DNA Alterations in Human Aberrant Crypt Foci and Colon Cancers by Random Primed Polymerase Chain Reaction

Liping Luo, Biaoru Li, and Theresa P. Pretlow

Institute of Pathology [L. L., T. P. P.] and Department of Biochemistry [B. L.], Case Western Reserve University, Cleveland, Ohio 44106

Abstract

Colon cancers are the result of the accumulation of multiple genetic alterations. To evaluate the role genomic instability plays during tumor development, we compared DNA fingerprints of 44 aberrant crypt foci (ACF; the earliest identified neoplastic lesion in the colon), 23 cancers, and normal crypts generated by random primers with PCR. The PCR products, separated by PAGE and viewed after silver staining, demonstrate altered fingerprints for 23.3% of the ACF and 95.7% of the cancers. In this first study of human ACF with this approach, the finding of altered DNA fingerprints in these microscopic lesions suggests that genomic instability can occur very early in human colon tumorigenesis.

Introduction

ACF (1) (Fig. 1A) are the earliest neoplastic lesions that can be detected microscopically in whole mounts of human colonic mucosa (1, 2). The prevalence of ACF is increased with familial adenomatous polyposis and colorectal cancer (1, 3, 4). Demonstrations of monoclonality (2) and similar genetic alterations (5, 6) in ACF suggest that ACF are precursors of cancer in human colon. Colorectal tumorigenesis is a stepwise process that involves multiple genetic alterations (7). Mismatch repair deficiency gives rise to microsatellite instability that characterizes hereditary nonpolyposis colorectal cancer. Microsatellite instability is also found in about 15% of sporadic colorectal cancers (8) and a similar proportion of ACF (9, 10). However, most colorectal cancers have multiple chromosomal abnormalities and a high frequency of loss of heterozygosity that are thought to be the result of general chromosomal instability or “CIN” (11). The RAPD method, which amplifies random DNA fragments with single primers of arbitrary nucleotide sequence, provides genomic profiles without prior sequence information and has been used to detect and localize allelic alterations in colon cancer (12–14). By comparing RAPD fingerprints of human ACF and colon cancers with those of normal crypts, genomic alterations were found in 22 of 23 colon cancers and in 10 (23.3%) of 43 ACF analyzed.

Materials and Methods

Samples. Human colon specimens were collected in 4°C saline by the Tissue Procurement Core Facility of the Comprehensive Cancer Center of Case Western Reserve University and University Hospitals of Cleveland. Strips of grossly normal mucosa (located between 4 and 28 cm from the cancer; mean, 14 cm) were separated from the submucosa, snap-frozen flat and viewed after silver staining, demonstrate altered fingerprints for 23.3% of the ACF and 95.7% of the cancers. In this first study of human ACF with this approach, the finding of altered DNA fingerprints in these microscopic lesions suggests that genomic instability can occur very early in human colon tumorigenesis.

Advances in Brief

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an average of the two standardized values was used. An allelic ratio of 2 or greater was considered a gain of a band; an allelic ratio of 0.5 or less was considered a loss of a band. This is in the same range as used by us and others to determine allelic loss (2, 18).

Results

Reproducible RAPD fingerprints from multiple patients (Fig. 1) were generated by PCR amplification of genomic DNA with each random primer or random primer pair. Samples 4004233 (Fig. 1C) and 4003641 (Fig. 1D) have similar RAPD fingerprints with multiple bands between 100 and 500 bp when amplified with the primer pair P4+P6. For sample 4004233 (Fig. 1C), there are multiple changes that occur in both the ACF and tumor, and additional alterations (gain of bands at a, b, and c) that occur only in the tumor when the RAPD bands are compared with those from normal crypts. For sample 4003641 (Fig. 1D), there are different alterations in both the ACF and the tumor; i.e., there is a loss of a band at d in the ACF that is not seen in the tumor, and there are two alterations in the tumor (a gain of a band at a, and a loss of a band at g) that are not seen in the ACF.

In addition, each random primer or random primer pair generated a unique RAPD fingerprint for each patient (Fig. 2). Amplifications were successful for 43 of 44 ACF samples; 10 (23.3%) of 43 ACF showed a gain and/or loss of RAPD bands compared with the fingerprints of their corresponding normal crypts. The cancer samples also had more altered RAPD fingerprints per primer than did the ACF (Fig. 3); 17 tumors exhibited genomic alterations with two or more random primers. The total number of altered RAPD fingerprints per successful amplification was low for ACF (12 of 309 or 3.9%) compared with cancer samples (77 of 134 or 57.5%; P < 0.01). The main genetic alterations are gain and/or loss of RAPD bands that are observed both in ACF and cancer samples compared with the fingerprints of normal crypts. In addition to these quantitative alterations, we also observed qualitative changes in the relative intensity of DNA bands between those from ACF or cancer samples and those from normal crypts (Fig. 1D, allele d in tumor).

Discussion

To our knowledge, this is the first report of altered DNA fingerprints in human ACF, the earliest identified neoplastic lesions in the colon (2). Genome-wide alterations identified with RAPD in 23.3% of

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5'-3'</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>CTT GCG CGC ATA CGC ACA AC</td>
<td>(13)</td>
</tr>
<tr>
<td>P2</td>
<td>AAC CCT CAC CCT AAC CCC AA</td>
<td>(13)</td>
</tr>
<tr>
<td>P3</td>
<td>AAC CCT CAC CCT AAC CCC GG</td>
<td>(22)</td>
</tr>
<tr>
<td>P4</td>
<td>CCC CAC CGG AGA GAA ACC</td>
<td>(23)</td>
</tr>
<tr>
<td>P5</td>
<td>GAT AGC CAG CAC AAA GAG AGC TAA</td>
<td>(23)</td>
</tr>
<tr>
<td>P6</td>
<td>CGA CGG TOT TTT GCA AGA TGT</td>
<td>(23)</td>
</tr>
<tr>
<td>P01</td>
<td>CCG GCT ACG G</td>
<td>(24)</td>
</tr>
<tr>
<td>P10A</td>
<td>ACG GTA CAC T</td>
<td>(12)</td>
</tr>
<tr>
<td>P10B</td>
<td>ACG GTA CAC G</td>
<td>(12)</td>
</tr>
<tr>
<td>PGKB</td>
<td>CCT ACA CGC GTA TAC TCC</td>
<td>(16)</td>
</tr>
</tbody>
</table>
ACF suggest that chromosomal instability is a very early event and might play a crucial role during the development of some colorectal cancers. There are previous reports of chromosomal instability as early as the polyp stage (19, 20), and some have suggested that genetic instability is required for the development of tumors (discussed in Refs. 20 and 21). Although RAPD was not used to identify genomic alterations in those polyp studies, Peinado et al. (13) used two arbitrary primers to identify genetic alterations in a large number of human colorectal cancers and a few polyps. By cloning and further analysis of the altered RAPD bands in tumors, they demonstrated that these altered bands are the result of losses or gains of DNA sequences in the original tumor (13). As compared with colorectal tumors, the smaller numbers of altered bands per human ACF and the smaller proportion of ACF with genomic alterations support the role of ACF as early precursors of some colorectal cancers. Luceri et al. (14), with 21 random primers, found genomic alterations in 16 of 16 colon tumors and 7 of 10 ACF induced in rats with azoxymethane. The finding of more alterations in rat ACF, compared with our study with human ACF, could be attributable to species differences, the use of more and/or different primers in the rat study, and/or the presence of more advanced lesions in rats treated with a carcinogen.

The advantages of RAPD for our studies are that it requires only small amounts of DNA to generate genome-wide fingerprints that can show multiple alterations and it does not require prior knowledge of DNA sequences. Two-stringency PCR was used in our first study as used previously in the study of human colon tumors (13). The initial, low-stringency condition allows a large number of hybridizations to take place throughout the genome; the second, high-stringency condition allows only the segments that closely match the primer to be amplified further. In addition we added primers in pair-wise combinations, which produce distinct genomic fingerprints different from those generated with either single primer alone (16). Whereas these conditions produced fingerprints that demonstrate multiple differences between normal mucosa and 95.7% of our cancer samples, they did not reveal as high a proportion (7 of 22, or 32%) of altered human ACF as had been reported in the rat study (14). In hopes of improving our results in the second experiment with 21 additional ACF, we used only four primers from our first experiment and added six new primers with a single stringency (12). This time only 3 of 21 or 14.3% of the ACF showed alterations. From these very limited experiments, it appears that the two-stringency PCR with the original primers, especially P4+P6 and P5, was more effective with our very small

Table 2. Human ACF with altered RAPD fingerprints

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of crypts in ACF</th>
<th>Size of ACF (mm²)</th>
<th>Location in colon</th>
<th>Dukes' stage</th>
<th>Age</th>
<th>Sex</th>
<th>Primer</th>
<th>Alteration of DNA bands</th>
<th>Gel in Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4002298</td>
<td>13</td>
<td>0.39</td>
<td>Left</td>
<td>B</td>
<td>75</td>
<td>F</td>
<td>P2</td>
<td>Gain</td>
<td>2A</td>
</tr>
<tr>
<td>4002483</td>
<td>58</td>
<td>1.78</td>
<td>Left</td>
<td>C</td>
<td>57</td>
<td>M</td>
<td>P5</td>
<td>Gain</td>
<td>2B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P2+P6</td>
<td>Gain</td>
<td></td>
</tr>
<tr>
<td>4002518</td>
<td>20</td>
<td>0.5</td>
<td>Left</td>
<td>B</td>
<td>70</td>
<td>M</td>
<td>P4</td>
<td>Gain/Loss</td>
<td>2C</td>
</tr>
<tr>
<td>4005933</td>
<td>109</td>
<td>3.25</td>
<td>Right</td>
<td>C</td>
<td>87</td>
<td>M</td>
<td>P2+P3</td>
<td>Gain/Loss</td>
<td>2D</td>
</tr>
<tr>
<td>4002293</td>
<td>41</td>
<td>1.17</td>
<td>Left</td>
<td>B</td>
<td>41</td>
<td>M</td>
<td>P4+P6</td>
<td>Loss</td>
<td>2E</td>
</tr>
<tr>
<td>4004233</td>
<td>48</td>
<td>2.28</td>
<td>Left</td>
<td>C</td>
<td>68</td>
<td>M</td>
<td>P4+P6</td>
<td>Loss</td>
<td>1C</td>
</tr>
<tr>
<td>4003641</td>
<td>40</td>
<td>1.18</td>
<td>Left</td>
<td>C</td>
<td>52</td>
<td>F</td>
<td>P4+P6</td>
<td>Loss</td>
<td>1D</td>
</tr>
<tr>
<td>4004689</td>
<td>33</td>
<td>1.2</td>
<td>Left</td>
<td>C</td>
<td>76</td>
<td>F</td>
<td>P4+P6</td>
<td>Loss</td>
<td>2F</td>
</tr>
<tr>
<td>4006039</td>
<td>26</td>
<td>0.61</td>
<td>Right</td>
<td>D</td>
<td>63</td>
<td>F</td>
<td>P5</td>
<td>Loss</td>
<td>2G</td>
</tr>
<tr>
<td>93-04-W219</td>
<td>65</td>
<td>3.23</td>
<td>Left</td>
<td>C</td>
<td>71</td>
<td>F</td>
<td>P5</td>
<td>Loss</td>
<td>2H</td>
</tr>
</tbody>
</table>
samples of human DNA. Consequently, our results likely underestimate the amount of chromosomal instability in human ACF.

The wide range of sizes of ACF, from 13 to 109 crypts, with altered fingerprints, also argues that chromosomal instability occurs very early in colon tumorigenesis. Some ACF (Fig. 2, A and C) have gains of DNA bands that are similar to those seen in the tumor samples from the same patient. These results suggest that these alterations observed in the tumors occurred early in the ACF and persisted in the final cancer. One ACF and tumor (Fig. 1C) show multiple similar losses of DNA bands, but the tumor shows additional alterations. This supports the hypothesis that ACF are early precursors that gain additional alterations to become cancer. However, several ACF (Figs. 1D and 2, B, C, E, and F) show losses or gains of DNA bands that are not seen in the cancers from the same patients. One possible explanation is that these changes observed in the ACF do not contribute to tumorigenesis, i.e., these ACF are not likely to persist. A second equally plausible explanation is that each tumor develops independently along its own unique pathway, and not every change observed in each ACF will be observed in all tumors.

In summary, the observations of altered fingerprints in microscopic lesions known as ACF suggest that chromosomal instability can occur very early in colon tumorigenesis and may be a driving factor of this process. Future studies of larger numbers of ACF and cancers with RAPD might aid in finding the earliest molecular changes that occur in colon tumorigenesis.

Acknowledgments

We thank Karen Stiffler and Erin Vittori for their technical assistance.

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