Somatic Instability of the APC I1307K Allele in Colorectal Neoplasia

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Abstract

The adenomatous polyposis coli (APC) gene is proposed to function as a gatekeeper of colorectal neoplasia. A germ-line variant of this gene, the APC I1307K allele, is present in ~6% of the Ashkenazi Jewish population. To assess the role in tumorigenesis of the variant (A)4 insertion produced by this allele, we undertook a somatic mutation analysis of the region surrounding codon 1307 in colorectal tumors from APC I1307K carriers. Somatic mutations involving the variant (A)4 tract were identified in 53 of 127 (42%) tumors from APC I1307K carriers compared with 5 of 127 (4%) mutations involving the wild-type allele of these tumors (P < 0.0001). Loss of heterozygosity of the wild-type allele was significantly more common in tumors with APC I1307K allele mutations (25 of 41, 61%) compared with APC I1307K carrier tumors without mutation of the variant (A)4 tract (12 of 53, 23%; P < 0.0005). This somatic biallelic APC inactivation further confirms the biological importance of the I1307K germ-line variant. The vast majority of APC I1307K somatic mutations consisted of a single adenine insertion (insA) involving the variant (A)4 tract. This insA mutation was mutually exclusive of the presence of microsatellite instability with 0 of 49 tumors with insA displaying BAT-26 instability compared with 9 of 78 tumors without insA (P = 0.01). These findings support a model where somatic instability of the (A)4 tract produced by the APC I1307K allele leads to increased APC gene inactivation and directly accounts for 42% of the colorectal neoplasms occurring in APC I1307K carriers.

Introduction

The APC I1307K polymorphism (codon 1307 isoleucine to lysine), carried by 6.1% of the Ashkenazi Jewish population, has been observed at an increased frequency in CRC patients with a family history of CRC (1). Analysis of a small number of colorectal tumors from carriers has suggested that the polymorphism may be a target of increased somatic mutation (1). The wild-type allele that gives rise to APC I1307K is a T to A transversion altering (A)3T(A)4 to an (A)g repeat. Mononucleotide repeat sequences have been shown previously to undergo somatic mutation in colorectal tumors with MSI (2). MSI has been observed in the majority of tumors from individuals with HNPPC (3) caused by MMR deficiency and in 10-20% of sporadic CRCs (4, 5). To clarify the mechanistic role of the APC I1307K polymorphism in colorectal carcinogenesis and investigate its association with MSI, we have analyzed a large number of CRCs and adenomas from APC I1307K carriers. Somatic mutation of the (A)4 mononucleotide repeat of the APC I1307K polymorphism was identified at an exceptionally high frequency and consisted almost exclusively of a single adenine frameshift insertion. This instability was observed to be mutually exclusive of the presence of tumor MSI. Thus, sequence-specific hypermutability and biallelic APC gene inactivation likely lead to colorectal tumor initiation in APC I1307K carriers.

Materials and Methods

Tumor Samples. A consecutive series of CRC patients was screened at our institution to identify APC I1307K carriers. In accordance with genetic testing guidelines (6) and institutional approval, tumor samples were obtained from all subjects, coded, and stripped of identifiers prior to genetic analysis. In total, we characterized 47 CRCs and 80 adenomatous polyps from germ-line APC I1307K carriers. Residual adenoma adjacent to CRC was available from 15 of the CRCs. In addition, nine hyperplastic polyps, a colorectal lesion not associated with significant neoplastic progression, were identified for analysis. Genomic DNA was isolated from microdissected formalin-embedded samples by standard proteinase K digestion. Only tumor samples with at least 50% neoplastic cellularity were used for somatic mutation analysis. We have demonstrated previously that this threshold allows for reliable mutation detection by direct sequencing (8).

Somatic Mutation Analysis. Codons 1303-1317 of the APC gene were PCR amplified from microdissected genomic tumor DNA template using forward primer (5'-AGATTCTGCTAATCCCTGC-3') and reverse primer (5'-GAACCTGCTCAGAGGATC-3'), and the 83-bp product was directly sequenced (ThermoSequenase; Amersham) using the reverse primer. Tumor DNA was also sequenced using the forward primer to confirm the allele affected by somatic mutation as needed. LOH was determined only in tumor samples of >70% neoplastic cellularity and was judged by eye or computerized scanning densitometry with ImageQuant software where ambiguous. LOH was considered to be present by densitometry if the ratio (A1/A2):(T1/T2) was greater than 2 (A1, the polymorphic A of I1307K; A2, the immediately adjacent A in the sequencing reaction; T1, the wild-type T at codon 1307; T2, the nearest T in the sequencing reaction). Tumor samples of <70% neoplastic cellularity were considered noninformative for LOH analysis.

MSI. Microdissected tumor DNA was analyzed for MSI at the polyadenine BAT-26 mononucleotide locus by PCR amplification and denaturing PAGE using primers and conditions published previously (2). This locus has been demonstrated to be 96% sensitive, 100% specific, and highly reproducible for MSI (9, 10), the hallmark of MMR deficiency. Furthermore, because BAT-26 is quasimonomorphic and alterations consist of large deletions, tumor DNA need not be paired with normal DNA for analysis (10).

Statistical Methods. Somatic mutation data proportions were compared by $\chi^2$ test or Fisher's exact test.

Results

In total, 127 separate colorectal tumors (47 CRCs and 80 adenomatous polyps) from APC I1307K carriers were analyzed. Somatic alterations of APC were identified in 76 (60%) of 127 tumors. Inactivation of both APC alleles was observed in 29 (31%) of 94 informative tumors. Mutations predicted to yield a truncated protein product were observed in 53 (42%) of the APC I1307K alleles, compared with 5 (4%) of the wild-type alleles (P < 0.0001; Table 1). Identical alterations in the APC I1307K allele were detected in 15 of 15 residual adenomas adjacent to CRCs, confirming that these mutations occurred...
SOMATIC INSTABILITY OF APC II307K

Table 1  Somatic mutations in APC II307K carrier colorectal cancers and adenomatous polyps

<table>
<thead>
<tr>
<th>Somatic alterations</th>
<th>APC codon</th>
<th>II307K allele</th>
<th>Wild-type allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frameshift</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>insA</td>
<td>1306–1308</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>delA</td>
<td>1306–1308</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>delGA</td>
<td>1306</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>delAAAG</td>
<td>1309</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Nonsense</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAA to TAA</td>
<td>1308</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>GAA to TAA</td>
<td>1306</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total intragenic mutations</td>
<td></td>
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<td>5</td>
</tr>
<tr>
<td>LOH</td>
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<td>10</td>
<td>37</td>
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<tr>
<td>Intact</td>
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<td>52</td>
</tr>
<tr>
<td>Noninformative</td>
<td></td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>127</td>
<td>127</td>
</tr>
</tbody>
</table>

*a,b* Significant differences between alleles: *a* P < 0.0001; *b* P < 0.0005.

>70% tumor cellularity in which no frameshift, substitution, or LOH was observed.

<70% tumor cellularity in which no frameshift or substitution was observed.

Forty-nine (92%) of 53 predicted truncating APC II307K mutations consisted of a single adenine insert (insA) frameshift in the (A)_8 repeat (Fig. 1). Inactivation of the wild-type allele was observed in 25 of 38 (66%) informative APC II307K tumors with insA somatic mutation. Wild-type allelic loss was observed in 25 of 41 (61%) informative tumors with intragenic II307K allele mutation compared with 12 of 53 (23%) informative tumors without II307K mutation (P < 0.0005). Furthermore, LOH of wild-type (n = 12) and II307K (n = 10) alleles was similar in 53 informative tumors that did not undergo intragenic mutation of the II307K allele.

BAT-26 MSI was present in 9 of 127 (7.1%) tumors (Fig. 2). Surprisingly, tumor MSI and somatic insA mutation of the APC II307K were mutually exclusive with MSI present in 9 of 78 (12%) tumors without somatic insA compared with 0 of 49 tumors with insA (P = 0.01). Interestingly, the only tumor with a single adenine deletion in the (A)_8 repeat tract demonstrated MSI. APC LOH was observed in 0 of 5 informative tumors with MSI compared with 47 of 89 (53%) informative tumors without MSI (P < 0.06).

Discussion

We have observed an exceptionally high rate of somatic mutation specifically targeting the APC II307K sequence. Although instability of some repeated sequences may occur without functional significance, particularly in tumors with MSI, there are five separate lines of evidence strongly supporting the biological functional significance of the APC II307K insA mutation: (a) the insA mutation predicts a protein truncation in the mutation cluster region, using the same termination codon as other common APC frameshift mutations (11); (b) the insA mutation is represented as a monoclonal alteration in tumors and is readily detected by sequencing bulk DNA without the necessity of subcloning; (c) LOH of the wild-type allele occurs at a very high rate in association with the insA mutation, confirming biallelic inactivation. This biallelic inactivation also suggests that the APC II307K variant has no functional effect per se, which is further supported by the similar rates of LOH of II307K and wild-type alleles in the absence of somatic II307K mutation; (d) identical alterations were identified in adenomas adjacent to carcinomas, consistent with APC inactivation during the noninvasive stage of colorectal neoplasia; and (e) no mutations were identified in hyperplastic polyps, a lesion shown previously to harbor clonal K-ras gene mutations but not APC mutations (12).

The mutation rate of the APC II307K variant is remarkable given that it may be attributed to a single bp substitution in an 8.5-kb gene. The relative increase in APC II307K mutability can be estimated by comparison with other mutation rates quantified in our study. The early during neoplastic progression, prior to the development of carcinoma. No differences were observed in the frequency or spectrum of mutations in CRCs compared with adenomas (data not shown). APC II307K did not appear to contribute to the development of hyperplastic polyps because no somatic mutations were detected in any of the nine hyperplastic polyps.

Fig. 1. Reverse primer sequence of APC II307K from carrier colorectal cancers. The arrowhead points to the germ-line APC II307K T to A polymorphism. The double arrowhead points to the start of the somatic frameshift single adenine insertion (insA) in the (A)_8 mononucleotide repeat of the APC II307K polymorphism. A, colorectal cancer with insA of II307K allele and no wild-type LOH. B, colorectal cancer with insA of the II307K allele and wild-type LOH. LOH of the wild-type allele is evident, with loss of the "A" corresponding to a thymine nucleotide in the forward sequence.

Fig. 2. BAT-26 microsatellite analysis of APC II307K carrier colorectal tumors. The arrowhead points to the normal BAT-26 PCR product size. Deletions can be seen in Lanes 8 and 12 (double arrowhead), both products of tumors without insA somatic mutation. Normal BAT-26 product in Lane 8 comes from residual normal tissue in the tumor sample.

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most frequent wild-type allele frameshift mutation observed in APC 11307K carrier tumors was codon 1309delAAAAG. This somatic alteration has been reported previously as one of the most common somatic frameshift APC mutations in CRC (11, 13), and our 1.2% mutation rate of alleles tested is similar to those reported previously. Our data suggest that the mononucleotide repeat (A)₈ of APC 11307K is approximately 32 times more mutable than one of the most mutable wild-type APC sequences (95% confidence interval, 10.4–102.8).

The mutual exclusivity of the APC 11307K insA mutation and the presence of MSI is a striking finding, suggesting that the tumorigenic effects of the APC 11307K variant are entirely separate from the MSI carcinogenic pathway. This finding is consistent with observations in both yeast and human experiments that mononucleotide tract deletions, rather than insertions, predominate in MMR deficiency (9, 13–15). The relatively low rate of frameshift deletion of the APC 11307K (A)₈ tract in tumors with MSI is also interesting. In MMR deficiency, (A)₈ repeats are ~75 times more mutable than (A)₈ tracts (16). Furthermore, somatic mutation rates of >90% have been described for the translated (A)₈ repeat of the transforming growth factor β receptor type II (TGF-βRII) gene in tumors with MSI (2, 13). Our low APC mutation rate and the absence of APC LOH in tumors with MSI provide further evidence that APC gene inactivation is a less common event in CRCs characterized by MSI, compared with those with chromosomal instability (13). These results are also consistent with recent findings that mutations in the APC binding protein β-catenin, rather than APC itself, are present in ~50% of tumors with MSI (17).

In addition to the APC 11307K insA mutation, we observed two tumors with nonsense mutations at codon 1308 of the 11307K allele, a mutation that has not been reported previously. In contrast, the two nonsense mutations on the wild-type allele were at codon 1306, a mutation that is reported to account for ~1% of somatic APC mutations (11). These findings provide some evidence that the variant (A)₈ tract could alter the rate or profile of specific nucleotide substitutions in addition to the profound effects on frameshift mutagenesis.

The APC 11307K polymorphism may be targeted for somatic mutation in specific at-risk individuals or may represent a sequence variant that is the target of mutation to a similar degree in most carriers. The predominance of a single (insA) mutation, as opposed to other alterations, raises the possibility that there could be an accompanying specific DNA repair deficiency. For instance, yeast deficient in polymerase delta proofreading are prone to bp insertions (16). Although we have not found evidence for involvement of MMR deficiency in APC 11307K instability, subtle alterations in other DNA repair pathways cannot be excluded.

At least two other possibilities may account for the predominance of a single bp insertion in the APC 11307K allele: (a) conformational structures of DNA and/or repair proteins could favor (stabilize) misalignment intermediates that lead to insertions. This possibility is supported by the observation that insertions predominate in mononucleotide repeats in DNA repair-proficient yeast (14). Accordingly, the hypermutability could merely be a reflection of the instability associated with this mononucleotide repeat, rather than a specific DNA repair deficiency; (b) it is possible that a functional difference exists between the insertion and deletion frameshift APC product. However, both the insertion and deletion mutations are predicted to produce a truncated protein of similar size. Interestingly, the common APC 1309delAAAAG mutation uses the same termination codon as the more prevalent APC 11307K insA mutant.

Hereditary factors contribute significantly to colorectal tumorigenesis (18), and APC 11307K represents a novel mechanism of cancer predisposition compared with other common syndromes (Fig. 3). In FAP, because one functionally inactivated copy of APC is inherited, any single inactivating mutation of the second allele leads to complete abrogation of APC. Inactivation of the APC gatekeeper gene is believed to initiate colorectal tumorigenesis, and thus FAP patients are characterized by the development of hundreds to thousands of adenomas (19). MMR gene inactivation, either due to germ-line inactivation of one allele followed by somatic loss of the second copy in HNPCC, or somatic biallelic inactivation in sporadic CRC, leads to a profoundly increased genomic mutation rate (20). Although adenoma formation may be slightly increased, there is an accelerated acquisition of mutations required for progression to carcinoma (19).

We propose a model in which the APC 11307K allele contributes a proportional increase in the potential mutability of APC via (A)₈ repeat instability. Forty-two % of tumors from APC 11307K carriers may be directly attributed to specific mutation of the polymorphic sequence. Compared with HNPCC, this increased mutation rate is modest and affects only the APC locus. Furthermore, in contrast to FAP, the APC 11307K variant must still undergo somatic biallelic APC inactivation. Thus, the APC 11307K variant may be thought of as a susceptible gatekeeper allele. With a much reduced likelihood of tumor initiation compared with FAP and a much slower rate of acquisition of other genetic alterations compared with HNPCC, it is not surprising that the penetrance of the APC 11307K allele should be modest compared with these other inherited colorectal cancer syndromes. The identification of the APC 11307K allele and other common low penetrance cancer predisposition alleles will allow intermediate risk target populations to be recognized both for cost-effective endoscopic screening programs and for risk modification by dietary, chemopreventive, or other measures.

References

Fig. 3. Gatekeeper inactivation in colorectal carcinogenesis. Germ-line FAP APC mutation (B) is followed by somatic inactivation of the second APC allele and the formation of hundreds to thousands of adenomatous polyps. Germ-line APC 11307K carriers undergo somatic mutation of both the hypermutable 11307K allele (B) and the remaining wild-type allele (C) and have an increased rate of adenoma formation compared with sporadic tumorigenesis in which biallelic APC inactivation occurs.
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