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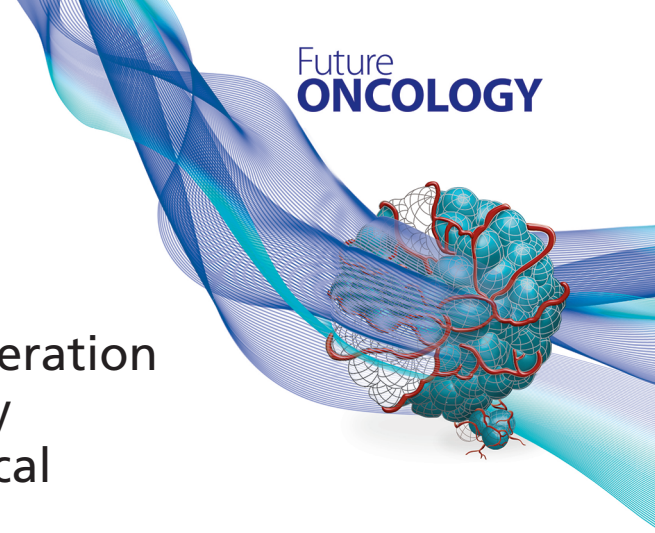

Real-world application of next-generation sequencing-based test for surgically resectable colorectal cancer in clinical practice

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Aim: To evaluate the significance of next-generation sequencing-based gene panel testing in surgically resectable colorectal cancer by analyzing real-world data. **Materials & methods:** A total of 107 colorectal cancer patients who underwent curative surgery were included, and correlations between next-generation sequencing data and clinicopathological findings were evaluated. **Results:** More combination patterns in gene alteration were identified in advanced-stage tumors than in early-stage tumors. The copy number alteration count was significantly lower in right-sided colon tumors and early-stage tumors. Homologous recombination deficiency was more often identified in advanced-stage tumors, and high homologous recombination deficiency status was useful for identifying high-risk stage II tumors. **Conclusion:** Homologous recombination deficiency was identified as a useful result of gene panel testing with novel utility in clinical practice.

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Keywords: colorectal cancer • copy number alterations • homologous recombination deficiency • next-generation sequencing • tumor mutational burden

Malignant tumors are traditionally diagnosed and classified based on the organ of origin and histological type, and treatments are selected according to such classification. However, it has become clear in recent years that malignant tumors are caused by the accumulation of various genetic mutations. Hence, treatment strategies against malignant tumors place more emphasis on targeting such genetic mutations. The concept of 'precision medicine', in which genetic mutations in individual malignant tumors are analyzed and individualized treatment targeting those mutations is employed, has also been gaining ground in recent years [1]. In the case of colorectal cancer (CRC), it has been reported that tumors are caused by carcinogenic pathways involving various genetic mutations, such as mutations caused by the adenoma–carcinoma sequence [2]. Accordingly, comprehensive next-generation sequencing (NGS) studies, such as those of The Cancer Genome Atlas, have been performed and have revealed that CRC can be classified into some molecular subtypes based on genomic events [3].

NGS-based genomic testing is currently used in clinical settings for the practice of precision medicine. Gene panel tests such as MSK-IMPACT™ (Memorial Sloan Kettering Cancer Center, NY, USA) and Foundation One® CDx (Foundation Medicine, Inc., MA, USA) have been approved by the US FDA, and their use is spreading to countries worldwide, including Japan. However, the indications for these gene panel tests are limited to locally advanced or metastatic solid tumors for which standard treatment has been completed or advanced solid tumors for which no standard treatment is available. Therefore, currently, very few patients can benefit from testing. In fact, although actionable gene mutations are identified in 37–86% of solid cancer patients, only 11–13% are actually

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identified as targetable mutations [4–6]. Extending the indications of gene panel testing to early-stage tumors may reveal its utility, but no such studies have been conducted thus far.

In the authors' institute, in-house NGS-based gene panel testing, which analyzes 160 oncogenes, was performed for all resectable solid cancer patients in a clinical trial setting [7]. The novelty of this trial is that gene panel testing was performed immediately after the primary curative surgery – timing that is thus far not available for other types of insurance-covered tests. In addition, the authors' in-house gene panel testing is cost-effective, making it cheaper than other tests. This trial was expected to explore any advantage of genetic information in the decision of treatment after curative surgery.

Here the authors report the real-world data collected prospectively from patients with CRC who underwent primary curative surgery at our hospital, including patients with early-stage cancers. The aim of this study was to investigate any additional information on genetic changes during CRC progression and to explore the significance of gene panel testing at primary surgery, which will lead to further expansion of testing.

Materials & methods

Patients

This study included patients with colorectal cancer (CRC) who underwent curative surgery from July 2018 to February 2020 at Keio University Hospital. The study protocol was approved by the ethics committee of the Keio University School of Medicine (approval number: 20180015). All study participants provided informed consent. This study was performed following all relevant guidelines and regulations. The Union for International Cancer Control tumor, node, metastasis classification was used for stage classification, and European Society for Medical Oncology clinical practice guidelines were used for high-risk stage II classification – specifically, lymph nodes <12, poorly differentiated tumor, presence of vascular/lymphatic or perineural invasion, pT4 stage and clinical presentation with intestinal occlusion or perforation [8].

Next-generation sequencing

Tumor tissue was collected from surgical specimens of CRC patients who provided consent to undergo comprehensive genomic testing. Details of the panel have been previously reported [7,9,10]. Briefly, genomic DNA was extracted from 10-μm thick formalin-fixed, paraffin-embedded tissue sections of tumor specimens using the Maxwell RSC FFPE Plus DNA Kit (AS1720; Promega Corporation, WI, USA) according to the manufacturer's instructions. DNA quality was checked by calculating the DNA integrity number (DIN) using a 4200 TapeStation (Agilent Technologies, Waldbronn, Germany); all analytes had DIN $\geq$2.0. Libraries were generated from 80 ng (DIN $\leq$2.5) or 160 ng (DIN $>$2.5) of DNA per sample using the Human Comprehensive Cancer Panel, GeneRead DNAseq Panel PCR Kit V2, GeneRead DNA Library I Core Kit and GeneRead DNA Library I Amp Kit (Qiagen, Hilden, Germany), and the library quality was assessed using a D1000 ScreenTape (Agilent Technologies). Targeted amplicon exome sequencing was performed using a 160 cancer-related gene panel as previously described. The targeted regions of all 160 genes were specifically enriched using oligonucleotide probes. The enriched libraries were sequenced with a paired-end (150 bp $\times$ 2) sequencing method using the NextSeq sequencing platform (Illumina, CA, USA), resulting in a mean depth of 500. The sequencing data were analyzed using the GenomeJack bioinformatics pipeline (Mitsubishi Space Software Co., Ltd., Tokyo, Japan; http://genomejack.net/) as previously described [11]. The proportion of tumor cells ranged from 5 to 80% (median: 45%). Tumor mutational burden (TMB) was defined as the number of nonsynonymous and synonymous mutations in the target. The estimated copy number (CN) of the tumor cells was calculated using the following formula: measured CN - 2/proportion of tumor cells + 2 = estimated CN.

Homologous recombination deficiency (HRD) was evaluated by determining the 'HRD score'. The score was calculated using an algorithm similar to the loss of heterozygosity (LOH) score in myChoice® CDx (Myriad Genetics, Inc., UT, USA). Although the LOH score is calculated by the sum of LOH, telomeric allelic imbalance and large-scale state transitions, the latter two factors cannot be calculated in targeted gene panel sequences because of the limited number of genes. Thus, a unique method of counting CN alterations (CNAs) has been used to ensure measurement sensitivity. In detail, the score is defined as the percentage of detected breakpoints in the whole genome and differences in the CNA status of adjacent probe genes. CNA status includes three categories: loss, neutral and amplification. LOH regions spanning $\geq$90% of a whole chromosome or chromosome arm are considered to be due to non-HRD mechanisms [12]. Thus, chromosomes with fewer than two probe genes (no. 8, no. 18, no. 21 and X in this test) were excluded from the calculation of the HRD score. Chromosomes with

Table 1. Summary of patient clinicopathological characteristics in each histological type.

Characteristic	WEL	POR	MUC	All
	n = 94	n = 2	n = 11	n = 107
Age, median (interquartile range)	70 (61–77)	57 (52–62)	69 (63–75)	70 (61–76)
Sex, n (%)				
Male	53 (56.4)	1 (50.0)	5 (45.5)	59 (55.1)
Tumor location, n (%)				
Right-sided colon	34 (36.2)	2 (100)	6 (54.5)	42 (39.3)
Left-sided colon	27 (28.7)	0 (0)	0 (0)	27 (25.2)
Rectum	33 (35.1)	0 (0)	5 (45.5)	38 (35.5)
Tumor stage, n (%)				
I	28 (29.8)	0 (0)	0 (0)	28 (26.2)
II	37 (39.4)	0 (0)	3 (27.3)	40 (37.4)
III	25 (26.6)	2 (100)	5 (45.5)	32 (29.9)
IV	4 (4.2)	0 (0)	3 (27.3)	7 (6.5)

MUC: Mucinous adenocarcinoma; POR: Poorly differentiated adenocarcinoma; WEL: Well–moderately differentiated adenocarcinoma.

the same CNA status on a single chromosome were also excluded. In this study, an 'actionable' gene alteration was defined as pathogenic variants and CNAs (CN >4 or homozygous deletions or LOH). The annotated and curated analysis report was discussed at a genome expert conference consisting of medical oncologists, molecular oncologists, pathologists, medical geneticists, clinical laboratory technicians, bioinformaticians, genetic counselors, pharmacists and nurses.

Statistical analysis

All statistical analyses were performed with Prism 9 (GraphPad Software, Inc., CA, USA). The Mann–Whitney U test and chi-square test were applied as appropriate. One-way analysis of variance (ANOVA) was applied for comparison among three groups. The significance level was set at 0.05. Co-alteration analysis results were plotted using Circos, a Perl language-based tool used to represent visual data in a circular form [13]. The Circos plot was generated using Circular Layout Interactive Converter Free Services. The usage details have been previously reported [14].

Results

Patient characteristics

The characteristics of CRC patients who were included in this analysis are shown in Table 1. Overall, sequencing was performed for 107 CRC cases – 94 well–moderately differentiated adenocarcinoma (WEL) cases, two poorly differentiated adenocarcinoma (POR) cases and 11 mucinous adenocarcinoma (MUC) cases – and the cohort included 59 (55.1%) males and 48 (44.9%) females. The median age was 70 years old (interquartile range: 61–76), and the tumor locations were as follows: right-sided colon, 42 (39.3%); left-sided colon, 27 (25.2%); and rectum, 38 (35.5%). The tumor stages were as follows: stage I, 28 (26.2%); stage II, 40 (37.4%); stage III, 32 (29.9%); and stage IV, seven (6.5%).

Actionable gene alterations

Among the 107 patients, actionable gene alterations were identified in a total of 104 (actionable gene rate: 97.2%) samples: WEL, 91 (96.8%); POR, two (100.0%); and MUC, 11 (100.0%). The frequent actionable gene alterations are summarized in Figure 1A and Table 2. Actionable gene variants in mismatch repair genes were identified in WEL only, with mutations in *MSH2* in 4.3% of cases and mutations in *MLH1* in 3.2% of cases. Among the genes related to HRD, an actionable gene variant in *ATM* was identified in 4.3% of WEL cases, 100% of POR cases and 9.1% of MUC cases; an actionable gene variant in *BRCA2* was identified in 6.4% of WEL cases and 9.1% of MUC cases; and an actionable gene variant in *PALB2* was identified in 5.3% of WEL cases. With regard to tumor suppressor genes, actionable gene variants were identified in various genes. Among the tumor suppressor genes, *APC* was the most frequently mutated gene and was mutated in 58.9% of all samples, 59.6% of WEL samples, 0% of POR samples and 63.6% of MUC samples. A *TP53* actionable gene variant was identified in 54.2% of all

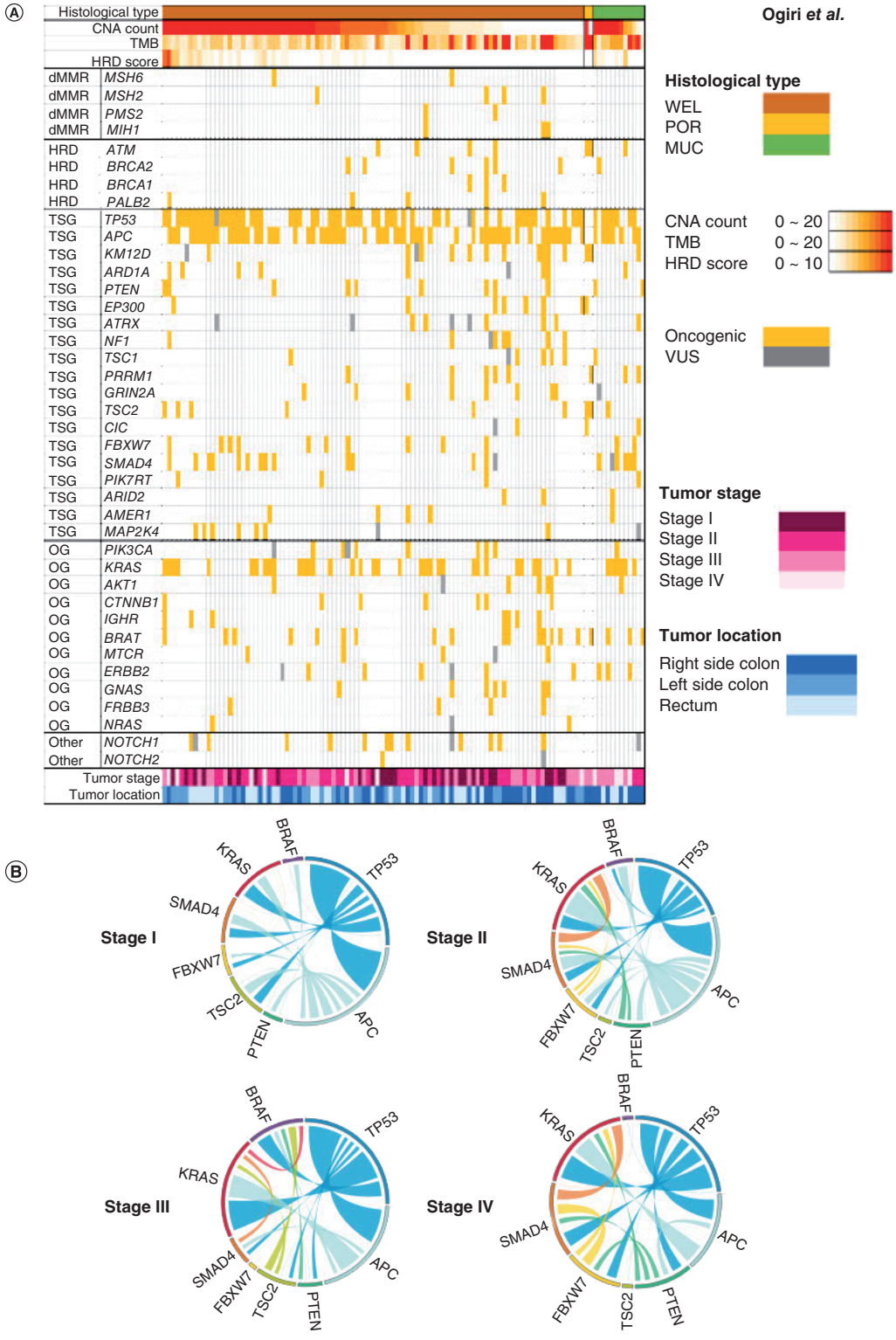

Figure 1. Actionable gene alterations. (A) Actionable gene variants identified in resectable colorectal cancer. Oncogenic variant and VUS are shown. **(B)** Circos plots of seven major mutated genes in stage I–IV colorectal cancer. CNA: Copy number alteration; dMMR: Deficient mismatch repair; HRD: Homologous recombination deficiency; MUC: Mucinous adenocarcinoma; OG: Oncogene; POR: Poorly differentiated adenocarcinoma; TMB: Tumor mutational burden; TSG: Tumor suppressor gene; VUS: Variant of unknown significance; WEL: Well–moderately differentiated adenocarcinoma.

Table 2. List of extracted actionable gene variants in each histological type in colorectal cancer.

Gene category	Gene	WEL, n (%)	POR, n (%)	MUC, n (%)	All, n (%)
		n = 94	n = 2	n = 11	n = 107
dMMR	*MSH2*	4 (4.3)	0 (0)	0 (0)	4 (3.7)
	MLH1	3 (3.2)	0 (0)	0 (0)	3 (2.8)
HRD	*ATM*	4 (4.3)	2 (100)	1 (9.1)	7 (6.5)
	BRCA2	6 (6.4)	0 (0)	1 (9.1)	7 (6.5)
	PALB2	5 (5.3)	0 (0)	0 (0)	5 (4.7)
TSG	*APC*	56 (59.6)	0 (0)	7 (63.6)	63 (58.9)
	TP53	52 (55.3)	0 (0)	6 (54.5)	58 (54.2)
	SMAD4	13 (13.8)	0 (0)	5 (45.5)	18 (16.8)
	KMT2D	11 (11.7)	2 (100)	1 (9.1)	14 (13.1)
	FBXW7	10 (10.6)	0 (0)	2 (18.2)	12 (11.2)
	PTEN	9 (9.6)	0 (0)	2 (18.2)	11 (10.3)
	TSC2	6 (6.4)	2 (100)	1 (9.1)	9 (8.4)
OG	*KRAS*	32 (34.0)	0 (0)	6 (54.5)	38 (35.5)
	BRAF	16 (17.0)	1 (50.0)	2 (18.2)	19 (17.8)
	ERBB2	5 (5.3)	0 (0)	3 (27.3)	8 (7.5)
	CTNNB1	7 (7.4)	0 (0)	0 (0)	7 (6.5)
	GNAS	7 (7.4)	0 (0)	0 (0)	7 (6.5)
	PIK3CA	7 (7.4)	0 (0)	1 (9.1)	8 (7.5)
Other	*NOTCH1*	12 (12.8)	0 (0)	0 (0)	12 (11.2)

dMMR: Deficient mismatch repair; HRD: Homologous recombination deficiency; MUC: Mucinous adenocarcinoma; OG: Oncogene; POR: Poorly differentiated adenocarcinoma; TSG: Tumor suppressor gene; WEL: Well–moderately differentiated adenocarcinoma.

samples, 55.3% of WEL samples, 0% of POR samples and 54.5% of MUC samples. A *SMAD4* actionable gene variant was identified in 16.8% of all samples and was very frequent in MUC samples; it was identified in 13.8% of WEL samples, 0% of POR samples and 45.5% of MUC samples. For oncogenes, a v-Ki-ras2 *KRAS* actionable gene variant was identified in 35.5% of all sample types, 34.0% of WEL samples, 0% of POR samples and 54.5% of MUC samples. In addition, a *BRAF* actionable gene variant was identified in 17.8% of all samples, 17.0% of WEL samples, 50.0% of POR samples and 18.2% of MUC samples. Figure 1B shows the co-alteration analysis results according to tumor stage. In stage I tumors, the majority of co-alterations were either *TP53*- or *APC*-related, whereas other combinations were identified in advanced tumors.

Copy number alterations

A heatmap of CNAs is shown in Figure 2A. Overall, frequent losses of *SMAD4* (36.4%), *TP53* (33.6%) and *FAS* (24.3%) and amplifications of *ASXL1* (36.4%), *GNAS* (33.6%) and *HSPH1* (33.6%) were identified. Based on the analysis of CNAs of the 160 cancer-related genes, frequent gains of genes in 13q and 20q were identified in WEL, but these were less frequent in MUC. There was no statistically significant difference in CNA count between histological types (Figure 2B). The median CNA counts were as follows: WEL 16.0 (0–96.0) versus POR 12.5 (1.0–24.0) versus MUC 21.0 (0–46.0; one-way ANOVA: $p = 0.57$). The median CNA counts for each tumor stage were as follows: stage I 11.0 (0–70.0) versus stage II 17.5 (0–82.0) versus stage III 14.0 (0–96.0) versus stage IV 30.0 (17.0–77.0; Figure 2C). Stage IV had a significantly higher CNA count than the others (one-way ANOVA: $p = 0.04$). There was a statistically significant difference between stages I and IV ($p = 0.008$) and between stages II and IV ($p = 0.02$). Moreover, tumor location was associated with CNA count: right-sided colon 7.0 (0–77.0) versus left-sided colon 17.0 (0–96.0) versus rectum 18.5 (0–55.0; Figure 2D). Right-sided colon had a significantly lower CNA count than the others (one-way ANOVA: $p = 0.045$). There were statistically significant differences between right- and left-sided colon ($p = 0.03$) and between right-sided colon and rectum ($p = 0.04$).

Tumor mutational burden

With regard to TMB, there was no significant difference in any of the comparisons (Figure 3A–C). Hypermutation, which was defined as TMB $\geq$10, was identified in a total of 29 (27.1%) cases. However, the proportion of hypermutation cases was not significantly different in any of the comparisons (data not shown).

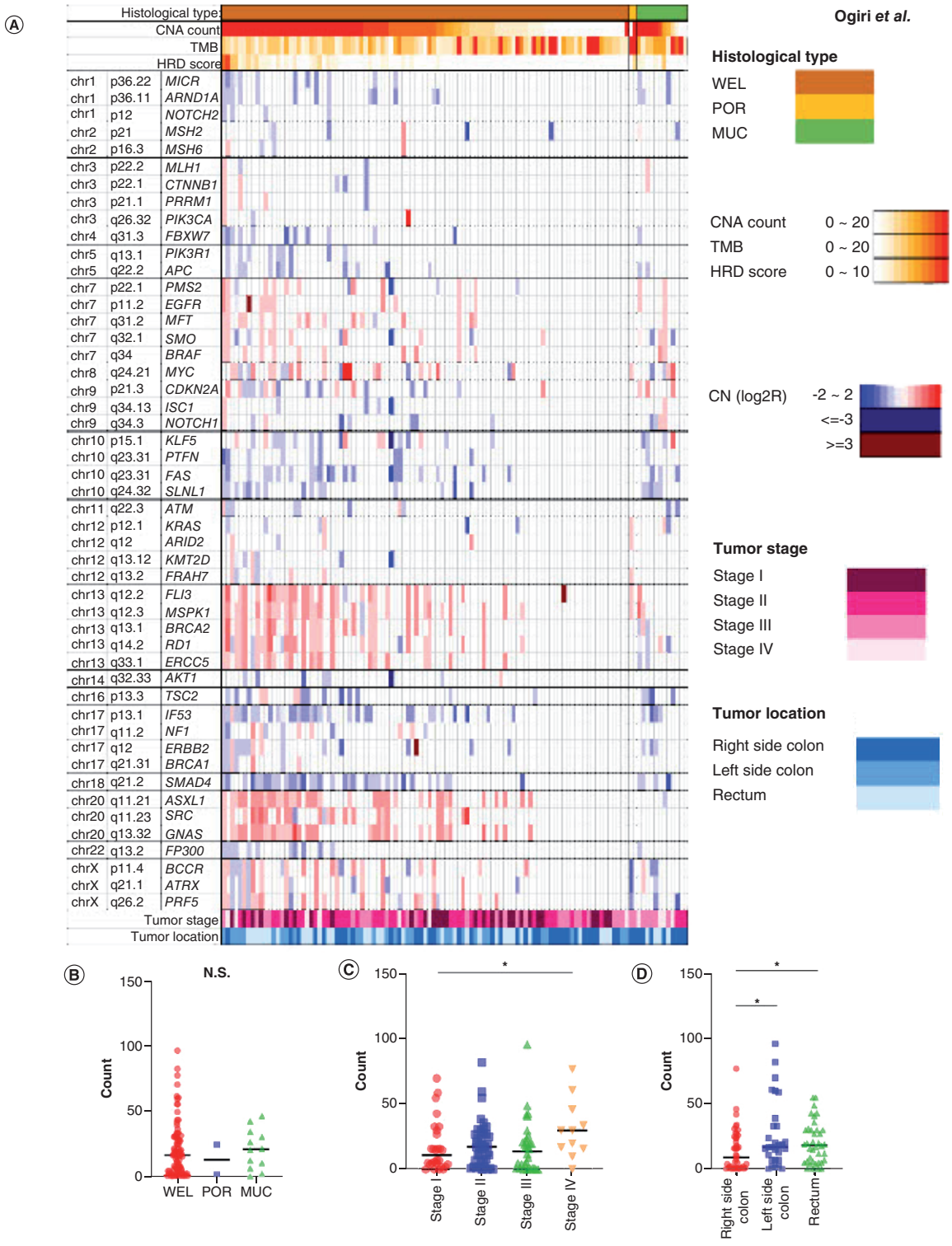

Figure 2. Copy number alteration. (A) CNA identified in resectable colorectal cancer. **(B)** CNA count in each histological type. **(C)** CNA count in each tumor stage. **(D)** CNA count in each tumor location. Horizontal bars represent median value.

*$p < 0.05$.

CN: Copy number; CNA: Copy number alteration; HRD: Homologous recombination deficiency; MUC: Mucinous adenocarcinoma; N.S.: Not significant; POR: Poorly differentiated adenocarcinoma; TMB: Tumor mutational burden; WEL: Well–moderately differentiated adenocarcinoma.

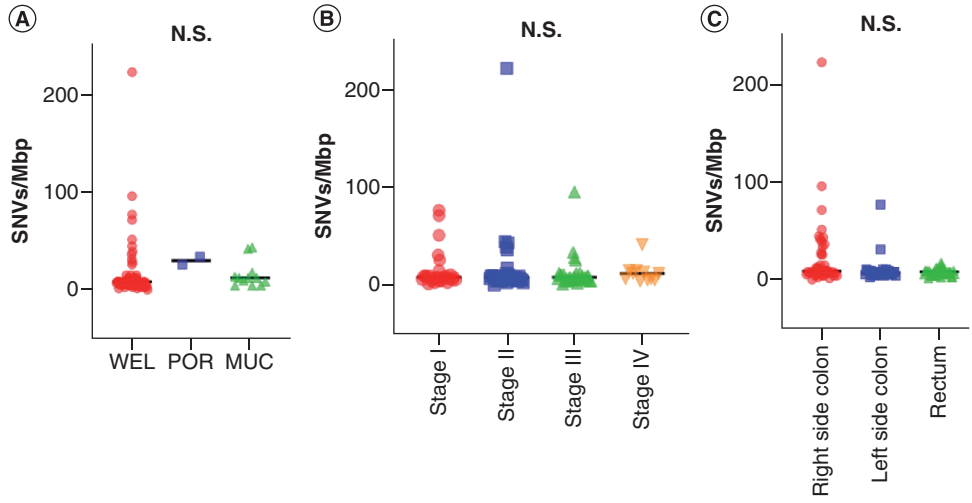

Figure 3. Tumor mutational burden. (A) Tumor mutational burden in each histological type. **(B)** Tumor mutational burden in each tumor stage. **(C)** Tumor mutational burden in each tumor location. Horizontal bars represent median value.
MUC: Mucinous adenocarcinoma; N.S.: Not significant; POR: Poorly differentiated adenocarcinoma; SNV: Single nucleotide variant; WEL: Well–moderately differentiated adenocarcinoma.

Homologous recombination deficiency

The median HRD scores for each tumor stage were as follows: stage I 0 (0–4.5) versus stage II 0 (0–7.5) versus stage III 0 (0–8.3) versus stage IV 1.5 (0–9.8; Figure 4B). Stage IV had a marginally higher score than the others (one-way ANOVA: $p = 0.05$). There were statistically significant differences between stages I and IV ($p = 0.02$), between stages II and IV ($p = 0.005$) and between stages III and IV ($p = 0.03$). The authors next attempted to use the HRD score as a recurrence risk predictor in stage II patients (Figure 4C). When an HRD score ≥ 1 was defined as a high HRD score, the HRD score correlated well with high-risk stage II tumors: low risk 0% versus high risk 30% ($p = 0.049$). By contrast, the HRD score was not associated with histological type or tumor location: WEL 0 (0–9.8) versus POR 0 (0–0) versus MUC 0.8 (0–4.1; one-way ANOVA: $p = 0.16$) and right-sided colon 0 (0–9.8) versus left-sided colon 0.4 (0–8.3) versus rectum 0 (0–4.1; one-way ANOVA: $p = 0.20$; Figure 4A & D).

Discussion

This study presents real-world NGS data obtained from samples prospectively collected from CRC patients who were eligible for curative surgery. Although there have been many reports on unresectable advanced or metastatic CRC, the results of this study, in which all cases were surgically resectable and over 60% were stage I or II cancer, are important for understanding the potential significance of genetic testing. According to this study, TMB and CNA count are associated with pathological histology and tumor location and represent the biological features of tumors, and a combination of altered genes represents tumor progression. By contrast, the HRD score is more associated with tumor stage and represents tumor progression, suggesting its possible utility in clinical practice.

Here the authors employed an in-house targeted amplicon exome sequencing-based panel that includes 160 cancer-related genes and has been validated in several solid tumors, such as ovarian cancer and pancreatic cancer [7,9,10]. The detection rate of actionable genes in CRC was higher than 90% with this panel, which is comparable to that observed with other NGS-based oncogene panels [15–17]. The major driver genes in CRC, including *APC*, *KRAS* and *SMAD4*, also had mutation rates comparable to those seen in other studies. The slightly lower mutation rate in *TP53* may be because the authors' study included more early-stage cancers than other studies. Interestingly, co-alteration analysis showed that most of the stage I tumors had alterations with either *TP53* or *APC* co-alterations. By contrast, more advanced-stage tumors (stages III and IV) had various gene combinations. This finding may explain the adenoma–carcinoma sequence, in which *APC* and *TP53* alterations leading to adenoma formation are the first to manifest and other gene alterations accumulate during malignant transformation [18–20]. Therefore, the authors' results indicate that stage I tumors are closer to adenomas.

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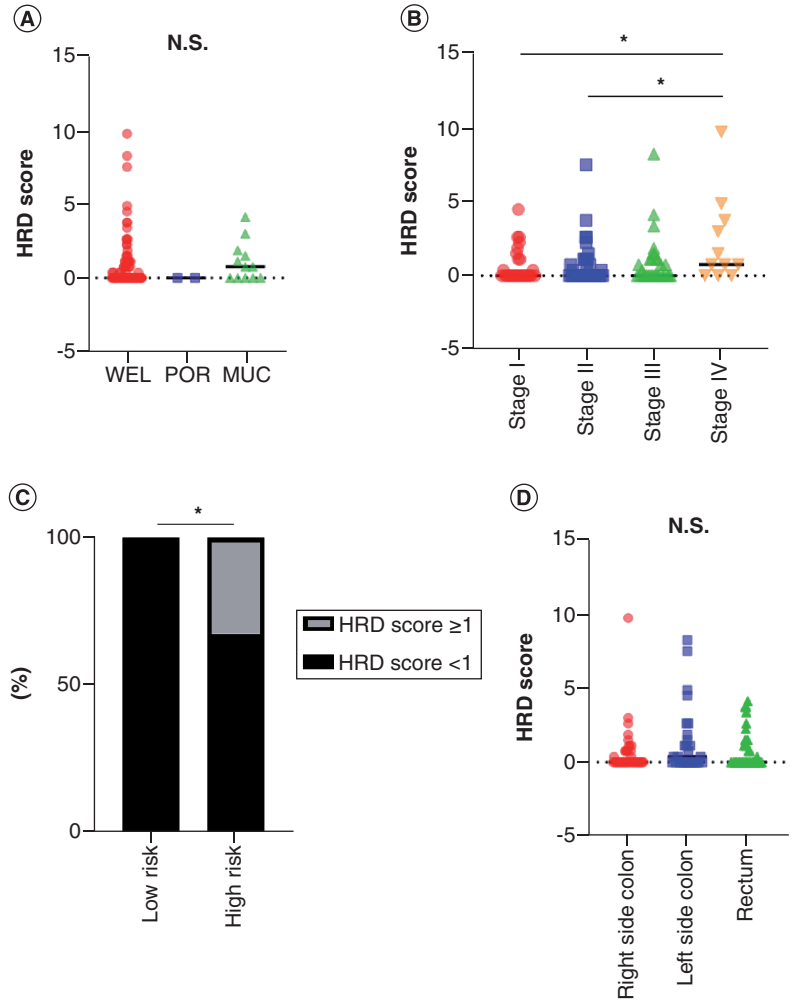

Figure 4. Homologous recombination deficiency. (A) HRD score in each histological type. **(B)** HRD score in each tumor stage. **(C)** Percentages of high HRD score in low- and high-risk stage II cases. **(D)** HRD score in each tumor location. Horizontal bars represent median value.
HRD: Homologous recombination deficiency; MUC: Mucinous adenocarcinoma; N.S.: Not significant; POR: Poorly differentiated adenocarcinoma; WEL: Well–moderately differentiated adenocarcinoma.

In this study, the CNA count was related to tumor location. Consistent with other reports, right-sided colon tumors had lower CNA counts than tumors in other locations [21]. This finding may suggest that cellular genomic instability is more pronounced in left-sided tumors than in right-sided tumors. In addition, the CNA count tended to increase as the tumor stage advanced. No clear results have been noted with regard to the existence of a gradual increase in the CNA count between early, invasive and metastatic CRC [22], but it has been suggested that progression from invasive cancer to metastasis is accompanied by an increase in the CNA count [23,24]. As a high CNA count most likely represents genomic instability, the authors' results suggest that genomic instability increases with cancer progression.

Although immunotherapy has proven to be effective in treating cancers and is being approved for various types of cancer, including CRC, the number of patients who can benefit from it is still limited [25]. TMB is an emerging biomarker of sensitivity to immune checkpoint inhibitors and has been shown to be more significantly associated with the response to PD-1 and PD-L1 blockade immunotherapy than PD-1 or PD-L1 expression [26]. In CRC, TMB is reported to be higher in right-sided colon tumors than in left-sided tumors [27]. Although not statistically significant, this study also showed that TMB was relatively higher in right-sided colon tumors. The distribution of

TMB and the subset of patients with high TMB have not been well characterized and are issues to be elucidated in the future.

HRD has received much attention, primarily in breast cancer treatment, since an underlying mechanism of breast cancer formation has been largely attributed to the HRD pathway [28]. Indeed, the importance of the breast and ovarian cancer susceptibility proteins BRCA1 and BRCA2 has been well documented [29,30]. Genomic tests such as myChoice CDx, which detects *BRCA1* and *BRCA2* mutants, have been approved by the FDA and are used to detect biomarkers for PARP inhibitor treatment [31]. Unfortunately, the relationship between CRC and HRD has not yet been fully studied. A few reports have shown that brain metastases of CRC and locally advanced rectal carcinomas exhibit elevated mutational signatures of HRD [32,33]. In this study, a higher HRD score was clearly correlated with tumor progression, and moreover, it was suggested to correlate well with the high-risk stage II classification. When considering postoperative chemotherapy, this finding could be utilized for patient and regimen selection. In fact, high HRD is associated with susceptibility to platinum agents in ovarian cancer [34].

Nevertheless, the results of this study indicate that the cost–effectiveness of performing NGS-based genomic testing in all patients with CRC who undergo curative surgery is still debatable. As is the case for HRD, many biomarkers can be measured by companion diagnostics. Additional studies are expected to further explore the potential of NGS-based genomic testing. In addition, the heterogeneity of tumors should be taken into account. CRC generally consists of multiple subclones, and these subclones have their own unique characteristics, resulting in intratumor heterogeneity [35]. In the presence of intratumor heterogeneity, the remained treatment-resistant clones after shrinkage of the tumor may have entirely different characteristics from the original tumor. The validity of the treatment strategy based on the original tumor rather than the recurrent tumor is another issue to be elucidated in the future.

Conclusion

Real-world NGS data represent significant biological features of cancer progression in resectable CRC. Evaluating HRD was considered useful in clinical practice as a novel significance of gene panel testing.

Future perspective

The indication for cancer gene panel testing should be reconsidered because the rate of patients who reach to targeted therapy is limited to only 11–13% of locally advanced or metastatic solid tumors. Extending the indication to early-stage tumors could be one of the options, and it is important to consider its utility in clinical practice.

Summary points

- In stage I colorectal cancer, the majority of co-alterations were either *TP53*- or *APC*-related, whereas other combinations were identified in advanced tumors.
- The copy number alteration count was significantly lower in right-sided colon tumors.
- Homologous recombination deficiency was more often identified in advanced-stage tumors.
- There was no significant difference in any of the comparisons with regard to tumor mutational burden.
- Homologous recombination deficiency status was useful for identifying high-risk stage II tumors.

Author contributions

M Ogiri designed the study, analyzed the data and wrote the manuscript. R Seishima collected tissues for next-generation sequencing, wrote the manuscript and supervised the study. K Okabayashi, K Shigeta, S Matsui and Y Kitagawa collected tissues for next-generation sequencing and clinicopathological data. K Nakamura, E Aimono, S Tanishima, T Chiyoda and H Nishihara performed the next-generation sequencing genomic testing and analyzed the data. All authors reviewed the manuscript.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the ethics committee of the Keio University School of Medicine (approval number: 20180015), and informed consent was obtained from the participants involved.

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•• **Showed that actionable gene mutations were found in approximately 36.7% of all patients and that the overall proportion of patients who were successfully enrolled in clinical trials involving molecularly targeted agents was 11%.**

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• **Revealed that potentially actionable gene mutations were identified in 86.3% of all solid cancers and that 13.0% of patients received subsequent therapy based on next-generation sequencing (NGS) assay results.**

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