Evaluation of A 55-Gene Classifier As A Prognostic Biomarker for Adjuvant Chemotherapy in Stage III Colon Cancer Patients

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Abstract

Background

Adjuvant chemotherapy reduces the recurrence risk in stage III colon cancer (CC). However, better prognostic and predictive biomarkers are required to stratify patients for treatment. We constructed a 55-gene classifier (55GC) and investigated its utility for classifying patients with stage III CC.

Methods

We retrospectively identified patients with stage III CC aged 20–79 years who received adjuvant chemotherapy with or without oxaliplatin during 2009–2012.

Results

Among 938 eligible patients, 203 and 201 patients who received adjuvant chemotherapy with and without oxaliplatin, respectively, were selected by propensity-score matching. Of these, 95 patients from each group were analyzed and their 5-year relapse-free survival (RFS) rates with and without oxaliplatin were 73.7% and 77.1%, respectively. The hazard ratios for 5-year RFS for adjuvant chemotherapy (fluoropyrimidine), with or without oxaliplatin, were 1.241 (95% CI, 0.465–3.308; $P = 0.67$) and 0.791 (95% CI, 0.329–1.901; $P = 0.60$), respectively. Stratification using the 55GC revealed that 52 (27.3%), 78 (41.1%), and 60 (31.6%) patients had microsatellite instability (MSI)-like, chromosomal instability (CIN)-like, and stromal subtypes, respectively. The 5-year RFS rates were 84.3% and 72.0% in patients treated with and without oxaliplatin, respectively, for the MSI-like subtype (HR, 0.495; 95% CI, 0.145–1.692; $P = 0.25$). No RFS rate differences were noted in CIN-like or stromal subtypes. Stratification by cancer sidedness for each subtype showed improved RFS only in left-sided primary cancer treated with oxaliplatin for the MSI-like subtype ($P = 0.007$). The 5-year RFS rates for the MSI-like subtype in left-sided cancer were 100% and 53.9% with and without oxaliplatin, respectively.

Conclusions

Subclassification using 55GC and tumor sidedness revealed increased RFS of patients within the MSI-like subtype with stage III left-sided CC treated with fluoropyrimidine with oxaliplatin relative to those treated without oxaliplatin. However, the predictive power of 55GC subtyping alone did not reach statistical significance in this cohort, warranting larger prospective studies.

Trial registration:

The study protocol was registered in the University Hospital Medical Education Network (UMIN) clinical trial registry (UMIN study ID 000023879).

Background

Colorectal cancer remains one of the most common causes of cancer-related deaths worldwide [1]. Adjuvant chemotherapy in stage III colon cancer (CC) after curative-intent resection prolongs survival and reduces the risk of recurrence [2]. Pivotal trials have shown superior outcomes for fluoropyrimidine in combination with oxaliplatin compared with fluoropyrimidine alone in most patient populations; however, the evidence is less well established in elderly patients. Emerging data have also led to debate over the optimal duration of chemotherapy, specifically in the context of increased toxicity [3]. Furthermore, subclassification of stage III CC is an ongoing process with revision based on the accumulating patient survival data and cancer presentation [4]. Therefore, better prognostic and predictive biomarkers are required to stratify patients for adjuvant therapies based on the chemotherapy regimen and duration.

The consensus molecular subtypes (CMS) are robust classification systems for colorectal cancer that include over 600 genes. DNA microarray analysis of CMS can provide prognostic information [5]. CMS is also potentially predictive, with different sensitivities to adjuvant chemotherapy. We previously simplified this classification by constructing a 55-gene classifier (55GC) focusing on genes located on the long arms of chromosomes 18 and 20 as well as stroma-related genes [6]. Using the 55GC, we categorized stage II/III CC into three subtypes with different recurrence rates: “microsatellite instability (MSI)-like,” “chromosomal instability (CIN)-like,” and “stromal” subtypes and showed prognostic utility in a single institutional study. We conducted a validation study by the 55-gene classifier for assessing stratification recurrence (55 STAR) risk. Further, 55GC-based subtyping was able to stratify stage II CC recurrence risk in a multi-institutional validation cohort study of 232 patients [7]. To expand on these previous findings, here we hypothesized that 55GC could be utilized to stratify survival of patients with stage III CC that received adjuvant chemotherapy with or without oxaliplatin.

Materials And Methods

Tissue samples

We retrospectively identified consecutive patients with stage III colon and rectosigmoid CC aged 20–79 years who underwent curative surgery (R0) and received adjuvant chemotherapy with or without oxaliplatin from 15 institutions in Japan between January 1, 2009 and December 31, 2012. Samples from patients who received neoadjuvant treatment, had multiple active cancers, died, or had recurrence within 60 days post-surgery were excluded from the study. Patient characteristics were recorded. The study protocol was approved by the institutional review boards of Kyushu University (study ID 28–69), National Defense Medical College (study ID 2477), Saitama Medical University International Medical Center (study ID 16–051), Tokyo Medical and Dental University (study ID G2016-007), Saiseikai Yokohamashi Nanbu Hospital (study ID 2017-D21), Niigata Cancer Center Hospital (study ID 796),

Saitama Medical University Saitama Medical Center (study ID 1812), National Hospital Organization Kyushu Medical Center (study ID 16C058), National Hospital Organization Kyushu Cancer Center (study ID 2016-48), Hyogo College of Medicine (study ID Hi326), Kanagawa Cancer Center (study ID 2017-8), Takano Hospital (study ID 16 – 04), Tochigi Cancer Center (study ID A432), Teikyo University School of Medicine (study ID 16–057), Tohoku University (study ID 2016-1-222) and Sysmex Corporation (study ID 2015-71), and was registered in the University Hospital Medical Education Network Clinical Trial Registry (UMIN study ID 000023879). All of the methods were carried out in accordance with relevant guidelines and regulations. Because this study was a retrospective observational study carried out in Japan, informed consent was obtained using the opt-out/opt-in approach according to each participating institution's policy (as per Japanese ethical guidelines for observational study, consent of the family is not required for dead participants). The Consolidated Standards of Reporting Trials (CONSORT) diagram is shown in Fig. 1.

Gene expression analysis

Formalin-fixed paraffin-embedded (FFPE) primary cancer tissue specimens containing the tumor invasive front with the greatest depth of invasion were collected from each institution, and a single 5- μ m section was sent to Takeda Pathology Center (Osaka, Japan) for assessment. Total RNA was extracted from fewer than four unstained 10- μ m sections for gene expression microarray assay profiling using the RNeasy FFPE Kit (Qiagen; Valencia, CA). Samples with insufficient RNA quality for microarray analysis were omitted from the cohort. Gene expression data were generated using the Affymetrix GeneChip Human Genome U133 Plus 2.0 Array (Thermo Fisher Scientific; Waltham, MA) and analyzed with the 55GC model as described previously [6]. DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen). *RAS* mutations were assessed using the MEBGEN RASKET KIT (Medical & Biological Laboratories; Nagoya, Japan).

Statistical analyses

The primary endpoint was RFS, defined as the time from surgery to first CC recurrence or death from any cause. A propensity score method was used to reduce selection bias, and a logistic regression model was used to calculate patient propensity scores. Propensity score matching was performed for the number of lymph node metastases, tumor location, sex, and age in a 1:1 ratio using a caliper width of 0.1.

Demographic characteristics are summarized using contingency tables. The RFS curve was estimated using the Kaplan–Meier method and compared between groups using log-rank tests. HRs and 95% CIs were calculated using a Cox proportional hazards model. Risk factors for RFS were assessed using a Cox proportional hazards model with a backward elimination method that included known clinicopathological prognostic factors and gene mutations as covariates. Subgroup analysis was performed for age (< 70 vs. $\geq$ 70 years), sex (male vs. female), carcinoembryonic antigen (< upper limit of normal [ULN] vs. $\geq$ ULN), tumor location (left vs. right), T stage (T1–T3 vs. T4), lymph node metastasis (N1 vs. N2–N3), tumor grade (poorly differentiated and mucinous adenocarcinoma vs. tubular adenocarcinoma), vascular invasion (v0 vs. v1–v3), subtype (CIN vs. MSI vs. stromal), and *RAS* (wild vs. mutant). Fisher's exact test was used to compare patient characteristics between groups. *P* values were two-sided, and *P* < 0.05 was

considered statistically significant. All statistical analyses were performed using the Statistical Analysis System (SAS) version 9.4 (SAS Institute, Cary, NC).

Results

Patient characteristics

Among 938 eligible patients, 203 and 201 patients receiving adjuvant chemotherapy with and without oxaliplatin, respectively, were selected using propensity score matching. After excluding patients with low-quality specimens and those who had received chemotherapy for < 3 months, 95 patients from each group were analyzed (Fig. 1). In the overall cohort of 190 patients (Table 1), 98 (51.6%) patients were men, 146 (76.8%) were aged < 70 years, and 126 (66.3%) had left-sided tumors. Regarding histopathological characteristics, there were more patients with T4 stage cancer in the cohort treated with oxaliplatin (44/95 patients, 46.3%) than in the cohort treated without oxaliplatin (34/95 patients, 35.8%) ($P = 0.022$). In addition, there were more patients with < 12 resected lymph nodes in the cohort treated with oxaliplatin (15/95 patients, 15.8%) than in the cohort treated without oxaliplatin (5/95 patients, 5.3%) ($P = 0.018$). The 5-year RFS rates were 73.7% and 77.1% in patients treated without and with oxaliplatin, respectively (Fig. 2; hazard ratio [HR]: 0.858; 95% confidence interval [CI]: 0.484–1.522).

Table 1
Patient characteristics

Factors		Oxaliplatin (-) (N=95)	Oxaliplatin (+) (N=95)	Total (N=190)	P value
		n (%)	n (%)	n (%)	
Sex	Male	46 (48.4)	52 (54.7)	98 (51.6)	0.3838
	Female	49 (51.6)	43 (45.3)	92 (48.4)	
Age (years)	< 70	71 (74.7)	75 (78.9)	146 (76.8)	0.4915
	≥ 70	24 (25.3)	20 (21.1)	44 (23.2)	
CEA	<ULN	63 (66.3)	50 (52.6)	113 (59.5)	0.6105
	≥ULN	31 (32.6)	20 (21.1)	51 (26.8)	
	Unknown	1 (1.1)	25 (26.3)	26 (13.7)	
Tumor location	Right side	31 (32.6)	33 (34.7)	64 (33.7)	0.7588
	Left side	64 (67.4)	62 (65.3)	126 (66.3)	
T stage	T1-T3	61 (64.2)	51 (53.7)	112 (58.9)	0.1403
	T4	34 (35.8)	44 (46.3)	78 (41.1)	
Tumor grade	por & muc	4 (4.2)	13 (13.7)	17 (8.9)	0.0222
	tub	91 (95.8)	82 (86.3)	173 (91.1)	
Lymphatic invasion	Negative	29 (30.5)	26 (27.4)	55 (28.9)	0.6313
	Positive	66 (69.5)	69 (72.6)	135 (71.1)	

CEA, carcinoembryonic antigen; ULN, upper limit of normal

Factors		Oxaliplatin (-) (<i>N</i> =95)	Oxaliplatin (+) (<i>N</i> = 95)	Total (<i>N</i> = 190)	<i>P</i> value
		n (%)	n (%)	n (%)	
Vascular invasion	Negative	28 (29.5)	23 (24.2)	51 (26.8)	0.4130
	Positive	67 (70.5)	72 (75.8)	139 (73.2)	
N stage	N1	39 (41.1)	36 (37.9)	75 (39.5)	0.0940
	N2	54 (56.8)	50 (52.6)	104 (54.7)	
	N3	2 (2.1)	9 (9.5)	11 (5.8)	
Number of resected lymph nodes	< 12	5 (5.3)	15 (15.8)	20 (10.5)	0.0181
	≥ 12	90 (94.7)	80 (84.2)	170 (89.5)	
CEA, carcinoembryonic antigen; ULN, upper limit of normal					

55-gene classifier subtype analysis

Of the total cohort, 55GC analysis revealed 52 (27.3%) patients with a MSI-like subtype, 78 (41.1%) patients with a CIN-like subtype, and 60 (31.6%) patients with a stromal subtype. The patient characteristics within each subtype are shown in Supplementary Table S1. MSI-like subtype tumors were more likely to be right-sided, whereas CIN-like subtype tumors were more likely to be left-sided compared with the overall cohort. All (100%) CIN-like subtype tumors had non-mucinous histology compared with 95.8% in the overall cohort. MSI-like subtype tumors had higher proportions of N2 (34/52 tumors, 65.4%) and N3 (5/52 tumors, 9.6%) stages compared with the overall cohort.

Survival analysis according to 55-gene classifier analysis and chemotherapy regimen

Comparisons of RFS in patients treated with and without oxaliplatin according to the 55GC subtype are shown in Fig. 3. The 5-year RFS rates were 84.3% and 72.0% in patients treated with and without oxaliplatin, respectively, for the MSI-like subtype (HR, 0.495; 95% CI, 0.145–1.692); however, the trend was not statistically significant (log-rank $P=0.25$). There was no difference in RFS in CIN-like subtype patients according to oxaliplatin treatment (HR, 1.241; 95% CI, 0.465–3.308; log-rank $P=0.67$). RFS was also unchanged in the stromal subtype patients regardless of oxaliplatin treatment (HR, 0.791; 95% CI, 0.329–1.901; log-rank $P=0.60$). Further subdivision into left- and right-sided primary cancer of the subtypes

showed improved RFS only in left-sided primary cancer of the MSI-like subtype treated with oxaliplatin (Fig. 4; log-rank $P=0.0071$). The 5-year RFS rates for the MSI-like subtype in left-sided cancer were 100% and 53.9% with and without oxaliplatin, respectively. No significant differences in RFS were noted between subtypes when stratified by treatment with (log-rank $P=0.23$) and without oxaliplatin (log-rank $P=0.37$; Supplementary Fig. S1).

Subgroup analysis is shown in Supplementary Fig. S2. Comparison of RFS in patients treated with or without oxaliplatin revealed no significant differences according to patient characteristics (age, sex) or histopathological findings (tumor location, T stage, N stage, tumor grade, and vascular invasion).

Discussion

Despite significant progress in the development of prognostic and predictive biomarkers in CC, particularly *RAS* mutation status and deficient mismatch repair (dMMR) status to guide therapy for metastases [8, 9], there is an ongoing need for progress in the molecular analysis of early stage CC to guide adjuvant therapy. While dMMR status may indicate a lack of efficacy of fluoropyrimidine-only regimens without oxaliplatin, there are no other validated predictive tumor biomarkers in early stage CC [10]. Several multigene expression profiling systems such as Oncotype DX (Genomic Health; Redwood City, CA) and ColoPrint (Agendia; Amsterdam, Netherlands), have been developed [11]. However, they are not subtyping systems; therefore, they have prognostic but no predictive value for recurrence risk. In contrast, the predictive potential of molecular subtypes in CC has been demonstrated recently in prospective trials [12–14]. While the classification of CMS is considered the most robust classification based on comprehensive gene expression profiling [5], other classifications have been developed and validated [15–18]. However, there is debate over the optimal method of identification of subtypes and difficulties in the practical widespread measurement of these genotypes in routine clinical practice [19]. Recently, other gene set classifiers obtained from microarray used in CMS were reported. Similar prognostic utility was shown using 99 or 200 gene sets [20]. In this study, we suggest the possible utility of a 55 gene set, especially when paired with other cancer properties such as sidedness, which may provide potentially prognostic and predictive information for adjuvant therapy in early stage CC after curative-intent resection.

Post-hoc analyses of tumor tissue from patients in large randomized trials of adjuvant chemotherapy have revealed the overall poor prognosis of certain molecular subtypes. For example, analysis of the National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07 clinical trial demonstrated poor prognosis in both stage II and III patients with a ‘stem-like’ subtype identified from three different subtyping methods [21]. This is consistent with our findings herein, which show a tendency for poorer prognosis of the stromal subtype in stage III patients as well as our previous results in stage II/III CC patients [6]. Furthermore, in the aforementioned NSABP C-07 retrospective analysis, the stem-like subtype from Colorectal Cancer Assigner classification (CRCA) predicted a lack of benefit for the addition of oxaliplatin [21]. Similar to the NSABP C-07 study, our cohort demonstrated a 3.4% improvement in 5-year RFS with the addition of oxaliplatin to adjuvant chemotherapy. Considering this result, the potential

additive benefit of oxaliplatin for adjuvant chemotherapy in the MSI-like subtype from our 55GC system requires further investigation for validation. Similar to the CMS classification, the 55GC system requires caution; the MSI-like subtype is not identical to MSI-high or dMMR CC.

Our study has the following major limitation: the relatively small number of patients in each subgroup, especially after accounting for propensity score matching. Nevertheless, the results suggest the potential benefit of adjuvant chemotherapy with oxaliplatin in the MSI-like subtype, when accounting for tumor sidedness. These findings require further prospective validation in an independent cohort to determine the true clinical significance. Combined with deeper analysis of genomic and histopathologic correlates, including the immune and tumor microenvironment, this would enhance our understanding of the biological underpinnings of each subtype and their predictive efficacy.

Conclusions

In conclusion, the current 55GC study illustrated that oxaliplatin may have an additive effect in adjuvant chemotherapy for the MSI-like subtype for left-sided primary CC. Hence, future study with a larger number of CC cases are warranted.

Abbreviations

55GC

55 gene classifier

CC

Colon cancer

CEA

Carcinoembryonic antigen

CI

Confidence interval

CIN

Chromosomal instability

CMS

Consensus molecular subtypes

CONSORT

Consolidated Standards of Reporting Trials

CRCA

Colorectal Cancer Assigner

DNA

Deoxyribonucleic acid

FFPE

Formalin-fixed paraffin-embedded

HR

Hazard ratio

MSI

Microsatellite instability

NSABP

National Surgical Adjuvant Breast and Bowel Project

P

P-value

RFS

Relapse-free survival

RNA

Ribonucleic acid

STAR

Stratification recurrence

ULN

Upper limit of normal

UMIN

University Hospital Medical Education Network

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the following institutional review boards: Kyushu University (study ID 28-69), National Defense Medical College (study ID 2477), Saitama Medical University International Medical Center (study ID 16-051), Tokyo Medical and Dental University (study ID G2016-007), Saiseikai Yokohamashi Nanbu Hospital (study ID 2017-D21), Niigata Cancer Center Hospital (study ID 796), Saitama Medical University Saitama Medical Center (study ID 1812), National Hospital Organization Kyushu Medical Center (study ID 16C058), National Hospital Organization Kyushu Cancer Center (study ID 2016-48), Hyogo College of Medicine (study ID Hi326), Kanagawa Cancer Center (study ID 2017-8), Takano Hospital (study ID 16-04), Tochigi Cancer Center (study ID A432), Teikyo University School of Medicine (study ID 16-057), Tohoku University (study ID 2016-1-222) and Sysmex Corporation (study ID 2015-71). All methods were carried out in accordance with relevant guidelines and regulations. Because this study was a retrospective observational study carried out in Japan, informed consent was obtained using the opt-out/opt-in approach according to each participating institution's policy (as per Japanese ethical guidelines for observational study, consent of the family is not required for dead participants).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The cDNA microarray datasets generated during the current study are available from the corresponding author upon reasonable request. Repository to Gene Expression Omnibus (GEO) database are not prepared by concern of the Japanese Act on the Protection of Personal Information.

COMPETING INTEREST

E.O. has been part of a speakers' bureau at Bayer, Chugai Pharmaceutical, Eli Lilly, Merck Serono, Ono Pharmaceutical, Taiho Pharmaceutical, Takeda Pharmaceutical, and Yakult Honsha. M.S. has a consulting/advisory role with Sysmex. S.Y. has been part of a speakers' bureau at Chugai Pharmaceutical, Covidien, Eli Lilly, Merck Serono, Johnson & Johnson, and Taiho Pharmaceutical. M.I. has received honoraria from Merck Serono, Taiho Pharmaceutical, and Yakult Honsha, and has a consulting/advisory role with Taiho Pharmaceutical and Yakult Honsha. N.T. has received research funding from Chugai Pharmaceutical and Taiho Pharmaceutical. Y.H. has received honoraria from Bayer, Chugai Pharmaceutical, Kaken Pharmaceutical, Merck Serono, Sanofi, Taiho Pharmaceutical, and Takeda Pharmaceutical, and has received research funding from Chugai Pharmaceutical, Kaken Pharmaceutical, Merck Serono, Sanofi, Taiho Pharmaceutical, and Takeda Pharmaceutical. S.T. and T.M. are employed at Sysmex and own stock in Sysmex. E.S., S.H., Y.T., H.I., T.K., M.M, M.S., M.T., H.O., S.O., and K.H. have no conflicts of interest to disclose.

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AUTHOR'S CONTRIBUTIONS

S.T. and T.M.: Quality control of data and algorithms. E.O., E.S., M.S., S.T., T.M., K.H.: Data analysis and interpretation. M.S.: Statistical analysis. E.O., E.S., M.S., S.T., T.M., K.H.: Manuscript preparation. E.O., E.S., S.Y., M.I., S.H., Y.T., H.I., T.K., M.M., N.T., Ma.S., M.T., H.O., Y.H., S.O., S.T., T.M., K.H.: Study concepts, Study design, Data acquisition, Manuscript editing, and Manuscript review.

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Figures

Figure 1

CONSORT diagram

Figure 2

Five-year relapse-free survival (RFS) curves of all patients treated with [I-OHP(+), indicated in red] and without oxaliplatin [I-OHP(-), indicated in blue].

Figure 3

Five-year relapse-free survival (RFS) curves of patients treated with [I-OHP(+), indicated in red] and without oxaliplatin [I-OHP(-), indicated in blue] according to 55-gene classifier (55GC) subtypes [top: microsatellite instability (MSI)-like; middle: chromosomal instability (CIN)-like; bottom: stromal].

Left-sided tumor

Right-sided tumor

Figure 4

Five-year relapse-free survival (RFS) curves of patients treated with [I-OHP(+), indicated in red] and without oxaliplatin [I-OHP(-), indicated in blue] according to 55-gene classifier (55GC) subtypes and primary tumor sidedness (top: left-side; bottom: right-side).

Supplementary Files

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