Fractional Genomic Alteration Detected by Array-Based Comparative Genomic Hybridization Independently Predicts Survival after Hepatic Resection for Metastatic Colorectal Cancer

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ABSTRACT

Purpose: Although liver resection is the primary curative therapy for patients with colorectal hepatic metastases, most patients have a recurrence. Identification of molecular markers that predict patients at highest risk for recurrence may help to target further therapy.

Experimental Design: Array-based comparative genomic hybridization was used to investigate the association of DNA copy number alterations with outcome in patients with colorectal liver metastasis resected with curative intent. DNA from 50 liver metastases was labeled and hybridized onto an array consisting of 2,463 bacterial artificial chromosome clones covering the entire genome. The total fraction of genome altered (FGA) in the metastases and the patient’s clinical risk score (CRS) were calculated to identify independent prognostic factors for survival.

Results: An average of 30 ± 14% of the genome was altered in the liver metastases (14% gained and 16% lost). As expected, a lower CRS was an independent predictor of overall survival (P = 0.03). In addition, a high FGA also was an independent predictor of survival (P = 0.01). The median survival time in patients with a low CRS (score 0-2) and a high (≥20%) FGA was 38 months compared with 18 months in patients with a low CRS and a low FGA. Supervised analyses, using Prediction Analysis of Microarrays and Significance Analysis of Microarrays, identified a set of clones, predominantly located on chromosomes 7 and 20, which best predicted survival.

Conclusions: Both FGA and CRS are independent predictors of survival in patients with resected hepatic colorectal cancer metastases. The greater the FGA, the more likely the patient is to survive.

INTRODUCTION

Resection is the primary curative therapy for colorectal cancer patients with isolated hepatic metastases (1). Long-term survival occurs in 20% to 40% of these patients (2) and may be enhanced by the delivery of postoperative systemic and regional chemotherapy (3). Several clinical variables have been found to be predictors of survival, including the five variables used in the validated clinical risk score (CRS): nodal status of the primary tumor, length of disease free interval from primary to metastasis, number of hepatic tumors, size of largest hepatic tumor, and preoperative carcinoembryonic antigen level (1). Long-term survival approaches 60% among individuals with a favorable low CRS, whereas it is <15% among those with a high CRS (1). However, significant uncertainty remains for the individual patient. As a result, there is a great deal of interest in evaluating molecular markers as predictors of outcome to help with clinical decisions.

The development and progression of colorectal cancer is a multistep process leading to the accumulation of genomic alterations that occur over the lifetime of a tumor (4). Central to that process is a loss of genomic integrity, with most colorectal cancers demonstrating gross chromosomal aberrations and abnormalities of nuclear DNA content (aneuploidy), whereas some tumors show microsatellite instability and have few cytogenetic abnormalities (5–7). Some studies have linked specific genomic alterations with clinical outcome in colorectal cancer patients, whereas others have examined the relationship of global measures of genomic integrity with outcome (8–10). A much smaller number of studies have focused these efforts on patients with hepatic metastases (11–17).

Array-based comparative genomic hybridization (array CGH) allows for high-throughput, high-resolution, genome-wide screening of DNA copy number changes in solid tumors (18–20). Array CGH can provide an overview of the extent of genomic damage in a tumor at the chromosomal and subchromosomal level. In addition, specific copy number alterations detected by array CGH can be associated with clinical outcome, and in turn these copy number alterations can

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be directly related to sequence information to aid in the localization and identification of genes with predictive and prognostic potential (21–25).

We previously have applied array CGH to primary colorectal tumors and were able to identify alterations in several small, previously uncharacterized genomic regions, such as on chromosomes 8 and 20 (26). In the current study, array CGH was used to investigate the association of DNA copy number alterations with clinical outcome in a set of colorectal liver metastases resected with curative intent.

**MATERIAL AND METHODS**

**Patients and Samples.** After approval by the University of California, San Francisco (UCSF) institutional review board, DNA was extracted from hepatic metastases of 50 patients with colorectal cancer removed with curative intent by a single surgeon (R.S.W.) at UCSF between 1989 and 2002. No cases of familial colorectal cancer were included. There was no evidence of extrahepatic disease, and in each case the hepatic resection margin was tumor free based on pathology review. The tumors were surgically dissected and immediately frozen at −80°C. All samples were reviewed histologically to assure that >70% tumor cells were present before DNA extraction. DNA was extracted as previously described (24, 25). Lymphocytic DNA from normal individuals was used as reference. Clinicopathologic data were obtained by written and telephone questionnaire and review of medical records. Patients are contacted at a minimum of every 3 to 6 months to assess disease-free and overall survival. The CRS was calculated using five preoperative variables with one point assigned to each of the following: node positive primary tumor, disease-free interval from primary to metastasis of <12 months, number of hepatic tumors >1, largest hepatic tumor >5 cm, and carcinoembryonic antigen level >200 ng/mL. A score of 0 to 2 was considered a low CRS score, whereas a score of 3 to 5 was considered a high score (1).

**Array-Based Comparative Genomic Hybridization.** The arrays used in the study were prepared and hybridized as described previously (24–26). Human 1.14 arrays were obtained from the UCSF Cancer Center Array Core. The arrays consisted of 2,463 bacterial artificial chromosome clones that covered the human genome at a 1.5-Mb resolution.

Each tumor DNA sample was hybridized to the array as described previously, with minor modifications (26). Five hundred nanograms of tumor and reference DNA were fluorescently labeled with Cy3- and Cy5-dUTP by random priming. Hybridization was done for 48 hours in a formamide-based buffer with Cot-1 DNA (Invitrogen, Carlsbad, CA) added to block repetitive sequences. Three, 16-bit fluorescence single-color intensity images (4′,6-diamidino-2-phenylindole, cy3, and cy5) were collected from each array using a charge-coupled device camera (Sensys, Photometric, equipped with a Kodak KAF 1400 chip) coupled to a 1× optical system.

**Data Analysis.** Image data were analyzed by Spot and Sproc software as previously described (24–26). Spot exclusion criteria included the removal of unmapped clones and clones appearing in fewer than 70% of the samples. Applying these selection criteria resulted in reducing the clone number from 2,463 to 2,170 clones. Removal of polymorphic clones further reduced the overall clone number to 2,151 clones.5

A series of 10 reference DNA versus reference DNA hybridizations were done to define the normal variation of the test to reference log2 intensity ratio for each target clone. A slight clone to clone variabiliry in the intensity ratios was observed, but the overall coefficient of variation was <10%. The log2 ratios for each case were median centered to zero. The threshold for determining chromosome gain or loss was defined as log2 ratio >0.225 or less than −0.225. This threshold corresponded to values between 2 and 3 SDs from the mean (see refs. 19, 21, 25). In addition, high-level amplifications were defined as a log2 ratio >0.9 and high-magnitude deletions as log2 ratio less than −0.75. These thresholds were derived from analyses of cell lines with known gene amplifications and homozygous loss at defined loci. The threshold for gain or loss of an entire chromosome arm was defined as a median log2 ratio of >0.14 or less than −0.14 for all clones on the chromosome arm. The fraction of the genome gained or lost for each case was calculated as the sum of genomic distances represented by each clone.

**Microsatellite Instability.** Tumor microsatellite instability status was determined using the BAT26 microsatellite marker using standard methods (27). Tumors were defined as having microsatellite instability if a change of length mutation of at least 3 bp was detected in the BAT26 locus when comparing normal with tumor DNA.

**Statistical Analysis.** The primary outcome of interest was overall survival, calculated from the date of hepatic resection to the date of death. Disease-free survival was considered as a secondary end point. Cox proportional hazards models were used for the univariate and multivariate analyses. All significant baseline variables from the univariate analysis (P < 0.10) were included. Model choice was done using a backward selection with P < 0.05 as the exit criterion. All survival probabilities were estimated using the Kaplan-Meier method. The association of CRS and fraction of genome altered (FGA) with outcome was assessed with the variables defined in either continuous or dichotomous fashion. When analyzed as a dichotomous variable, low CRS was defined as a score of 0 to 2, and high CRS as a score of 3 to 5. Low FGA was defined as <20% of the genome altered and high FGA as ≥20%, based on the greatest separation between survivors and nonsurvivors.

Differentially expressed clones between tumor subgroups were selected using Significance Analysis of Microarrays (SAM) analysis, and Prediction Analysis of Microarrays (PAM) was used to aid in the identification of clones that best predicted class (28, 29). For these analyses, patients were categorized as having either a good prognosis (alive for ≥24 months) or a bad prognosis (death before 24 months). Clone subsets were identified by limiting the false discovery rate to ~25% for SAM or optimizing the cross-validated misclassification error rate for PAM to limit the number of clones in the predictive sets. Clones sets in common between the two analyses were then further analyzed by PAM to determine the overall classification.

5 Clone list is available in Mehta.polymorphisms found at: http://cc.ucsf.edu/people/waldman/colon/mehta.html.
success rate, using a threshold of 0. Because multiple comparisons may result in a large number of individual clones appearing significant by chance alone, significance for individual clones was determined by a MaxT test using permutation analysis to control for family-wise false positive error rates (30).

RESULTS

Clinical Characteristics and Outcomes. Fifty patients who underwent resection of colorectal cancer hepatic metastases with curative intent were the subjects of this study. None of the patients had evidence of extrahepatic disease at the time of their hepatic resection, all had resection margins free of tumor, and all survived a minimum of 30 days after their operation. The clinicopathologic and treatment characteristics of the study population are listed in Table 1.6 The median follow-up for these patients was 30 months. At last follow-up, 42% of the patients were alive and 26% were without recurrent disease. The median time to death after hepatic resection was 24 months and the median time to disease recurrence was 10 months. Five-year survival after hepatic resection was 37% (95% confidence interval, 22-52).

Array-Based Comparative Genome Hybridization in Colorectal Cancer Hepatic Metastases. Array CGH was done on hepatic metastases from all 50 patients. An average of 660 clones were gained or lost in tumor DNA samples, comprising 30% of the genome (312 clones gained representing 14 ± 8% of the entire genome and 348 clones lost representing 16 ± 7% of the genome; Fig. 1A and B).7 The most commonly altered regions (gained or lost in ≥50% of cases) were gains within chromosome arms 7p, 7q, 8q, 13q, and 20q and losses within 4p, 4q, 8p, 17p, 18p, 18q, and 22q (Fig. 2). The mean number of clones demonstrating high-level amplifications and very low level deletions were 16 and 17 per case, respectively (representing 0.8 ± 1% of the entire genome amplified and 0.8 ± 1% of the genome deleted). High-level amplifications were found most frequently among clones mapping to 20q, whereas clones demonstrating very low level deletions mapped most commonly to 8p and 18q. The six clones amplified more than 20% of the time were all on 20q (RP11-13418, 32.3 Mb; RP11-138A15, 37.8 Mb; RP11-29H19, 44.3 Mb; RP11-169A6, 45.4 Mb; RP11-15M15, 53.4 Mb; and RMC 20P073, 57.8 Mb). The four clones that showed very low-level deletion ~15% of the time mapped to 8p (GS21 77L23, 0 Mb; RP11-51C1, 23.5 Mb) and 18q (RP11-104N111, 40.4 Mb; RP11-60P1, 70.9 Mb).

Microsatellite Instability. Three cases (6%) were judged to be microsatellite unstable based on the presence of change of length mutations of BAT26. The average FGA in these three microsatellite instability cases was 21.0 ± 10.5%, whereas the FGA in the microsatellite stable cases was 29.9 ± 13.2%.

Predictors of Clinical Outcome Clinical Risk Score. The CRS was predictive of both overall and disease-free survival in the Cox univariate analysis (P = 0.03; Table 1). The median overall survival among patients with a low CRS (score of 0-2) was 33 months (mean, 40.2 months), compared with 24 months (mean, 29.3 months) among those with a high CRS (score of 3-5). The median disease-free survival among patients with a low CRS was 14 months (mean, 28.0 months), compared with 12 months (mean, 16.3 months) among those with a high CRS.

Genomic Alterations. In the univariate analysis, analyzed as either a continuous or dichotomous variable, the greater the FGA, the greater the likelihood of both overall (P = 0.01) and disease-free survival, although the latter did not reach statistical significance. The median overall survival for individuals with tumors with ≥20% of the genome altered was 33 months (mean, 39.6), compared with 21 months (mean, 20.8) among those with a low FGA (<20% FGA). The median disease-free survival was 14 months (mean, 25) among patients with a high FGA, compared with 12 months (mean, 14.1) among those with a low FGA.

To validate the association between high FGA and longer survival, the total number of chromosome arms gained or lost was determined and tested against outcome. The total number of chromosomal arms altered was predictive of overall survival (Cox P = 0.01), with a greater number of chromosomal arm alterations associated with better outcome. Individual alteration of three chromosome arms, gain of 7p (P = 0.02), gain of 7q (P = 0.03), and loss of 8p (0.03), were predictive of greater overall survival in the univariate analysis.

The microsatellite instability status of the tumors was not predictive of overall or disease-free survival.

Supervised analyses using PAM and SAM was done to identify a set of clones that best predicted which patients survived for ≥24 months and which patients died within 24 months. A set of 111 clones was identified that best distinguished between the two groups, with an overall classification success rate of 81%. These clones were predominantly located on chromosomes 7 and 20.

Further analysis was undertaken to determine if any individual bacterial artificial chromosome clones showed a significant association with overall survival. The MaxT test, using permutation analysis to correct for the multiple comparisons problem, was used. No individual clones were found to be significantly associated with overall survival.

Multivariate Analysis. Both CRS and FGA were independent predictors of overall survival when analyzed as either continuous (P = 0.01) or dichotomous variables (Table 2). Patients with a low CRS or a high FGA also had a longer disease-free survival, although the association of CRS and FGA with disease-free survival did not reach statistical significance in the multivariate analysis (Table 2). The median survival time in patients with a low CRS and a high FGA was 38 months (mean, 47) compared with 18 months (mean, 20) for low CRS and a low FGA (P = 0.005; Fig. 3). If the total number of chromosome arms altered was substituted for the FGA in the multivariate analysis, both total number of arms altered and CRS were independent predictors of overall survival (P = 0.03 and P = 0.02, respectively). The greater the number of

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6 Data set is available in Mehta.clinicaldata found at: http://cc.unc.edu/people/waldman/colon/mehta.html.
7 Data set is available in Mehta.data found at: http://cc.unc.edu/people/waldman/colon/mehta.html.
chromosome arms altered, the greater the likelihood of survival. No individual chromosome arm, or clone, was an independent predictor of overall or disease-free survival when analyzed together with CRS and/or FGA.

Interaction between Fraction of the Genome Altered and Clinicopathologic Variables. The FGA showed no association with the clinicopathologic variables tested, CRS, and its component parts. To test whether the association between FGA and survival was dependent on the delivery of systemic and/or regional chemotherapy, we compared outcomes in those patients with high versus low FGA, and in patient subsets who did or did not receive therapy. No interaction was seen between treatment status and FGA with respect to overall survival.

**DISCUSSION**

The CRS is predictive of survival in patients with colorectal cancer hepatic metastases who undergo potentially curative resection (1). However, much heterogeneity remains with respect to outcome, even among those with similar scores. Evaluation of molecular markers may help in the assessment of prognosis and affect future treatment strategies. In the current study, array CGH was applied to a series of 50 hepatic metastases resected for cure to determine if specific genomic loci or the degree of genomic instability provide prognostic information beyond that available from the assessment of routine clinicopathologic variables. The chromosomal regions altered in these metastases were similar to those already described in our previous study of primary colorectal tumors (26). In the current study we show that FGA

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**Table 1** Clinicopathologic predictors of overall and disease-free survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (%)</th>
<th>Overall survival, mean ± SD (mo)</th>
<th>P</th>
<th>Disease-free survival, mean ± SD (mo)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>64.2 ± 11.6 y (mean ± SD)</td>
<td>35.2 ± 23.63</td>
<td>NS*</td>
<td>23.0 ± 24.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>1.9 ± 14.1</td>
<td></td>
<td>19.7 ± 17.1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (50%)</td>
<td>36.4 ± 24.7</td>
<td>NS</td>
<td>24.4 ± 26.5</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>25 (50%)</td>
<td>31.5 ± 15.2</td>
<td></td>
<td>19.0 ± 16.4</td>
<td></td>
</tr>
<tr>
<td>Met number</td>
<td>2.6 ± 2.5 (mean ± SD)</td>
<td>41.3 ± 24.1</td>
<td>0.02</td>
<td>26.8 ± 28.0</td>
<td>NS</td>
</tr>
<tr>
<td>1</td>
<td>20 (40%)</td>
<td>29.1 ± 16.2</td>
<td></td>
<td>18.3 ± 16.6</td>
<td></td>
</tr>
<tr>
<td>≥1</td>
<td>30 (60%)</td>
<td>37.7 ± 21.1</td>
<td>0.03</td>
<td>24.7 ± 23.9</td>
<td>NS</td>
</tr>
<tr>
<td>Met size (cm)</td>
<td>5.4 ± 2.9 cm (mean ± SD)</td>
<td>33.5 ± 16.5</td>
<td>NS</td>
<td>19.6 ± 16.7</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;5</td>
<td>29 (58%)</td>
<td>34.8 ± 27.1</td>
<td></td>
<td>25.9 ± 29.9</td>
<td></td>
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<tr>
<td>≥5</td>
<td>21 (42%)</td>
<td>37.8 ± 18.8</td>
<td></td>
<td>17.6 ± 18.9</td>
<td></td>
</tr>
<tr>
<td>Met timing</td>
<td></td>
<td>33 (66%)</td>
<td></td>
<td>19.6 ± 16.7</td>
<td>NS</td>
</tr>
<tr>
<td>Synchronous</td>
<td>33 (66%)</td>
<td>34.8 ± 27.1</td>
<td></td>
<td>25.9 ± 29.9</td>
<td>NS</td>
</tr>
<tr>
<td>Metachronous</td>
<td>17 (34%)</td>
<td>38.7 ± 21.5</td>
<td></td>
<td>26.0 ± 24.8</td>
<td></td>
</tr>
<tr>
<td>Preoperative CEA (ng/mL)</td>
<td>111 ± 227 ng/mL (mean ± SD)</td>
<td>33.3 ± 21.2</td>
<td>NS</td>
<td>19.9 ± 20.8</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;200</td>
<td>44 (88%)</td>
<td>41.5 ± 34.5</td>
<td></td>
<td>29.3 ± 38.0</td>
<td></td>
</tr>
<tr>
<td>≥200</td>
<td>6 (12%)</td>
<td>32.9 ± 18.1</td>
<td>NS</td>
<td>20.7 ± 19.4</td>
<td>NS</td>
</tr>
<tr>
<td>Node status</td>
<td></td>
<td>33.3 ± 21.2</td>
<td></td>
<td>19.9 ± 20.8</td>
<td>NS</td>
</tr>
<tr>
<td>Node +</td>
<td>15 (30%)</td>
<td>35.6 ± 19.1</td>
<td>NS</td>
<td>26.0 ± 24.8</td>
<td></td>
</tr>
<tr>
<td>Node –</td>
<td>35 (70%)</td>
<td>38.7 ± 21.5</td>
<td></td>
<td>26.0 ± 24.8</td>
<td></td>
</tr>
<tr>
<td>CRS</td>
<td>2.5 ± 0.9 (mean ± SD)</td>
<td>40.2 ± 23.4</td>
<td>0.03</td>
<td>28.0 ± 29.2</td>
<td>NS</td>
</tr>
<tr>
<td>0-2</td>
<td>20 (44%)</td>
<td>29.3 ± 18.2</td>
<td></td>
<td>16.3 ± 14.7</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>25 (56%)</td>
<td>38.7 ± 21.5</td>
<td>NS</td>
<td>31.1 ± 26.7</td>
<td></td>
</tr>
<tr>
<td>Primary stage</td>
<td></td>
<td>33.3 ± 20.5</td>
<td>NS</td>
<td>19.7 ± 20.9</td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>9 (18%)</td>
<td>38.7 ± 21.5</td>
<td>NS</td>
<td>31.1 ± 26.7</td>
<td></td>
</tr>
<tr>
<td>III-JV</td>
<td>40 (82%)</td>
<td>33.3 ± 20.5</td>
<td></td>
<td>19.7 ± 20.9</td>
<td></td>
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<tr>
<td>Tumor grade</td>
<td></td>
<td>38.7 ± 21.5</td>
<td>NS</td>
<td>31.1 ± 26.7</td>
<td></td>
</tr>
<tr>
<td>Well/moderate</td>
<td>46 (92%)</td>
<td>35.3 ± 20.7</td>
<td>0.01</td>
<td>22.9 ± 22.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Poor</td>
<td>4 (8%)</td>
<td>19.3 ± 8.8</td>
<td></td>
<td>8 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Systemic/regional chemotherapy</td>
<td></td>
<td>34.0 ± 15.2</td>
<td>NS</td>
<td>20.4 ± 16.7</td>
<td>NS</td>
</tr>
<tr>
<td>post resection</td>
<td>Yes</td>
<td>34.0 ± 26.9</td>
<td></td>
<td>23.8 ± 28.5</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20 (40%)</td>
<td>34.0 ± 26.9</td>
<td></td>
<td>23.8 ± 28.5</td>
<td></td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td></td>
<td>34.2 ± 20.2</td>
<td>NS</td>
<td>21.5 ± 21.2</td>
<td>NS</td>
</tr>
<tr>
<td>Stable</td>
<td>44 (94%)</td>
<td>33.5 ± 28.7</td>
<td>NS</td>
<td>24.5 ± 33.7</td>
<td>NS</td>
</tr>
<tr>
<td>Unstable</td>
<td>3 (6%)</td>
<td>34.2 ± 20.2</td>
<td>NS</td>
<td>21.5 ± 21.2</td>
<td>NS</td>
</tr>
<tr>
<td>FGA (%)</td>
<td>30.3 ± 13.7% (mean ± SD)</td>
<td>20.8 ± 10.9</td>
<td>0.01</td>
<td>14.1 ± 12.1</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;20</td>
<td>15 (30%)</td>
<td>39.6 ± 21.1</td>
<td>NS</td>
<td>25 ± 24.5</td>
<td></td>
</tr>
<tr>
<td>≥20</td>
<td>35 (70%)</td>
<td>39.6 ± 21.1</td>
<td></td>
<td>25 ± 24.5</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NS, not significant; Met, metastasis; CEA, carcinoembryonic antigen.

*Not significant, P ≥ 0.10.
is an independent predictor of overall survival and adds important predictive information to that available when using the CRS alone.

Some previous reports have found that greater genomic alteration in a tumor is associated with poorer outcome (31–38), whereas others have either seen no such relationship (13, 15, 39–41) or, as in this study, have found that greater genomic damage is associated with better outcome (42). Rooney et al. showed that a greater number of chromosomal arm aberrations detected by conventional CGH was associated with improved survival in patients with node positive colorectal cancer (42). Scott et al. did not observe an increase in aneuploidy in higher stage tumors, but rather observed a decrease in DNA index in lymph node metastases (39). In that study, 40% of higher stage cancers were diploid tumors, whereas all low-stage tumors were aneuploid. Crowe et al. found no correlation between overall or disease-free survival and the degree of aneuploidy in a set of 71 hepatic colorectal cancer metastases removed for cure (15). On the other hand, Risques et al. found that greater aneuploidy correlated with tumor stage, metastasis, and poor outcome in colorectal cancers, but, interestingly, they also found a subset of diploid tumors with no evidence of microsatellite or chromosomal instability that were highly aggressive and had a poor prognosis (43).

Some of the differences reported in the relationship between genomic instability and outcome in colorectal cancer may stem from differences in the stage of the tumors studied. Across all studies, the extent of genomic alteration increases as one moves from adenoma to invasive cancer to metastasis, regardless of the methodology used to assess it (44–47). Most studies also find that there is an increased extent of genomic alteration with progression in early-stage tumors, and that extent of genomic alteration is a marker for poor outcome in these early cancers (31–34, 36). However, the association between the extent of genomic alteration and outcome is much less clear in studies, including ours, that have focused on advanced tumors (13, 15). The results of the current study are consistent with the hypothesis that genomic instability is required for cancer progression. However, too much instability may result in accumulated damage that exceeds a threshold for cell viability (48). Instability leading to increased genomic diversity may be especially critical for the progression of early-stage tumors (44, 49, 50), but ongoing instability may even be counterproductive for continued growth and further metastatic spread of advanced tumors (48). In the current study, the average FGA was 30%, higher than previously found in a set of primary tumors also analyzed by array CGH (26). Among the current set...
of advanced tumors, however, instability was associated with less aggressive behavior.

The hypothesis that the association between the extent of genomic instability and survival may depend on the stage of colorectal cancer is supported by data regarding microsatellite unstable tumors found in patients with hereditary nonpolyposis colorectal cancer (HNPCC). Early high-level genomic instability, in this case microsatellite instability rather than chromosomal instability, which occurs in HNPCC leads to an increased rate of adenoma formation and transition from adenoma to invasive carcinoma (51–53). However, invasive cancers in HNPCC are less likely to metastasize, and survival is better, than in sporadic microsatellite stable tumors matched for stage and location (54–56). One possible explanation is that once invasive cancer has developed, ongoing high rates of genomic instability, regardless of the type, may decrease tumor growth or viability.

In the current study, PAM and SAM analyses did define a set of ~100 clones, predominantly located on chromosomes 7 and 20, whose alteration was associated with better survival. The results of our PAM and SAM analyses are not dissimilar to those recently reported by Knosel et al. (38). In the latter study, using conventional CGH, loss of 18q or gain of 20q was associated with significantly longer disease-free survival. Our study did not confirm the often reported association between genomic alterations involving 8p, 8q, 17p, 18q, or 20q and worse outcome in primary colorectal cancer (8–10), as well as a report showing allelic imbalance of 17p was an independent predictor of worse overall and disease-free survival in a set of 141 hepatic metastases from colorectal cancer resected for cure (13).

Limitations of the current study include the limited sample size, the fact that the extent of genomic alteration was assessed using only a single experimental method, and the fact that this is a retrospective study of patients followed outside of a clinical trial, and thus had no established protocol for tumor surveillance. The results need to be confirmed in an independent and larger set of metastatic colorectal tumors, using both array CGH and alternate techniques to measure global genomic alteration. Establishing whether a similar association between genomic instability and outcome also holds in earlier stage tumors would be of obvious clinical interest. A larger set of patients will allow a more complete exploration of the relationship between specific genomic alterations and outcome, as well as confirm the finding that FGA is an independent predictor of survival. The choice of overall survival as the primary end point in the study was because of the certainty with which that end point could be assessed in this retrospective analysis. Although disease-free survival was greater in patients with a low CRS or a high FGA, the inability to find a statistically significant association may be related to the lack of an established protocol to detect tumor recurrence in the study population.

In summary, the greater the total FGA in resected hepatic colorectal cancer metastases, as determined by array CGH, the more likely patients were to survive. The improved survival in patients with a greater FGA was independent of their CRS. In addition to confirming this finding, future research will explore the biological mechanisms, such as the relationship between proliferation and apoptosis and the FGA, which may account for this association. The ultimate translation of these findings, once validated, to the clinical environment will require modifications of these research techniques to allow for timely and robust assessment of genomic instability of tumor samples in the clinical laboratory.

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REFERENCES


**Table 2** Multivariate analysis

<table>
<thead>
<tr>
<th>End point</th>
<th>Hazard (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGA*</td>
<td>0.37 (0.17-0.82)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRS†</td>
<td>2.12 (0.91-4.91)</td>
<td>0.08</td>
</tr>
<tr>
<td>Disease-free survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGA*</td>
<td>0.71 (0.34-1.48)</td>
<td>0.35</td>
</tr>
<tr>
<td>CRS†</td>
<td>1.49 (0.73-3.02)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

**Abbreviation:** CI, confidence interval.

*High FGA defined as ≥20%.
†High CRS defined as 3 to 5.


Fractional Genomic Alteration Detected by Array-Based Comparative Genomic Hybridization Independently Predicts Survival after Hepatic Resection for Metastatic Colorectal Cancer

Kshama R. Mehta, Kentaro Nakao, Marlene B. Zuraek, et al.


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