DNA copy number profiles of primary tumors as predictors of response to chemotherapy in advanced colorectal cancer

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Background: Colorectal cancer (CRC) is biologically a heterogeneous disease, which may affect response to drug therapy. We investigated the correlation of genome-wide DNA copy number profiles of primary tumors with response to systemic chemotherapy in advanced CRC.

Patients and methods: DNA was isolated from formaldehyde-fixed paraffin-embedded primary tumors of 32 patients with advanced CRC, which were selected based on either a good response (n = 16) or a poor response (n = 16) to first-line combination therapy with capecitabine and irinotecan. High-resolution DNA copy number profiles were obtained by means of 30 K oligonucleotide-based array comparative genomic hybridization (aCGH).

Results: Unsupervised hierarchical cluster analysis of the aCGH data revealed two clusters of 19 and 13 tumors, respectively, and cluster membership showed a significant correlation with response status (P < 0.03). The nonresponders had fewer chromosomal alterations compared with the responders, in particular less losses were found (P < 0.03). Most prominent differences between the two groups were losses of regions 18p11.32–q11.2 (P < 0.02) and 18q12.1–q23 (P < 0.03), which were more frequently observed in responders.

Conclusions: Differences in DNA copy number profiles of primary CRCs are associated with response to systemic combination chemotherapy with capecitabine and irinotecan. Responders overall had more chromosomal alterations, especially loss of chromosome 18.

Key words: array comparative genomic hybridization, chemotherapy, chromosomal alterations, colorectal cancer, predictive marker

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer death in the western world with nearly 204 000 deaths per year in Europe [1]. Clinical outcome mainly depends on the tumor stage at time of diagnosis. In patients with advanced disease, the response to systemic therapy is a major determinant of prognosis.

Approximately 50% of all CRC patients develop distant metastases and will ultimately die of the disease. In patients with advanced CRC, 5-fluorouracil (5-FU) in combination with leucovorin (LV) has been the standard drug therapy for many years. During the past decade, median overall survival (OS) times have further increased by the incorporation of new cytotoxic drugs like irinotecan and oxaliplatin. Furthermore, the oral fluoropyrimidine prodrug capecitabine has been proven a useful alternative to i.v. 5-FU [2]. Recently, incorporation of the antiangiogenic drug bevacizumab has increased survival as well [3]. While overall these new drug regimens have resulted in increased response rates and prolonged median survival, treatment strategies are still based on a ‘one size fits all’ approach to which only a subset of patients will respond. Therefore, predictive markers are needed to identify those patients who will maximally benefit from the available treatment options [4].

For predicting response to 5-FU, most research has been focused on enzymes involved in its mechanism of action like thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase, which plays a role in the activation of capecitabine [5–9]. Recently, selection of advanced CRC patients with a low expression of TS and DPD doubled response rate to 5-FU/LV compared with no selection. Yet, still 60% of the patients did not respond [9].

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addition, microsatellite instability status maybe a predictor of 5-FU-based therapy [10].

Response to DNA-topoisomerase I inhibitor irinotecan has been associated with expression levels of the target Top-1 [11, 12] and of uridine diphosphate-glucuronosyltransferase (UGT1A1), which metabolizes SN-38, the active metabolite of irinotecan [13–15].

In addition to these specific enzymes, other factors are likely to influence response to therapy. CRC is a heterogeneous disease, and this biological diversity can be determined at the DNA level, messenger RNA level and protein level. CRC also shows heterogeneous phenotypes and differences in clinical behavior, including the risk of metastasis and the response to therapy. We are just starting to understand the interactions between this biological and clinical heterogeneity. A substantial part of the biological diversity in CRC occurs at the level of chromosomes, giving rise to DNA copy number alterations [16]. Moreover, recent data show that DNA copy number profiles maybe indicative for the response to therapy [17–20]. The aim of the present study was to test whether genome-wide DNA copy number profiles of primary tumors can predict the response to combination therapy with capecitabine and irinotecan in advanced CRC.

patients and methods

patients
Thirty-nine patients with previously untreated advanced CRC, who received first-line combination chemotherapy with capecitabine and irinotecan, were selected from the series of the CAIRO study, a randomized phase III study of sequential versus combination chemotherapy of the Dutch Colorectal Cancer Group. Details on patient eligibility, study design and results have been published [21]. The study was approved by the Central Committee of Human-Related Research and by the local ethics committees of all participating centers.

chemotherapy
Patients received capecitabine (1000 mg/m²) twice daily for 14 days plus irinotecan (250 mg/m²) on day 1 at intervals of 3 weeks. Tumor response was confirmed by CT scan after 4 weeks and in case of stable disease (SD) (less than a 20% increase in the sum of the longest diameter of all measured lesions and the appearance of no new lesions), after a period of 6 weeks. Treatment was continued until disease progression or unacceptable toxicity, whichever came first.

For the present study, best observed response was used to classify patients into two groups. Of the 39 patients, 19 achieved CR or PR as best response (responder group) and 20 patients had progressive disease (nonresponder group). Median progression-free survival (PFS) in first-line treatment for responders was 11.6 months (range 8.9–23.2) and for nonresponders 2.1 months (range 1.5–4.1).

DNA isolation
DNA from primary tumors and matched normal tissue, resected before chemotherapy, was isolated from formaldehyde-fixed paraffin-embedded tissue, using an extensively validated protocol [16]. Tumor tissue was microdissected from marked tumor rich areas on hematoxylin-stained sections, by scraping off tumor tissue with a surgical blade, as described before. Two of 39 samples were excluded because of tumor cell content <80% [23].

array comparative genomic hybridization
High-resolution DNA copy number profiles were obtained by means of oligonucleotide-based array comparative genomic hybridization (aCGH) as described by van den Ijssel et al. [24]. Arrays contained 60mer oligonucleotides, representing 28 830 unique genetic locations designed by Compugene (San Jose, CA).

Normal DNA of the same patient was hybridized as reference for every aCGH experiment to avoid contamination of somatic DNA copy number alterations in the tumor with copy number variations and segmental duplications present in germline DNA [25] since the current high-resolution aCGH platforms are more sensitive to this bias when (pooled) reference DNA of other sources is used than previously used classical CGH or bacterial artificial chromosome array platforms.

aCGH data analysis
Log2 ratios (tumor/normal reference signal) of each aCGH experiment were normalized by subtraction of the mode value of the log2 ratios of all the oligonucleotides on chromosomes 1–22.

The quality of the DNA isolated from formalin-fixed paraffin-embedded tissue may vary, which can affect the signal quality of aCGH data. Therefore, a measure of variation, the median absolute deviation (MAD) value, was calculated as a quality measure of the final aCGH data. In the present study, 32 of 37 experiments had MAD values ≤0.22 and were accepted for analysis. Mean MAD value of these 32 tumors was 0.16 (range 0.10–0.22); the five tumors excluded had considerably higher MAD values (mean of 0.29, range 0.23–0.32) [26].

CGHcall software [27] was used for the calling of gains, high-level amplifications and losses and converting log2 ratios into ordinal data, i.e. ‘+1’ for gains, ‘+2’ for high-level amplifications, ‘−1’ for losses and ‘0’ if no DNA copy number alterations were present.

We used the CGH regions algorithm to reduce our dataset to chromosomal regions of losses, gains and high-level amplifications, using a threshold of 0.01 [28].

unsupervised hierarchical cluster analysis
To analyze the distribution of whole-genome DNA copy number profiles in these advanced CRC samples, unsupervised hierarchical cluster analysis was carried out using Weighted clustering of called aCGH data (WECCA) [29].

Clustering of aCGH data as discrete levels rather than continuous log2 ratios greatly improves the sensitivity and the specificity [30]. WECCA was applied to the region data, and weight was assigned to each region depending on the number of oligonucleotides (1 = 1–50 oligonucleotides; 2 = 51–150 oligonucleotides; 3 = 151–250 oligonucleotides, until 11 = 1051–1150 oligonucleotides).

statistical analysis
To determine differences in clinicopathological features between responder and nonresponder patients, the Mann–Whitney U test for comparing means of continuous variables between the two groups and the two-sided Fisher’s exact test and the chi-square test for testing significance of differences in distribution of categorical variables were used, respectively, where applicable. Survival analysis, applied on region data, was carried out by applying the Kaplan–Meier method.
Kaplan–Meier survival analysis with log-rank testing. SPSS version 15.0 statistical software package (SPSS Inc., Chicago, IL) was used.

To calculate differences in DNA copy numbers between tumors of responders and nonresponders, we carried out a Wilcoxon test with ties, the P values of which were corrected for multiple testing using a permutation version of false discovery rate (FDR). To gain statistical power and allow easier interpretation of the results, calculations were carried out using regions of gains and losses (defined as described above) [28], rather than the individual 30 K oligonucleotides. P values of <0.05 (after FDR correction where applicable) were considered statistically significant.

**results**

**patients**

Patient and tumor characteristics were well balanced between 16 responders and 16 nonresponders. Responder

Table 1. Clinical and pathologic characteristics of patients in relation to their response status

<table>
<thead>
<tr>
<th></th>
<th>All eligible patients (n = 32)</th>
<th>Responders (n = 16)</th>
<th>Nonresponders (n = 16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year (mean ± SD)</td>
<td>60.5 ± 10.2</td>
<td>60.9 ± 9.3</td>
<td>60.1 ± 11.4</td>
<td>0.79</td>
</tr>
<tr>
<td>Sex, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 (66)</td>
<td>10 (62.5)</td>
<td>11 (69)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>11 (34)</td>
<td>6 (37.5)</td>
<td>5 (31)</td>
<td></td>
</tr>
<tr>
<td>Site of primary tumor, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>16 (50)</td>
<td>9 (56)</td>
<td>7 (44)</td>
<td>0.50</td>
</tr>
<tr>
<td>Rectum</td>
<td>7 (22)</td>
<td>4 (25)</td>
<td>3 (19)</td>
<td></td>
</tr>
<tr>
<td>Tumor grade, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>1 (3)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0.59</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>16 (50)</td>
<td>8 (50)</td>
<td>8 (50)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>15 (47)</td>
<td>7 (44)</td>
<td>8 (50)</td>
<td></td>
</tr>
<tr>
<td>Predominant localization of metastases, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>26 (81)</td>
<td>14 (87.5)</td>
<td>12 (75)</td>
<td>0.65</td>
</tr>
<tr>
<td>Extrahepatic</td>
<td>6 (19)</td>
<td>2 (12.5)</td>
<td>4 (25)</td>
<td></td>
</tr>
<tr>
<td>Performance status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>16 (100)</td>
<td>15 (94)</td>
<td>15 (94)</td>
<td>1.00</td>
</tr>
<tr>
<td>PR</td>
<td>31 (97)</td>
<td>16 (100)</td>
<td>15 (94)</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td></td>
</tr>
<tr>
<td>Serum LDH, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23 (72)</td>
<td>12 (75)</td>
<td>11 (69)</td>
<td>1.00</td>
</tr>
<tr>
<td>Abnormal</td>
<td>9 (28)</td>
<td>4 (25)</td>
<td>5 (31)</td>
<td></td>
</tr>
<tr>
<td>MRR status, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMMR</td>
<td>31 (97)</td>
<td>16 (100)</td>
<td>15 (94)</td>
<td>1.00</td>
</tr>
<tr>
<td>dMMR</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td></td>
</tr>
<tr>
<td>Survival, months (median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>4.1</td>
<td>12.9</td>
<td>2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OS</td>
<td>17.3</td>
<td>31.8</td>
<td>8.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase; MMR, proficient mismatch repair system; pMMR, proficient mismatch repair system; dMMR, deficient mismatch repair system; PFS, progression-free survival; OS, overall survival.

patients had a significantly better PFS and OS, both P < 0.001 (Table 1).

unsupervised hierarchical cluster analysis divides tumors into two clusters

Unsupervised hierarchical cluster analysis of the aCGH data revealed two clusters of 19 and 13 tumors. Thirteen of 19 tumors of cluster 1 consisted of nonresponders, while 10 of 13 tumors of cluster 2 were responders (Figure 1). Cluster membership showed a significant correlation with response status (P < 0.03).

differences in DNA copy number profiles between responders and nonresponders

The average number of chromosomal alterations per tumor was 6.3 (median 5.0, range 0–19), with a mean number of 3.2 gains (median 3.0, range 0–9) and 3.2 losses (median 1.5, range 0–12). Less alterations were observed in the 16 nonresponders (P < 0.2) compared with the 16 responders, especially for losses (P < 0.03). The median number of chromosomal alterations of the 16 responders was 7.0 (range 1–19), with a median number of 3.0 gains (range 1–8) and 3.5 losses (range 0–12). For the 16 nonresponders, the median chromosomal alterations was 4.0 (range 0–14), with a median number of 2.5 gains (range 0–9) and 0 losses (range 0–12). The number of chromosomal alterations per response status (CR, PR and PD) is presented in Table 2. Eight tumors (three responders and five nonresponders) had high-level amplifications, which were distributed over the whole genome (Table 3).

In Figure 2 frequencies of gains and losses per oligonucleotide are plotted. Most frequently observed chromosomal alterations (i.e. >30%) in the 16 responders were loss of 1p36.33–36.23 (31%), 3p21.31 (31%), 17p13.3–p11.2 (50%), 18p (50%), and 22q13.21–q13.33 (38%) and gain of 8q12.22–q24.13 (31%), 8q24.13–q24.33 (38%), 13q (63%), 20p13–p12.11 (31%), 20q11.21–q13.32 (50%) and 20q13.33 (44%). For the 16 nonresponders the most frequent alterations were gain of 13q (median of 38%), 20q11.21–q13.32 (56%) and 20q13.33 (38%).

The 16 nonresponders had more gain of the regions 16p11.2–q12.1 and 16q12.2, whereas the 16 responders had more loss of the same regions (P = 0.03). However, the FDR value of 0.31 for both regions on chromosome 16 indicates that the relevance of this finding should be interpreted with caution (Table 4). Loss of whole chromosome 18 was significantly more frequent in the 16 responders. Loss of the region 18p11.32–q11.2 was observed in eight responders, whereas in nonresponders, a loss was found only once (P < 0.02, FDR = 0.06). Loss of 18q12.1–q23 was observed in nine responders, while in the group of nonresponders, two losses of this region were found (P < 0.03, FDR = 0.06).

Given the fact that these patients were treated with DNA-topoisomerase I inhibitor irinotecan, the response status was checked against the called DNA copy number ratio for TOP1 on chromosome 20q. Responders and nonresponders did not show significant differences in frequency of DNA copy number changes of TOP1. Gain of TOP1 was found in eight responders
Table 3. High-level amplifications per tumor in three responder patients and five nonresponder patients

<table>
<thead>
<tr>
<th>Samples</th>
<th>Regions</th>
<th>Amplicon size (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR-4</td>
<td>16p11.2</td>
<td>0.46</td>
</tr>
<tr>
<td>PR-3</td>
<td>8q24.3</td>
<td>0.23</td>
</tr>
<tr>
<td>PR-7</td>
<td>17p11.2</td>
<td>1.24</td>
</tr>
<tr>
<td>CR, complete response; PR, partial response; PD, progressive disease.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Unsupervised hierarchical cluster analysis of 32 advanced colorectal cancer samples, based on chromosomal gains and losses detected by array comparative genomic hybridization. Rows represent chromosome 1 till 22 (different chromosomes are indicated by alternating blue and yellow colors) and every column represents a separate tumor. Green cells represent gains, red cells represent losses, white cells represent amplifications and black cells indicate no abnormality. Two clusters emerged, cluster 1 containing mainly nonresponders and cluster 2 responders.

Table 2. Chromosomal alterations of the 32 tumors according to response status

<table>
<thead>
<tr>
<th>Response Status</th>
<th>Total</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>8.3</td>
<td>6.0</td>
<td>2–19</td>
</tr>
<tr>
<td>Losses</td>
<td>19</td>
<td>4.8</td>
<td>3.5</td>
<td>0–12</td>
</tr>
<tr>
<td>Gains</td>
<td>14</td>
<td>3.5</td>
<td>3.0</td>
<td>1–7</td>
</tr>
<tr>
<td>Ampl.</td>
<td>2</td>
<td>0.5</td>
<td>0.0</td>
<td>0–2</td>
</tr>
<tr>
<td>Partial response (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>7.8</td>
<td>7.0</td>
<td>1–18</td>
</tr>
<tr>
<td>Losses</td>
<td>53</td>
<td>4.4</td>
<td>3.5</td>
<td>0–12</td>
</tr>
<tr>
<td>Gains</td>
<td>40</td>
<td>3.3</td>
<td>3.0</td>
<td>1–8</td>
</tr>
<tr>
<td>Ampl.</td>
<td>3</td>
<td>0.3</td>
<td>0.0</td>
<td>0–2</td>
</tr>
<tr>
<td>Progressive disease (n = 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>4.8</td>
<td>4.0</td>
<td>0–14</td>
</tr>
<tr>
<td>Losses</td>
<td>29</td>
<td>1.8</td>
<td>0.0</td>
<td>0–12</td>
</tr>
<tr>
<td>Gains</td>
<td>48</td>
<td>3.0</td>
<td>2.5</td>
<td>0–9</td>
</tr>
<tr>
<td>Ampl.</td>
<td>10</td>
<td>0.6</td>
<td>0.0</td>
<td>0–5</td>
</tr>
</tbody>
</table>

Ampl., high-level amplifications.
and nine nonresponders. Exact DNA copy number ratios for UGT1A1, an enzyme that metabolizes the active metabolite of irinotecan SN-38, were not available because no oligonucleotide for this gene was spotted on the array. DNA copy number changes of the oligonucleotides flanking the locus of UGT1A1 on chromosome 2q37 were uncommon overall. Only one patient from the responder group showed a gain of this region.

**Table 4.** Chromosomal areas which are significantly different between responder and nonresponder patients

<table>
<thead>
<tr>
<th>Region</th>
<th>Size (Mb)</th>
<th>Responder (n)</th>
<th>Nonresponder (n)</th>
<th>Difference between responder and nonresponder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gain</td>
<td>Ampl.</td>
<td>Loss</td>
<td>Gain</td>
</tr>
<tr>
<td>16p11.2–16q12.1</td>
<td>19.1</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>16q12.2</td>
<td>0.4</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>18p11.32–18q11.2</td>
<td>22.5</td>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>18q12.1–23</td>
<td>52.2</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>

Most significant regions, based on the criteria of P < 0.05 and low FDR value, are indicated in bold. Significance of the regions 16p11.2-16q12.1 and 16q12.2 was reached only when the data were dichotomized in ‘gains’ and ‘losses’. For the regions 18p11.32-18q11.2 and 18q12.1-23, data were dichotomized in losses and ‘no losses’.

Ampl., high-level amplification; FDR, false discovery rate.

**Figure 2.** Frequency plot of DNA copy number gains and losses throughout the genome in (A) 16 responders and (B) 16 nonresponders. x-axis displays clones spotted on the array sorted by chromosome and base pair position. y-axis displays frequency of tumors with gains (above zero) or losses (below zero). The boundaries of individual chromosomes are indicated by black vertical lines and location of centromeres are delineated by gray-dotted vertical lines.
survival analysis

Patients who showed a good response to chemotherapy had a significantly better PFS and OS than patients who did not respond to treatment, both \( P < 0.001 \) (Figure 3A and B).

Comparing chromosomal alterations of all 32 CRCs with the OS status of the patients showed that tumors with loss of 1p36 or gain of 13q have a significantly better OS than tumors without these alterations. On chromosome 1p36 loss of four

![Figure 3. Kaplan–Meier survival analysis of 32 patients with advanced colorectal cancer. Patients with a good response to combination therapy with capecitabine and irinotecan had a significantly better progression-free survival (PFS) (A) and overall survival (OS) (B) than nonresponders. Cumulative survival curves (C) till (F) show the chromosomal alterations that gave a significantly better OS: loss of 1p36.23–p36.32 (C) and loss of 1p36.11–p36.13, 1p36.21–p36.22 and 1p36.32–p36.33 had the same survival curve (D) and gain of 13q13.1–q14.3 (E) and also gain of 13q12.11 and 13q14.3–q14 had the same survival curve (F).]
regions, 1p36.33–p36.32, 1p36.32–p36.23, 1p36.22–p36.21 and
1p36.13–p36.11 had a significantly better OS, respectively,
P < 0.05, P < 0.01, P < 0.05 and P < 0.05 (Figure 3C and D).
The three regions with a significantly better OS distributed over
whole chromosome 13q were 13q12.11, 13q13.1–q14.3 and
13q14.3–q34, with a P value of P < 0.03, P = 0.02 and P < 0.03,
respectively (Figure 3E and F). Table 5 shows the size of these
regions and the number of oligonucleotides per region.

The most frequently observed alterations in the responders,
loss of regions 18p11.32–q11.2 and 18q12.1–q23, had no
significant impact on PFS (P < 0.06 and P = 0.11, respectively)
or OS (P = 0.11 and P = 0.35, respectively).

discussion

We demonstrate that genome-wide DNA copy number profiles
of primary tumors of advanced CRC patients significantly
correlate with response to combination therapy with
capcitabine and irinotecan. Unsupervised hierarchical cluster
analysis of the tumors yielded two clusters, one of which
contained mainly nonresponders and the other cluster
responders. Although unsupervised hierarchical cluster analysis
in a small dataset like the present harbors the risk of false-
positive findings, these results indicate that advanced CRC
patients are a heterogeneous group at the level of genome-wide
DNA copy number status and that these differences are relevant
for the response to chemotherapy. A large-scale validation
study is presently ongoing. This will also allow working out in
more detail which candidate genes at loci with discriminating
DNA copy number alterations are causally involved in the
response to drug therapy. A further point to be kept in mind is
that the current study design does not allow to discriminate
between DNA copy number alterations that provide an a priori
better prognosis in patients with advanced CRC and DNA copy
number alterations that reflect a better responsiveness of tumor
cells to the drug therapy given. To this end, a control arm
would be required that has not received any drug therapy at all,
and the CAIRO study did not contain such a control arm.

Analysis of the specific DNA copy number alterations that
differ between responders and nonresponders may unravel the
biology behind the response phenotype. In the present series,
we did not find any specific narrow DNA copy number
alterations that could point to a single specific gene involved.
However, the DNA copy number alterations that differ

Table 5. Chromosomal alterations which give a better overall survival,
when present in the tumor

<table>
<thead>
<tr>
<th>Region</th>
<th>Size (Mb)</th>
<th>Oligonucleotides (n)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of 1p36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1p36.33–32</td>
<td>0.54</td>
<td>15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1p36.32–23</td>
<td>6.16</td>
<td>55</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1p36.22–21</td>
<td>6.65</td>
<td>76</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1p36.13–11</td>
<td>9.53</td>
<td>139</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Gain of 13q</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13q12.11</td>
<td>3.91</td>
<td>27</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>13q13.1–14.3</td>
<td>21.81</td>
<td>176</td>
<td>0.02</td>
</tr>
<tr>
<td>13q14.3–34</td>
<td>61.23</td>
<td>230</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

Mb, megabase.

considerably between both response phenotypes involve large
alterations, such as loss of chromosome 18, which was
significantly more present in the responders. Furthermore,
tumors with loss of 1p36 or gain of 13q are associated with
a significantly better OS compared with tumors without these
alterations. This suggests that if gene dosage effects influence
response to drug therapy, this probably will be caused by
altered expression of multiple genes rather than one or a few
individual genes. There is ample evidence that low-level
chromosomal gains change the expression of many genes at
these altered loci [26, 31]. This may complicate the attempts to
unravel the mechanisms involved, but may yield novel
biomarkers, which will help to explain the effects of the drugs.
Chromosomal alterations have given more insight in the
process of tumor progression, e.g. chromosomal gain of 13q
and loss of 1p36 and 18q, and especially the tumor
suppressor genes on 18q, DCC and Smad4 (DPC4), have been
widely studied in colorectal carcinogenesis. DCC at 18q21.2 is
lost in 50%–70% of CRCs and plays a role in apoptosis
induction [32, 33] and Smad4 (DPC4) at 18q21 encodes
intracellular transducers of the transforming growth factor-β
apoptosis pathway. In adjuvant 5-FU-based chemotherapy,
retention of heterozygosity of 18q and positive expression of
DCC are predictors of positive outcome [32, 34–36]. This is
in contrast to the role in treatment of advanced disease such as
in the present study, where we show that loss of 18q is
associated with the response phenotype to therapy. A
possible explanation for this paradox is that the predictive
effect of 18q loss in adjuvant setting is blurred by the prognostic
effect of these alterations which is independent of adjuvant
therapy.

TYMS, the gene encoding TS, is located on 18p11.32 and low
TS expression levels in metastatic CRC are associated with
better response to 5-FU-based therapy [5, 8, 9]. Our
observations that advanced CRC patients with loss of the region
18p11.32–q11.2 that harbors the TYMS gene had a better
response to combined therapy with capcitabine and irinotecan
seem consistent with these results because loss of TYMS may
give low TS expression levels. Wang et al. [18] describe that
TYMS gene amplification is responsible for 5-FU resistance.
Amplification of the TYMS gene was found in metastases of
advanced CRC patients only after treatment with 5-FU and
these patients had a significantly worse OS in comparison to
patients without this amplification. This suggests that tumors
with loss of 18p11.32 could have a good response to
capcitabine treatment because no TYMS gene amplification
was possible.

Loss of 1p36 has been found to be associated with a lower
percentage of stroma in CRC [37], while CRCs with a lower
percentage of stroma have also been found to have a better
prognosis [38]. However, loss of 1p36 has also been correlated
with poor prognosis [39, 40] and metastasis [41]. In our series
of CRCs, loss of 1p36 was found in 19% of all primary tumors
and the OS in patients with tumors showing a loss of four
regions, 1p36.11–p36.13, 1p36.21–p36.22, 1p36.23–p36.32 and
1p36.32–p36.33, was significantly better compared with
patients with tumors without these alterations. In particular,
the frequencies of 1p36 deletion in responders and
nonresponders were 31% and 6%, respectively.
Table 6. Sensitivity, specificity, PPV and NPV for response to combined capecitabine and irinotecan therapy in a selected series of 32 advanced colorectal cancers, based on DNA copy number variables

<table>
<thead>
<tr>
<th>Cluster 2 membership</th>
<th>Responders</th>
<th>Nonresponders</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of 18p11.32–q11.2</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>13</td>
<td>0.63</td>
<td>0.81</td>
</tr>
<tr>
<td>Loss of 18q12.1–q23</td>
<td>8</td>
<td>8</td>
<td>1</td>
<td>15</td>
<td>0.50</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>14</td>
<td>0.56</td>
<td>0.88</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value.

To our knowledge, a correlation between 13q gain and survival has not been presented before.

Del Rio et al. [42] have found a gene expression signature of 14 genes in advanced CRC patients that maybe of value in the prediction of the response to chemotherapy. However, these data mainly concerned genes on other chromosomal regions than found in the present study. Their results were based on a relatively small sample size (n = 21) and this gene expression signature has to be still validated and improved with a larger cohort of patients. Moreover, overexpression of the 14 genes found by Del Rio et al. [42] could be due to other mechanisms than DNA copy number changes.

Although the aim of the present study rather was meant to provide proof of concept that differences in genome-wide DNA copy number profiles are associated with response to systemic therapy in advanced CRC, it is tempting to obtain an impression of the diagnostic potential of such markers. Therefore, sensitivity, specificity and predictive value, based on the current data, were computed for loss of 18p11.32–q11.2, loss of 18q12.1–q23 and cluster membership, acknowledging the limitations of this exercise (Table 6). Loss of 18p11.32–q11.2 and 18q12.1–q23 as markers of response to combined capecitabine and irinotecan had sensitivities of 50% and 56%, respectively, and specificities of 94% and 88%, respectively. Positive predictive values for response were 89% and 82%, respectively, and negative predictive values were 65% and 67%, respectively. When cluster membership was taken as test, sensitivity and specificity were 63% and 81%, respectively, with positive and negative predictive values for response of 77% and 68%, respectively.

In conclusion, in this pilot study, genome-wide DNA copy number profiling of primary tumors of advanced CRC patients revealed genomic loci, of which the copy number status may serve as predictive markers of response to systemic chemotherapy. Tumors of patients with a good response to chemotherapy had an increased number of chromosomal alterations, in particular loss of regions 18p11.32–q11.2 and 18q12.1–q23. A large-scale validation study, also using a higher-resolution aCGH platform, is presently ongoing and will learn whether the findings of the present study can be of actual value in the selection of patients for chemotherapy.

funding

references