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 APC 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 population (5, 6).

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 (10). Normal colon tissue or EBV-transformed lymphoblasts obtained from each patient were used as control. Genomic 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 TGC AGA GCT GGG AAC G) 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 32P primers and SequiTherm polymerase (Epicerin Technologies, Madison, WI) following the recommended conditions. The primers were PL-2 (5'-AAA CTA AGG GAC AAG AGT GC) for exon 2, PL-3 (5'-AGG AGA GTA GCA GAG AGG G) for exon 3, PL-4 (5'-TCT TGC TTT GTG TTC CCC ACC ACA G) for exon 4, and PL-5 (5'-TTC TAC CCT AGG GGT TCC AC) for exon 5.

LOH Analysis. Intragene 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 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 intragene polymorphism was scored by sequence analysis as described above.

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 GGA GGT AGG CCA GTC C) and PL-8 (5'-GTT GAG GGA TGC TTT CGT C), which amplify the entire coding region of sPLA₂. The presence or absence of a single product at the expected size was determined by gel electrophoresis.

Results and Discussion

To determine whether sPLA₂ was within a region undergoing LOH in CR cancers, we performed LOH analysis in 23 CR carcinoma xenografts using polymorphic markers within the sPLA₂ locus. A (CA)₁₃ repeat located within the first intron was amplified and found to be polymorphic with 6 different alleles observed. This (CA)₁₃ repeat was heterozygous in the normal tissue of 15 of the 23 patients.

In 4 of the 15 informative patients, LOH in the tumor tissue was observed (Fig. 1). In addition, another LOH was determined by using the single base pair polymorphisms discovered during sequence analysis of the sPLA₂ gene in the tumors (described below). DNA from 16 of the 23 normal patients were heterozygous at one or more of three
sPLA2 polymorphisms (Table 1). Of these, 16 (531%) 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 effects 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 are 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


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