. *J Natl Compr Canc Netw.* F eb 2 2021;19(3):307-318.
68. Malesci A, Basso G, Bianchi P, et al. Molecular heterogeneity and prognostic implica tions of synchronous advanced colorectal neoplasia. *Br J Cancer.* Mar 4 2014;110 (5):1228-1235.
69. Cereda M, Gambardella G, Benedetti L, et al. Patients with genetically heterogeneou s synchronous colorectal cancer carry rare damaging germline mutations in immune-related genes. *Nature Communications.* 2016/07/05 2016;7(1):12072.
70. Cereda M, Gambardella G, Benedetti L, et al. Patients with genetically heterogeneou s synchronous colorectal cancer carry rare damaging germline mutations in immune-related genes. *Nat Commun.* Jul 5 2016;7:12072.
71. Simmer F, van der Linden RLA, Ligtenberg MJL, Ylstra B, van der Post RS, Nagteg aal ID. Multifocal Colorectal Cancer-Do Intraluminal Metastases Occur? *Gastroente rology.* Apr 2021;160(5):1853-1855.
72. Backes Y, Seerden TCJ, van Gestel R, et al. Tumor Seeding During Colonoscopy as a Possible Cause for Metachronous Colorectal Cancer. *Gastroenterology.* Nov 2019; 157(5):1222-1232.e1224.
73. Samuel N, Hudson TJ. Translating genomics to the clinic: implications of cancer het

erogeneity. *Clin Chem.* Jan 2013;59(1):127-137.

74. Jia Y, Jiang T, Li X, et al. Characterization of distinct types of KRAS mutation and it s impact on first-line platinum-based chemotherapy in Chinese patients with advance d non-small cell lung cancer. *Oncol Lett.* Dec 2017;14(6):6525-6532.

75. Gao L, Shen W. Light at the end of the tunnel: Clinical features and therapeutic pros pects of KRAS mutant subtypes in non-small-cell lung cancer. *Front Genet.* 2022;1 3:890247.

76. Jones JC, Renfro LA, Al-Shamsi HO, et al. (Non-V600) BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. *J Clin Oncol.* Aug 10 2017;35(23):2624-2630.

동시성 다발성 대장암의 면역유전체 발현양상 분석

연구목적: 동시성 다발성 대장암은 첫 진단 당시에 하나 이상의 대장직장암이 있는 것으로 정의된다. 동시성 다발성 대장암의 분자병리학적 특성은 면역관문 억제제와 같은 표적 치료를 결정하는데 매우 중요함에도 불구하고 아직 완전히 밝혀지지 않았다. 따라서 본 연구는 현미부수체 불안정성(microsatellite instability, MSI), 체성변이 등 동시성 다발성 종양 간 분자병리학적 특성에 대해 비교하고자 하였다.

연구방법: 2012 년부터 2014 년까지 동시성 다발성 대장암으로 수술적 절제를 받은 100 명의 환자를 후향적으로 분석하였다. 한 환자 내의 각각의 종양에 대해서 MSI, 종양 침윤 림프구를 포함한 각종 임상적, 분자병리학적 특성을 분석하였다. MSI 상태를 평가하기 위해 Bethesda panel 이 사용되었고 2 개 이상의 현미 부수체 표지자에 변화가 있는 경우를 High MSI (MSI-H)로 분류하였다. 종양 침윤 림프구의 밀도는 International TILs Working Group 의 지침에 근거하여 간질 영역에서 단핵 세포가 발견되는 비율로 결정되었다. Whole exome sequencing (WES)은 MSI-H 종양을 최소 하나 이상 가지는 9 명의 환자의 18 개 종양에 대해 시행되었고, 이를 통해 동시성 다발성 대장암의 종양간 이질성을 평가하였다.

연구결과: 동시성 다발성 대장암은 단일 대장암에 비해 남성에 호발하고 높은 빈도의 MSI-H, 그리고 우측 대장에 위치하는 경향을 보였다. MSI-H 종양은 MSS 종양에 비해 우측 대장에 더 잘 발생하고(68.4% vs 33.7%, $p = 0.011$), 점액선암종의 비율이 높았으며(15.8% vs 0%, $p < 0.001$), 종양 침윤 림프구의 밀도 또한 높은 것(57.9% vs 25.0%, $p < 0.001$)으로 나타났다. 전체 100 명의 동시성 다발성 대장암 환자 중에 12 명이 최소 하나 이상의 MSI-H 종양을 가졌고, 이 중 5 명은 MSI 불일치를 보였다. MSI 불일치를 보인 환자들은 무병생존율 및 전체 생존율에서 MSI 일치를 보인 환자들과 유의한 차이를 보이지 않았다. WES 분석에서 대부분의 환자들은 종양 간 매우 적은 분율(0.09–0.36%)의 변이를 공유하였는데, 한 명의 환자에서만 6.5%로 높은 분율의 변이를 공유하는 것으로 확인되었다. 같은 환자 내에서 동시성 종양간 *BRAF, KRAS, PIK3CA, NRAS* 유전자의 일치율은 각각 66.7%, 66.7%, 55.6%, 66.7%였다. 그러나 대부분의 동시성 종양이 같은 유전자 변이를 공유하더라도 서로 다른 하위 유형의 변이를 가지고 있었다.

결론: 동시성 다발성 대장암은 MSI 상태에 있어서는 5%의 불일치율을 보였고, 체성변이에 있어서는 상당히 높은 불일치율을 보였다. 종양간 이질성이 표적 치료에 대한 반응에 영향을 줄 수 있으므로, 한 환자 내에 존재하는 여러 개의 종양에 대해 분자병리학적 분석을 시행하는 것은 동시성 다발성 대장암의 치료 전략 수립을 위해 반드시 권장된다.

중심단어: 동시성 다발성 대장암, 현미부수체 불안정성, 전체 엑솜 분석, 종양 이질성