Absence of Secretory Phospholipase A2 Gene Alterations in Human Colorectal Cancer

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Abstract

A potent modifying locus of intestinal tumorigenesis in the mouse was recently identified as secretory phospholipase A2 (sPLA2). The human homologue of sPLA2 maps to chromosome 1p35, a region frequently lost in human tumors. To evaluate the possibility that sPLA2 was a tumor suppressor gene that was the target of the 1p loss events, we identified polymorphisms within the human sPLA2 gene. Using these polymorphisms, 31% of 16 colorectal carcinomas were found to lose a sPLA2 allele. However, sequence analysis of the complete coding region of sPLA2 revealed no somatic mutations in the remaining allele of those tumors with allelic loss, nor in 18 additional colorectal cancers. Thus, sPLA2 is within the chromosomal region often lost during colorectal tumorigenesis, but mutations of this gene do not appear to play a major role in colorectal cancer development, and sPLA2 is unlikely to be the 1p35 tumor suppressor.

Introduction

Inherited mutations in the APC3 gene are responsible for familial adenomatous polyposis, an autosomal dominant disorder (1–4). Patients with germline APC mutations develop thousands of CR adenomas, some of which, if left untreated, progress to carcinomas. In a similar manner, somatic APC mutations in single cells within the CR epithelium appear to initiate adenoma formation in the general population (5, 6).

As in humans, mice with germline alterations of APC develop multiple intestinal adenomas (7–9). The first example of such mice was the MIN strain, with a chemically induced nonsense mutation at codon 850 of mAPC (7). It was observed that the number of tumors developing in MIN mice depended on the genetic background of the strain (10). A gene that was partially responsible for modifying the effects of APC MIN mutation was mapped to mouse chromosome 4 (5'-GOT GAG GGA TGC CTF Cr0 C), which amplify the entire coding region of sPLA2. The presence or absence of a single product at the expected size of 4.4 kb for the 4.4-kb allele was scored by sequence analysis as a polymorphism, primers PL-9 and PL-2 (sequences above) were used as PCR primers to amplify a 2.3-kb genomic fragment encompassing the four coding exons: PL-9 (5'-TCC TTC TTG AGC GCC GAC AGG) for exon 3, PL-4 (5'-TCT TGC AGG GGA AGC 0) for exon 3, PL-4 (5'-TCT TGC AGG GGA AGC 0) for exon 3, and PL-5 (5'-TTC TGC AGG GGA AGC 0) for exon 5.

LOH Analysis. Intragenic single base pair polymorphisms identified during mutation analysis and a 26-bp CA repeat located 202 bp upstream from the translational start site (14) were used for LOH analysis. For the CA repeat polymorphism, primers PL-9 and PL-2 (sequences above) were used as PCR primers to amplify a 370-bp fragment containing the repeat. Primer PL-9 was end labeled in a kinase reaction with [γ-32P]ATP. PCR conditions were as above, except cycling times were shortened to 1 min for annealing and 1.5 min for extension. The different length alleles were separated on a sequencing gel. LOH at the intragenic polymorphism was scored by sequence analysis as described above.

Materials and Methods

Patient Samples. A panel of 25 genomic DNA samples from sporadic colorectal tumors and 25 corresponding normal tissue DNA samples were used for this study. Tumor tissue was obtained from nude mouse xenografts of human colorectal carcinomas as described (20). Normal colon tissue or EBV-transformed lymphoblasts obtained from each patient were used as controls. DNA from tumor and normal tissue was purified as described (21).

Sequencing. The published genomic sequence of the sPLA2 gene (13, 14) was used to design primers for PCR amplification of a 3.4-kb genomic fragment encompassing the four coding exons: PL-9 (5'-TTC TTC TTG AGC GCC GAC AGG) and PL-10 (5'-GCC AAG GAA CTG GGT TAG GG). PCR was performed as described (22) for 38 cycles of 95°C for 30 s, 63°C for 2 min, followed by 70°C for 4.5 min. Initial denaturation was for 2 min, and final extension was for 5 min. The product was purified from the reaction components by isopropanol precipitation as described (23).

Sequencing of the genomic PCR product was performed with γ-[32P]ATP primers and SequiTherm polymerase (Epigenic Technologies, Madison, WI) following the recommended conditions. The primers were PL-2 (5'-AAA CTA AGG AGC AGT AGG C) for exon 2, PL-3 (5'-AGG AGA GTA GCA GAC AGG) for exon 3, PL-4 (5'-TCT TAC CCT AGG GTT CCC AC) for exon 4, and PL-5 (5'-TTC TAC CCT AGG GTT CCC AC) for exon 5.

Reverse Transcription-PCR. First strand cDNA synthesis was performed using 4 μg of random hexamers, 5 μg of xenograft RNA, and Superscript II (Life Technologies, Gaithersburg, MD) in a 20-μl reaction, following the manufacturer’s conditions. PCR amplification of cDNA was performed as above using primers PL-1 (5'-AGA GAG GAC CCT GGT TTT TGT) and PL-8 (5'-GTT GAG GGA TGC TTT TGT C). Amplified products were cloned into the pBluescript vector (Stratagene, La Jolla, CA) and sequenced using SequiTherm polymerase. Sequencing reactions were performed on the DNA isolated from transformed lymphoblasts obtained from each patient were used as controls. DNA from tumor and normal tissue was purified as described (21).

Received 9/7/95; accepted 10/2/95.

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1 This work was supported by NIH Grants CA57345 and CA09243.

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3 The abbreviations used are: APC, adenomatous polyposis coli gene; CR, colorectal; LOH, loss of heterozygosity; MIN, multiple intestinal neoplasia; mAPC, murine adenomatous polyposis coli gene; sPLA2, secretory phospholipase A2 gene.
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sPLA2 polymorphisms (Table 1). Of these 16, 5 (31%) showed LOH, consistent with previous observations on LOH in this region (16-19).

The complete sPLA2 coding region and splice junctions from all 23 carcinomas were then sequenced to search for somatic mutations. Three different sequence variations were observed (Table 1; examples in Fig. 2A). The first two nucleotide variations (codons 32 and 44) were observed in multiple tumors, did not change the encoded amino acid, and were present in the corresponding normal tissue, suggesting that they represent common polymorphisms. The third variation was observed in only one case (MX22) and changed codon 143 from an arginine to a histidine codon. However, when the normal DNA from MX22 was sequenced, it too showed the rare allele (Fig. 2B), indicating that this mutation was not acquired during the development of the tumor. Furthermore, both alleles of the sPLA2 gene for MX22 appeared to be retained and unaltered (other than the codon 143 variant). Additional studies will be required to determine whether this variation affects sPLA2 activity or modifies colorectal cancer risk.

Although no acquired somatic mutations were observed in the tumors, the possibility remained that lack of sPLA2 transcription was responsible for inactivating the second allele in tumors undergoing LOH at this locus. However, reverse transcription-PCR from all five tumors with LOH, as well as five without, demonstrated presence of sPLA2 transcripts in all cases (data not shown). Furthermore, amplification included 5'- and 3'-untranslated regions, indicating normal splicing of the entire coding region.

The results described above demonstrate that sPLA2 is within a region undergoing LOH in a significant number of CR carcinomas. However, the target of the LOH event is likely to be a gene other than sPLA2 because no somatic mutations of sPLA2 were observed. A hallmark of tumor suppressor genes is that the allele remaining in the tumor is mutant, or when both alleles are present, each is mutant. The sequence analysis reported here shows that sPLA2 does not fit this paradigm. Although it is possible that mutations in the promoter region or epigenetic events such as DNA methylation could inactivate sPLA2 alleles in CR tumors (24), these mechanisms have not been found to be common in other suppressor genes involved in CR tumorigenesis, like APC or p53. In addition, sPLA2 appears to be transcribed in the 10 cases of sporadic CR tumors tested. We conclude that sPLA2 is not involved in the development of most sporadic colorectal cancers. The paucity of variations affecting the coding sequence of sPLA2 (Table 1) also makes it unlikely that germline variations of this gene play a role in tumor predisposition.

Reasons why the sPLA2 gene could modify tumor number in MIN mice but does not play a role in human colorectal cancers is easy to envision. One likely explanation is that the effects of sPLA2 are not cell autonomous and are manifest through the action of the secreted enzyme on lipids within the lumen of the bowel, as suggested (12). Alternatively, modifiers of APC mutations may be different in humans and mice.

References


Table 1 Sequence variations and loss of heterozygosity for sPLA2

<table>
<thead>
<tr>
<th>Location and nucleotide change</th>
<th>Coding change</th>
<th>PIC*</th>
<th>LOH</th>
</tr>
</thead>
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<tr>
<td>Intron 1: GT repeat</td>
<td>Noncoding</td>
<td>0.49</td>
<td>4/13 (31%)</td>
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<tr>
<td>Codon 32: ACG → ACC</td>
<td>Silent</td>
<td>0.11</td>
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<td>Codon 44: TAC → TAT</td>
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<td>Codon 143: CGT → CAT</td>
<td>Arg → His</td>
<td>0.04</td>
<td>0 / 1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5/16 (31%)</td>
<td></td>
</tr>
</tbody>
</table>

* Polymorphism information content.

Fig. 1. LOH at the sPLA2 locus. The first four patients show loss of one of the CA repeat alleles in the tumor (T) compared to the normal (N) tissue. Patient 5 does not show LOH.

Fig. 2. Examples of sequence alterations at the sPLA2 locus. Panel A, mutation screening showing the sequence polymorphisms at codon 32 (sample 3, bottom arrow) and codon 44 (sample 4, top arrow). Lanes 1-4 correspond to four different CR tumor samples. The reactions were loaded by groups of nucleotide termination reactions (labeled A, C, G, T) to facilitate alteration identification. Panel B, sequence of MX22 showing the Arg to His heterozygous change in both tumor (T) and normal (N) tissue.


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