Microsatellite Instability in Colorectal Adenocarcinoma Cell Lines That Have Full-Length Adenomatous Polyposis Coli Protein

Christopher D. Heinen, Diane Richardson, Ray White, and Joanna Groden

Abstract

Almost 20% of colon cancers are characterized by genomic instability at simple repeated sequences. This instability is the result of a defective DNA mismatch repair system. Sporadic, as well as hereditary carcinomas of the proximal colon display this effect. In this study, we examined colorectal adenocarcinoma cell lines, with or without wild-type adenomatous polyposis coli (APC) protein, for the presence of microsatellite instability. The three cell lines that maintained full-length APC protein also displayed the highest level of instability, suggesting a negative correlation between APC mutations and microsatellite instability. This data, in addition to other studies that show a negative correlation between microsatellite instability and mutations in p53 and K-ras, support the idea of a second pathway for colorectal cancer development.

Introduction

A widely accepted model of tumorigenesis in colon cancer involves the accumulation of mutations in oncogenes and tumor suppressors, which include K-ras, p53, and APC. Mutations in the APC gene are present in individuals with familial APC and in most sporadic colorectal adenomas and carcinomas (2, 3). Overall, APC mutations have been identified in about 80% of colon tumors studied (4); virtually all of these mutations result in the absence or truncation of the full-length APC protein (5).

A subset of colorectal tumors is characterized by the addition or deletion of bases within simple dinucleotide repeat sequences. This microsatellite instability occurs most frequently in tumors of the proximal colon (6–8). These tumors are classified as replication error positive or RER+ and have a biochemical defect in strand-specific mismatch repair. This defect leads to cells with a mutator phenotype that can result in the accumulation of a high frequency of somatic mutations (9). These mutations may affect genes involved in the control of cell growth and differentiation. Interestingly, other studies have shown that the presence of microsatellite instability correlates negatively with mutations in p53 and K-ras (8).

In this study, the correlation between microsatellite instability and mutation in the APC gene was analyzed. Because microsatellite instability occurs in approximately 20% of colon cancers (6–8) and full-length APC is present in about 20% of colon cancers (4), the question was asked whether this microsatellite instability occurs in the same tumors that maintain full-length APC. Western blotting was used to examine APC protein in 12 colorectal carcinoma cell lines. These cell lines were then assessed for the presence of genomic instability by examining loci containing dinucleotide repeat sequences. Three of the 12 lines contained full-length, presumably wild-type APC protein: HCT116, SW48, and LS 174T. These same three cell lines displayed dinucleotide repeat instability at most of the loci tested, suggesting a negative correlation between APC mutations and microsatellite instability.

Materials and Methods

Twelve colorectal adenocarcinoma cell lines obtained from the American Type Culture Collection were analyzed: LS-174T, WiDr, DLD-1, HCT-15, SW620, SW480, SW48, Caco-2, HT-29, SK-Co-1, SW837, and HCT116. APC protein from total cell lysates was visualized by Western blotting by using mAb APC (Ab-1) (Oncogene Sciences, Cambridge, MA) as described (4).

DNA extracted from cell lines was amplified by PCR at microsatellite repeat loci D2S123, D5S392, D5S346, D7S471, D7S472, D7S473, D14543, ACTC, D17S261, and D18S34. PCR conditions consisted of 35–40 cycles at 95°C for 60 s, 52–58°C for 60 s, and 72°C for 60 s. PCR was performed by using 3 μCi of [32P]dATP incorporated into a 25-μl reaction. PCR products were electrophoresed on denaturing 7% polyacrylamide (19:1) gels and visualized by autoradiography.

Results and Discussion

Analyses of germline mutations in APC patients have revealed that almost all disease-causing mutations are frameshifts or premature stop codons that result in a truncated or deleted APC protein (5). Therefore, to analyze APC mutations in 12 colorectal adenocarcinoma cell lines, APC protein length from the cancer cell lines was compared to APC obtained from a normal lymphoblastoid cell line. The full-length APC protein electrophoresed at Mr, 312,000 as seen in Fig. 1a, Lane 6.

A similar length APC protein also was observed in three adenocarcinoma cell lines, SW48, LS-174T, and HCT116 (Fig. 1a). The APC protein from the remaining nine cell lines, however, ran noticeably shorter, indicating that mutations existed in the gene (Fig. 1, a and b). The APC gene from the HCT116 cell line was sequenced to confirm the wild-type status of APC in this cell line; no mutations were found in the coding region between exons 1 and 15 (10).

The APC mutation status of the 12 cell lines was compared to the presence or absence of microsatellite instability. Each cell line was tested for instability at ten dinucleotide repeat loci by using PCR primers that flanked the entire repeat region. In a stable cell line, either one or two alleles were expected for each locus depending on whether the cell line was homozygous or heterozygous. The presence of more than two alleles indicated instability at that locus. HCT116 was unstable at the D18S34 locus as evidenced by the appearance of multiple extra alleles (Fig. 2a, Lane 3). Again, an extra allele appeared in HCT116, as well as SW48 at the D7S473 locus (Fig. 2b, Lanes 3 and 5). The remaining cell lines displayed only one or two alleles and were scored as stable.

The number of alleles identified for each cell line at ten loci is shown in Table 1. Three cell lines clearly displayed instability at a relatively higher level than the rest. HCT116 was unstable at six of ten...
Fig. 1. Western blot of APC protein in colorectal adenocarcinoma cell lines. a. Lanes 1-6 contain protein from DLD-1, HCT116, SW48, LS-174T, 3407, and yeast cells transfected with a normal APC gene. b. Lanes 1-8 contain protein from HT-29, WiDr, LS-174T, SW48, SW40, SW837, SK-C0-1, and Caco-2. 3407 is a cell line derived from lymphocytes taken from an APC patient with one normal and one mutant copy of APC. Full-length APC is indicated by an arrowhead.

Table 1 Number of alleles identified at each dinucleotide repeat loci for 12 different colorectal adenocarcinomas

<table>
<thead>
<tr>
<th>Line/marker</th>
<th>D14S43</th>
<th>D18S34</th>
<th>DSS392</th>
<th>D7S471</th>
<th>D7S572</th>
<th>D7S473</th>
<th>D7S472</th>
<th>ACTC</th>
<th>DSS346</th>
<th>D17S261</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caco-2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DLD-1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>HCT-15</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HCT116</td>
<td>7</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HT-29</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>LS-174T</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>SK-C0-1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SW48</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SW480</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SW620</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SW697</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

- an unobtainable PCR product at that locus.

Dinucleotide repeat loci assayed by PCR to determine the number of alleles present at a particular marker for these colorectal cancer cell lines. PCR products were visualized by autoradiography. The presence of more than two distinct alleles was scored as unstable.

Although a large number of tissue samples from multiple tumors would need to be analyzed for microsatellite instability and APC protein content to confirm this data, the negative correlation between APC mutations and microsatellite instability suggests that a gene commonly involved in colorectal tumorigenesis may not be affected in tumors characterized by microsatellite instability. This association also has been observed for p53 and K-ras (8). The studies are consistent with an alternate pathway of genes involved in the development of tumors characterized by microsatellite instability (7).

This alternative pathway begins with mutations in DNA mismatch repair genes; hMSH2 (11), hMLH1 (12), PMS1 (13), or PMS2 (13). These genes have been shown to be mutated in sporadic colon cancers and hereditary nonpolyposis colon cancers characterized by microsatellite instability (6). As a result of insufficient DNA mismatch repair, a mutator phenotype arises in a cell that leads to the accumulation of further mutations throughout the genome, involving as yet, unidentified key genes for tumorigenesis. These mutations would circumvent the need for APC, p53, or Ras mutations in tumorigenesis. There has been at least one report of APC mutations in RER+ tumors (14). These authors identified two tumors with APC mutations in two hereditary nonpolyposis colon cancers families. However, one might predict that APC mutations could occur in RER+ tumors, although other genes are greatly favored as targets by the established mutator phenotype. Alternatively, the frequency of APC mutations in colorectal tumors is so high (>80%) that it would not be unusual to find some tumors with both mutation in APC and one of the DNA mismatch repair genes.

Four cell lines with truncated APC display microsatellite instability, albeit at a lower level than HCT116, SW48, and LS-174T. These include DLD-1 and HCT-15, reported previously to have a low level of microsatellite instability yet a high rate of mutation at the HPRT locus (15). DLD-1 and HCT-15 do not carry mutations in hMSH2 or hMLH1, rather they harbor mutations in the exonuclease domain of pol δ (16). The observations parallel studies of Saccharomyces cerevisiae where pol δ mutants have a lower level of microsatellite instability than yMSH2 and yMLH1 mutants (17).

That DLD-1 and HCT-15 contain only truncated APC may suggest that the mutation in pol δ in these cells is insufficient to initiate the novel tumorigenesis pathway observed in tumors with the RER*
phenotype. Mutations in the RER+ pathway may involve more than just bp mismatches, such as those observed in HPRT mutability assays, but include the strand slippage events that generate microsatellite instability as well. Fitting this description are mutations identified recently in the type II TGF-ß receptor in RER+ tumors. Small deletions were identified in simple repeat sequences within the TGF-ß receptor gene that resulted in frameshift mutations (18).

The idea of a mutator phenotype in cancer cells is widely accepted, as a cancer cell must accumulate mutations in several genes during its development. Mutations in genes involved in the early stages of tumorigenesis might affect the genomic stability of that cell, increasing the chance of acquiring subsequent key mutations. Although the instability seen in an RER+ tumor is not evident in every colon cancer case, mutations of other genes such as p53 (19, 20) and K-ras (21) are known to result in gene amplification and other chromosomal aberrations. This suggests a more general mechanism of tumor formation. Rather than a gradual accumulation of specific alterations in growth-controlling genes, the disrupted genes lead to an increase in the overall mutation frequency. APC mutations, early events in the progression of a cell from a normal to cancerous state, thus, may also affect genomic instability.

References

Microsatellite Instability in Colorectal Adenocarcinoma Cell Lines That Have Full-Length Adenomatous Polyposis Coli Protein

Christopher D. Heinen, Diane Richardson, Ray White, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/55/21/4797

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://cancerres.aacrjournals.org/content/55/21/4797. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.