Evaluation of Cancer-Associated DNA Copy Number Events in Colorectal (Advanced) Adenomas

Beatriz Carvalho1, Begoña Diosdado1, Jochim S. Terhaar Sive Droste2, Anne S. Bolijn1, Malgorzata A. Komor1, Meike de Wit1, Linda J.W. Bosch1, Myrthe van Burink3, Evelien Dekker4, Ernst J. Kuipers5, Veerle M.H. Coupee6, Nicole C.T. van Grieken3, Remond J.A. Fijneman1, and Gerrit A. Meijer1

Abstract

About 5% of colorectal adenomas are estimated to progress to colorectal cancer. However, it is important to identify which adenomas actually carry a high risk of progression, because these serve as intermediate endpoints, for example, in screening programs. In clinical practice, adenomas with a size of ≥10 mm, villous component and/or high-grade dysplasia, called advanced adenomas, are considered high risk, although solid evidence for this classification is lacking. Specific DNA copy number changes are associated with adenoma-to-carcinoma progression. We set out to determine the prevalence of cancer-associated events (CAE) in advanced and nonadvanced adenomas. DNA copy number analysis was performed on archival tissues from three independent series of, in total, 297 adenomas (120 nonadvanced and 177 advanced) using multiplex ligation-dependent probe amplification or low-coverage whole-genome DNA sequencing. Alterations in two or more CAEs were considered to mark adenomas as high risk. Two or more CAEs were overall present in 25% (95% CI, 19.0–31.8) of advanced adenomas; 23% (11/48), 36% (12/33), and 23% (22/96) of the advanced adenomas in series 1, 2, and 3, respectively, and 1.7% (1/58) and 4.8% (3/62) of the nonadvanced adenomas, in series 1 and 2, respectively. The majority of advanced adenomas do not show CAEs, indicating that only a subset of these lesions is to be considered high risk. Nonadvanced adenomas have very low prevalence of CAEs, although those with CAEs should be considered high risk as well. Specific DNA copy number alterations may better reflect the true progression risk than the advanced adenoma phenotype.

Introduction

Colorectal cancer is a major health care problem. Over 600,000 people worldwide die from this disease every year (1). Removal of colorectal cancer at an early, or even precursor, stage, that is, secondary prevention, is the most realistic approach to reducing colorectal cancer mortality rates. The two methods for colorectal cancer screening that are currently most widely applied include colonoscopy and fecal occult blood test, or fecal immunochemical test (FIT; refs. 2–4). The vast majority of these studies have not used colorectal cancer–related death as an endpoint, as this is reached only after many years. Instead, detection of colorectal cancer and/or advanced adenomas, collectively referred to as advanced neoplasia, is mostly used as an intermediate endpoint. In fact, in these studies, advanced adenomas outnumber cancers, making these lesions the dominant intermediate endpoint used in colorectal cancer screening studies. With an overall death rate of approximately 50%, colorectal cancer appears to be a realistic intermediate endpoint, but for advanced adenomas, this is much less clear.

Clinical, histopathologic, and molecular studies have established that colorectal adenomas are the major form of dysplasia or intraepithelial neoplasia of the colorectal mucosa and are precursor lesions of colorectal carcinomas (5–9). Yet, studies identifying the proportion and

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerpreventionresearch.aacrjournals.org/).

Corresponding Author: Beatriz Carvalho, Department of Pathology, Netherlands Cancer Institute, Amsterdam, the Netherlands. Phone: +31-20-661-2121; Fax: +31-20-661-2759; E-mail: b.carvalho@nk.nl
doi: 10.1158/1940-6207.CAPR-17-0317

©2018 American Association for Cancer Research.
specific category of adenomas progressing to cancer are not available and will not be performed as the required longitudinal study design would be unethical. We are therefore left with cross-sectional observational studies. On the basis of the prevalence of focal cancer in removed adenomas, it has been estimated that about 5% of colorectal adenomas may progress to cancer (10). Histopathologic features statistically associated with the presence of focal cancer in adenomas include size ≥10 mm, high-grade dysplasia, and tubulovillous or villous histology (5, 10). With reference to these observations, the concept of advanced adenomas has been introduced in the National Polyp Study (11), where adenomas with at least one of these histopathologic features are classified as advanced adenomas. Since then, the scientific community has adopted the concept of advanced adenoma as the surrogate marker for evaluating the effect of colorectal cancer screening. Because of the high prevalence of advanced adenomas, colorectal cancer screening yields many screened individuals who are enrolled in surveillance programs to such an extent that it takes up to 20% to 25% of the whole colonoscopy capacity (12). Using advanced adenoma as a proxy for the risk of dying from colorectal cancer, therefore, is an important cause of overdiagnosis and overtreatment.

As an alternative to formal longitudinal evidence, our increasing understanding of the biology of colorectal adenoma-to-carcinoma progression may serve as a substitute and provide biomarkers for a more precise identification of adenomas that are at truly high risk of malignant transformation.

The first molecular adenoma-to-carcinoma progression model included alterations in APC (5q loss), KRAS, TP53 (17p loss), and 18q loss (7). Some of these alterations occur already in adenomas at a much higher frequency than 5%, which is why they cannot discriminate progressive from nonprogressive adenomas (6, 7). For instance, constitutional loss of APC in the context of familial adenomatous polyposis can lead to hundreds to thousands of adenomas while only a few of these progress to cancer, making APC an adenoma gene rather than a cancer gene. On the other hand, the onset of aneuploidy, which reflects chromosomal instability, has been found to be associated with late-stage dysplasia and progression to cancer in multiple epithelial neoplasias. This includes tumor types in which progression can be followed longitudinally, like cervical intraepithelial neoplasia (13).

Detailed analyses of DNA copy number aberrations in colorectal neoplasia identified gains of 8q, 13q, and 20q, and losses of 8p, 15q, 17p, and 18q to be closely associated to adenoma-to-carcinoma progression, which is the reason why these are referred to as cancer-associated events (CAE; refs. 14–17). The presence of two or more of these CAEs was found to be significantly associated with a cancer phenotype. Moreover, in malignant polyps, that is, adenomas that actually had demonstrated progression from adenoma to cancer, these specific CAEs appeared to be as prevalent in the adenoma (i.e., premalignant) component of these lesions as in the invasive component (16). These CAEs were also demonstrated to affect the expression of important oncogenes and tumor suppressor genes located on those loci, such as TP53, SMAD4, AURKA, TPS2, as well as cancer-associated miRNAs (18–21).

In this study, we set out to characterize the prevalence of CAEs in nonadvanced and advanced adenomas, and to define their association with clinicohistopathologic features.

Materials and Methods

Study population and tissues

Three series of adenoma samples were analyzed in this study. Two series are from referral populations (series 1 and 2) and one series is from a screening population (series 3). All samples are from archival material (formalin-fixed paraffin-embedded tissue). Both series 1 and 2 were retrospectively collected from the archives of the Department of Pathology of the VU – University Medical Center (VUmc; Amsterdam, the Netherlands). Series 1 comprises 106 samples from 100 patients (mean age, 64.8 ± 11.7; 52.8% male) with colorectal adenomas (58 nonadvanced adenomas and 48 advanced adenomas) collected in the period between 1992 and 2008. Series 2 comprises 95 samples from 71 patients (mean age, 65 ± 10.2; 59% male) with colorectal adenomas (62 nonadvanced adenomas and 33 advanced adenomas) collected in the period between 2007 and 2011. Series 3 comprises 96 advanced adenomas from 78 patients (mean age, 62 ± 5.5; 59% male) collected during the Dutch invitational colorectal cancer screening trial COCOS (22). Two senior gastrointestinal pathologists (G.A. Meijer and N.C.T. van Grieken) revised the histology of all adenomas. Adenomas ≥10 mm with high-grade dysplasia and/or a villous component were defined as advanced adenomas, whereas tubular adenomas <10 mm with low-grade dysplasia were classified as nonadvanced adenomas. An overview of the samples based on size and histology is presented in Fig. 1. Collection, storage, and use of tissue and patient data were performed in agreement with the "Code for Proper Secondary Use of Human Tissue in The Netherlands" (available at: http://www.federa.org/).

DNA isolation

From each tissue block, 8 to 20 sections of 10 μm, depending on the tumor area available, were used for DNA extraction. DNA was isolated using the Qiagen QIAamp DNA Micro Kit (Qiagen Benelux B.V.) as described previously (23).
DNA copy number analysis: multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) was performed as described previously (24). In brief, two oligonucleotide probe mixes of previously described DNA copy number CAEs were used (16). The first probe mix was a custom-made mix focusing on copy number losses (X-006-CRC LOSS probemix, MRC-Holland BV) and included 22 genes on chromosomes 8p, 15q, 17p, and 18q. The second probe mix was designed to detect copy number gains (P146 - CRC gain probe mix, MRC-Holland BV) and contained probes for 34 genes on chromosomes 8q, 13q, and 20q. Reference probes (probes on chromosomes 2, 4, 12, and 16) were included.

Figure 1.
Overview of adenoma characteristics based on size and histology in series 1 (A), series 2 (B), and series 3 (C).

Cancer-Associated DNA Copy Numbers in Colorectal Adenomas

www.aacrjournals.org

Published OnlineFirst April 23, 2018; DOI: 10.1158/1940-6207.CAPR-17-0317
in both probe mixes for normalization purposes and quality control (Supplementary Table S1). A control sample containing no DNA, and positive and negative controls were included in each MLPA reaction; for probe mix X-006-CRC LOSS, these were cell lines Caco-2 (losses in chromosome 18q) and HCT116 (no losses) as positive and negative control, respectively. For probe mix P146 CRC gain, cell line HT29 (gains in chromosomes 8q, 13q, and 20q) was used as positive control.

From each sample, 100 ng DNA was used as input for the MLPA reaction. Each sample was run at least in duplicate. Coffalyser (version 8) was used for data analysis, using standard normalization method (25). In every sample, for every probe, a tumor-to-normal DNA copy number ratio was calculated using the default settings. A ratio <0.7 was considered a deletion, and >1.3 a gain. A chromosomal alteration (gain or loss) was considered when at least 50% of the probes of a specific chromosome arm were altered (26).

DNA copy number analysis: low-coverage whole-genome sequencing

Low-coverage whole-genome sequencing (WGS) was performed as described previously (27). Briefly, DNA was fragmented by sonication (Covaris S2) and run on the HiSeq 2000 (Illumina) on a 50-bp single-read modus using the Illumina TrueSeq Nano kit. Low-coverage WGS copy number data were analyzed using the Bioconductor package QDNAseq (27).

Raw sequence reads were uniquely aligned to the human reference genome build GRCh37/hg19 with Burrows–Wheeler alignment (28). Reads with mapping qualities lower then Q37 and PCR duplicated were filtered out. QDNAseq (27) was used to divide the human reference genome into nonoverlapping fixed-sized bins of 100 kb, and for each sample, estimates of the copy number were determined by counting the number of reads in each bin. Noise of the copy number profiles was reduced by a two-dimensional Loess correction for mappability and GC. Also, problematic genomic regions and common copy number variants were filtered out by a blacklist generated using germline sequence data from the 1000 Genomes Project (29). Genomic waves caused by replication timing were smoothed using NoWaves (30).

Binned copy number data from sequencing and aCGH were segmented using circular binary segmentation algorithm (31). CGHcall is a Bioconductor/R-package used to discretize the log2 ratios of the segments back to three states: loss, normal, and gain (32). By using CGHregions, the data were further reduced to common regions, with exclusion of regions smaller than 5 Mbp (33). Downstream analysis was focused on the DNA copy number aberrations described previously to be associated with adenoma-to-carcinoma progression (16), the CAEs.

Data are made available in the European Genome-Phenome Archive (EGA; https://www.ebi.ac.uk) under EGAS00001002953/ EGAD00001004075 and EGAS00001002952/ EGAD00001004078, for series 2 and 3, respectively.

Statistical analysis

Frequencies of CAEs were determined and differences between subgroups evaluated using a χ² test using two-sided testing, or Fisher exact test when there were less than 5 data points per variable. Correction for multiple testing was done by the Benjamin–Hochberg method (presented as q-value).

To investigate the relation between the presence of two or more CAEs on the one hand, and size, grade of dysplasia, and villous architecture on the other hand, we carried out a multivariate regression analysis using backward variable elimination. The P value cutoff for variable exclusion from the model was set at 0.1.

Data analysis was performed using SPSS version 24 (IBM SPSS Statistics). q-values (and P values from multivariate analyses) below 0.05 were considered to be statistically significant.

Results

CAEs are present in only a subset of colorectal advanced adenomas

Two or more CAEs, which indicates risk of progression, were present in 22.9% (11/48), 36.4% (12/33), and 22.9% (22/96) of the advanced adenomas in series 1, 2, and 3, respectively. In contrast, two or more CAEs were present in 1.7% (1/58) and 4.8% (3/62) of the nonadvanced adenomas in both series 1 and 2 (Supplementary Table S2). In addition, in series 1, loss of 17p was significantly more common in advanced adenomas compared with nonadvanced adenomas in series 1 and 2, respectively (Table 1). While acknowledging that the three series differ in, for example, setting (referral vs. screening population) and techniques used, the overall frequency of CAEs in advanced adenomas was 25.4% (45/177; 95% CI, 19.0–31.8; Fig. 2).

Gains of 13q and 20q were significantly more common in advanced adenomas compared with nonadvanced adenomas in both series 1 and 2 (Supplementary Table S2). In addition, in series 1, loss of 17p was significantly more common in advanced adenomas compared with nonadvanced adenomas (Supplementary Table S2). As series 3 contained advanced adenomas only, no comparison between advanced and nonadvanced adenomas was possible.

Specific CAEs are associated with histologic features of advanced adenomas

We investigated associations of CAEs with the respective histologic features, which define advanced adenomas (size ≥ 10 mm, high-grade dysplasia, and/or villous component), in more detail.

Univariate analyses showed that gains of 13q and 20q were more frequently present in colorectal adenomas
Cancer-Associated DNA Copy Numbers in Colorectal Adenomas

Table 1. Prevalence of cancer associated events (CAEs) according to histological advanced features in the adenomas analyzed

<table>
<thead>
<tr>
<th>Adenoma</th>
<th>≥2 CAEs n (%; 95% CI)</th>
<th>≥2 CAEs n (%; 95% CI)</th>
<th>≥2 CAEs n (%; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series 1 (n = 106)</td>
<td>Series 2 (n = 95)</td>
<td>Series 3 (n = 96)</td>
</tr>
<tr>
<td></td>
<td>Advanced</td>
<td>Nonadvanced</td>
<td>Advanced</td>
</tr>
<tr>
<td>Ade</td>
<td>11/48 (22.9; 12.0–37.3)</td>
<td>12/33 (36.4; 20.4–54.9)</td>
<td>22/96 (22.9; 15.0–32.6)</td>
</tr>
<tr>
<td>Nonadvanced</td>
<td>1/58 (1.7; 0–9.2)</td>
<td>3/62 (4.8; 1.0–15.5)</td>
<td>P = n.a.</td>
</tr>
<tr>
<td>Adjusted P</td>
<td>q = 0.01</td>
<td>q = 0.001</td>
<td>q = 0.01</td>
</tr>
<tr>
<td>Size ≥10 mm</td>
<td>10/39 (25.6; 13.0–42.1)</td>
<td>9/24 (37.5; 18.8–59.4)</td>
<td>17/76 (22.4; 13.6–33.4)</td>
</tr>
<tr>
<td>Nonadvanced</td>
<td>2/67 (3.0; 0.4–10.4)</td>
<td>6/71 (8.5; 3.2–17.5)</td>
<td>P = 0.8</td>
</tr>
<tr>
<td>Adjusted P</td>
<td>q = 0.01</td>
<td>q = 0.008</td>
<td>q = 0.9</td>
</tr>
<tr>
<td>Grade of dysplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>3/3 (33.3; 7.5–70.1)</td>
<td>1/3 (33.3; 0.8–90.6)</td>
<td>10/23 (43.5; 23.2–65.5)</td>
</tr>
<tr>
<td>Low</td>
<td>9/97 (9.3; 4.3–16.9)</td>
<td>14/92 (15.2; 8.6–24.2)</td>
<td>12/75 (16.4; 8.8–27.0)</td>
</tr>
<tr>
<td>Nonadvanced</td>
<td>P = 0.06</td>
<td>P = 0.4</td>
<td>P = 0.007</td>
</tr>
<tr>
<td>Adjusted P</td>
<td>q = 0.1</td>
<td>q = 0.5</td>
<td>q = 0.08</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular</td>
<td>6/76 (7.9; 3.0–16.4)</td>
<td>5/72 (6.9; 2.3–15.5)</td>
<td>8/44 (18.2; 8.2–32.7)</td>
</tr>
<tr>
<td>(Tubulo) villos</td>
<td>6/30 (20.0; 7.7–38.6)</td>
<td>10/23 (43.5; 23.2–65.5)</td>
<td>14/52 (26.9; 15.6–41.0)</td>
</tr>
<tr>
<td>Nonadvanced</td>
<td>P = 0.08</td>
<td>P = 0.001</td>
<td>P = 0.1</td>
</tr>
<tr>
<td>Adjusted P</td>
<td>q = 0.1</td>
<td>q = 0.001</td>
<td>q = 0.6</td>
</tr>
</tbody>
</table>

NOTE: Statistically significant at: P < 0.05 (uncorrected P value), q < 0.05 (adjusted P value).
Abbreviation: CI, confidence interval.

≥10 mm than in adenomas <10 mm in both series 1 and 2. Also, losses on 17p were more frequently present in adenomas ≥10 mm than in adenomas <10 mm, in series 1 (Supplementary Table S2).

Adenomas with high-grade dysplasia presented significantly more 20q gains than adenomas with low-grade dysplasia, in series 1. Gains of 13q were also significantly more frequent in adenomas with high-grade dysplasia in series 2. Similarly, 18q losses were significantly more frequent in adenomas with high-grade dysplasia than in adenomas with low-grade dysplasia in series 1 (Supplementary Table S2).

Adenomas with a villous component showed significantly more 20q gain than adenomas with tubular histology in both series 1 and 2. Moreover, this was also the case for gains on 13q, only in series 2 (Supplementary Table S2).

Two or more CAEs were significantly associated with adenomas ≥10 mm in series 1 and 2 and with villous component in series 2 (Table 1).

Multivariate analysis with backward selection showed that the presence of two or more CAEs was significantly associated with large size of the adenomas (P = 0.003) in series 1, with high-grade of dysplasia in series 3 (P = 0.01), and with villous component in series 2 (P < 0.001; Table 2).

Discussion

Colorectal adenoma-to-carcinoma progression occurs in about 85% of cases via the chromosomal instability pathway (16–18). The few studies investigating DNA copy number changes in colorectal adenomas have shown that such colorectal cancer-associated copy number aberrations are uncommon in adenomas (16, 18, 34). These data are consistent with clinical studies, which estimate the life risk of adenomas progressing into colorectal cancer to be approximately 5% (10).

We set out to determine in colorectal adenomas the prevalence of chromosomal events previously shown to be associated with adenoma-to-carcinoma progression (CAEs), namely gains of 8q, 13q, and 20q and losses of 8p, 15q, 17p, and 18q (16). Three sets of well-defined colorectal adenomas, including both nonadvanced and advanced adenomas, were used to investigate the correlation between the presence of CAEs and the defined clinical risk classification of colorectal adenomas. This revealed that 23% to 36% of advanced adenomas and 1.7% to 4.8% of nonadvanced adenomas presented two or more CAEs, which implies that only a minority of advanced adenomas...
and very few of the nonadvanced adenomas could be considered to actually be at risk of progressing to cancer. The fact that biological high-risk features such as CAEs occur in only a subset of advanced adenomas, which make up about 20% of all adenomas (22), is consistent with the estimated risk of progression in adenomas of around 5% (10). In cervical and breast cancers, in which the natural history of premalignant lesions can be better followed over time than for colorectal adenomas (which are removed once detected), it has also been observed that only a small proportion of these progress to invasive cancer, and that this is associated with the presence of DNA copy number changes (13, 35). Moreover, in earlier studies, DNA aneuploidy in premalignant lesions has been reported to be associated with progression to cancer (36, 37). Two recent studies have modeled colorectal adenoma-to-carcinoma progression by perturbing organoids with the molecular events from the Vogelstein model (9, 38). Interestingly, only organoids derived from adenomas that acquired DNA copy number changes were able to produce tumors in mice that generated distant metastases (9). Along the same line, a recent study on molecular characterization of 25 adenomas derived from 12 familial adenomatous polyposis patients showed that adenomas with 20q gain had a higher mutational rate than the average mutational rate of all adenomas. This supports the notion that these adenomas are at higher risk of progressing to cancer (39). Two recent studies have modeled colorectal adenoma-to-carcinoma progression by perturbing organoids with the molecular events from the Vogelstein model (36, 37). Two recent studies have modeled colorectal adenoma-to-carcinoma progression by perturbing organoids with the molecular events from the Vogelstein model (36, 37). Two recent studies have modeled colorectal adenoma-to-carcinoma progression by perturbing organoids with the molecular events from the Vogelstein model (36, 37). Two recent studies have modeled colorectal adenoma-to-carcinoma progression by perturbing organoids with the molecular events from the Vogelstein model (36, 37).

In this study, we also investigated to which clinicohistological features that define advanced adenomas the CAEs were associated. In univariate analyses, we observed that 13q and 20q gains, and 17p losses were associated with large adenomas (≥10 mm). Gains of 8q, 13q, and 20q as well as 18q losses were associated with adenomas with high-grade dysplasia. In addition, 8q, 13q, and 20q gains were associated with the presence of a villous component.

### Differences in associations were observed between the three series of samples. This suggests that these clinicohistological features are not very strong predictors of the presence of CAEs, which is in line with what is expected, that not all advanced adenomas would progress.

Through which mechanisms the genes located on these chromosomal arms contribute to adenoma-to-carcinoma progression requires further exploration. Functional studies to validate the relevance of these changes in adenoma-to-carcinoma progression will help identifying those adenomas that are actually at risk of progressing to cancer and consequently guide on the most appropriate screening tests to diagnose these lesions. Recently, a consensus molecular subtype classification of colorectal cancers was put forward (40), which showed to have implications for the clinical stratification of colorectal cancer. Such a molecular classification, based on DNA copy number changes, would also be desirable in the adenoma setting. Initiatives like the Pre-Cancer Genome Atlas will create the tools to achieve a comprehensive molecular knowledge on colorectal adenomas (41).

Our results show that colorectal cancer–associated DNA copy number changes in adenomas are likely to be a more specific intermediate endpoint for the risk of dying from colorectal cancer than the advanced adenoma phenotype. This may have substantial impact in screening, as in most colorectal cancer screening studies, advanced adenomas outnumber colorectal cancers as an intermediate endpoint. Depending on the screening modality, the effect of the intermediate endpoint used can differ. For example, colonoscopy has a high sensitivity for detecting advanced adenomas. However, as the majority of detected adenomas are not at high risk of progressing, the impact on the reduction of colorectal cancer–related death may be overestimated. On the other hand, FIT and molecular stool testing (42) have a relatively low sensitivity for detecting advanced adenomas.

### Table 2. Multivariate regression analysis using backward variable elimination for association of the presence of ≥2 CAEs with clinicohistologic advanced features in the adenomas analyzed

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>Mv. analysis bw. selection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Size</td>
<td>11.2 (2.3–54.4)</td>
<td>0.003</td>
<td>9.8 (1.8–55.2)</td>
</tr>
<tr>
<td>Grade</td>
<td>4.9 (1.0–23.0)</td>
<td>0.04</td>
<td>2.0 (0.3–16.6)</td>
</tr>
<tr>
<td>Histology</td>
<td>2.9 (0.9–9.9)</td>
<td>0.09</td>
<td>1.0 (0.2–4.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Series 2</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>Mv. analysis bw. selection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Size</td>
<td>6.5 (2.0–21.1)</td>
<td>0.002</td>
<td>3.4 (0.9–15.2)</td>
</tr>
<tr>
<td>Grade</td>
<td>2.8 (0.2–12.8)</td>
<td>0.4</td>
<td>0.4 (0.03–5.0)</td>
</tr>
<tr>
<td>Histology</td>
<td>10.3 (3.0–35.2)</td>
<td>0.007</td>
<td>7.1 (1.8–27.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Series 3</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>Mv. analysis bw. selection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Size</td>
<td>0.9 (0.3–2.7)</td>
<td>0.8</td>
<td>1.8 (0.5–6.5)</td>
</tr>
<tr>
<td>Grade</td>
<td>3.9 (1.4–11.0)</td>
<td>0.01</td>
<td>5.9 (1.8–19.6)</td>
</tr>
<tr>
<td>Histology</td>
<td>0.6 (0.2–1.6)</td>
<td>0.3</td>
<td>0.4 (0.1–1.2)</td>
</tr>
</tbody>
</table>

**NOTE:** Dependent: CAE ≥2. Mv. analysis bw. Selection = Multivariate analysis _backward selection._

Abbreviation: CI, confidence interval.
but it is unknown whether the detected adenomas are the ones with a high risk of progression. If this is the case, a seemingly low sensitivity would in fact be higher if molecular high-risk lesions are considered. Therefore, in the light of the results of the current study, it would be desirable to add molecular markers alongside advanced adenoma classification in studies evaluating colorectal cancer screening tests that use cross-sectional endpoints.

Defining adenomas that carry a high risk of progression is not only important for screening purposes, but also for surveillance after the detection and removal of adenomas. Surveillance program guidelines categorize individuals as low risk (one to two adenomas <10 mm) or high risk (villous histology, high-grade dysplasia, ≥10 mm in size or ≥3 adenomas) and suggest to follow up patients in 5 to 10 years, or in 3 years, respectively (43, 44). In this scenario, independent of the number of polyps found, and considering the results of the current study, 64% to 77% of the adenomas from high-risk individuals to be followed up in 3 years do not show molecular features of malignancy (two or more CAEs).

Well-organized studies on surveillance populations with molecular characterization of the lesions removed during surveillance will help clarify whether patients with adenomas harboring two or more CAEs are the ones that later again develop molecularly aggressive adenomas and/or cancers.

Although the sample size of the different series analyzed in the current study is not very large, the results show that, independent of the type of series (referral or screening population) or the type of methodology used to determine DNA copy number changes, the majority of advanced adenomas do not present with cancer-associated molecular features, and therefore are not at risk of progressing to cancer.

Genomic instability is a hallmark of cancer, and it appears to be a critical step in the progression from premalignancy to cancer in many epithelial cancers, including colorectal cancer. Next to chromosomal instability, markers of which were investigated in the current study, microsatellite instability (MSI) occurs in a subset of about 15% of colorectal cancer. This type of genomic instability was not investigated here. Although features of chromosomal instability, as demonstrated in the current study, occur in a sizable fraction of adenomas, MSI however is rarely observed in colorectal adenomas. Therefore, the MSI phenotype does not appear to be a suitable intermediate endpoint biomarker.

In conclusion, the current study showed that cancer-associated DNA copy number alterations (CAEs) are present in only a subset of advanced adenomas and are rare in nonadvanced and diminutive lesions. These CAEs may serve as more precise markers to assess the risk of a given adenoma progressing to cancer than the advanced adenoma phenotype. Adding molecular markers of the adenomas identified in colorectal cancer screening studies as additional intermediate endpoint markers is likely to increase the precision with which the performance of colorectal cancer screening tests can be estimated.

Disclosure of Potential Conflicts of Interest

E. Dekker reports receiving a commercial research grant from Fujifilm and is a consultant/advisory board member for Fujifilm, Olympus, and Tillots. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: B. Carvalho, B. Diosdado, R.J.A. Fijneman, G.A. Meijer

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.S. Terhaar Sive Droste, A.S. Boliijn, M. de Wit, L.J.W. Bosch, M. van Burink, E. Dekker, N.C.T. van Grieken

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B. Carvalho, B. Diosdado, M.A. Komor, M. de Wit, L.J.W. Bosch, V.M.H. Coupé, G.A. Meijer

Writing, review, and/or revision of the manuscript: B. Carvalho, B. Diosdado, J.S.T.S. Droste, A.S. Boliijn, M.A. Komor, M. de Wit, L.J.W. Bosch, E. Dekker, V.M.H. Coupé, N.C.T. van Grieken, R.J.A. Fijneman, G.A. Meijer

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Dekker

Study supervision: B. Carvalho, B. Diosdado, E.J. Kuipers, G.A. Meijer

Acknowledgments

This research was performed within the framework of the SU2C-DCS Dream Team Translational Cancer Research Grant (SU2C-AACR-DT1415; project MEOCC). Stand Up To Cancer is a program of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the Scientific Partner of SU2C. This research was also supported within the framework of the Center for Translational Molecular Medicine (CTMM), project DeCoDe (CTMM-03O-101), of the Dutch Cancer Society (KWF) project number 2006-4279 and KWF-fellowship (to B. Diosdado) NKI 2014-6635.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 6, 2017; revised March 4, 2018; accepted April 16, 2018; published first April 23, 2018.

References


www.aacrjournals.org
Carvalho et al.


Evaluation of Cancer-Associated DNA Copy Number Events in Colorectal (Advanced) Adenomas

Beatriz Carvalho, Begoña Diosdado, Jochim S. Terhaar Sive Droste, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-17-0317

Supplementary Material
Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2018/04/21/1940-6207.CAPR-17-0317.DC1

Cited articles
This article cites 44 articles, 12 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/11/7/403.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerpreventionresearch.aacrjournals.org/content/11/7/403. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.