Intron 4 mutation in APC gene results in splice defect and attenuated FAP phenotype

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Abstract

The adenomatous polyposis coli (APC) protein is a tumor suppressor frequently involved in the development of inherited and sporadic colon cancers. Somatic mutations of the APC gene are found in 80% of all colon cancers. Inherited mutations result in familial adenomatous polyposis (FAP) as well as an attenuated form of this syndrome. FAP is characterized by the early age onset of hundreds to thousands of colonic adenomatous polyps and a virtual certainty of colon cancer unless the colon is removed. The attenuated form of FAP (AFAP) is characterized by fewer adenomas, later onset of adenomas and cancer, and a decreased lifetime cancer risk. We report a 37-year-old man with a history of more than 50 colonic adenomatous polyps, located predominately in the right colon. An insertion of a single thymidine between the second and third base pairs of intron 4 of the APC gene was identified (c.531+2_531+3insT). Monoallelic hybrid cells harboring a single copy of human chromosome 5 were generated from patient lymphoblasts. Sequencing of the APC cDNA product from these cells revealed a single RNA transcript with aberrant splicing in the mutant mRNA whereby exon 4 is deleted. The translational reading frame is shifted after codon 140 and a translational stop is generated predicting a truncated protein of 147 amino acids, thus indicating that the intronic mutation is disease causing. The lack of a secondary transcript from the mutant allele suggests that incomplete exon skipping is not the molecular mechanism behind the attenuated phenotype.

Introduction

The adenomatous polyposis coli (APC) protein resides on chromosome 5q21 and inherited mutations result in familial adenomatous polyposis (FAP), a syndrome characterized by the early age onset of hundreds to thousands of adenomatous polyps distributed throughout the colon and a virtual certainty of colon cancer unless the colon is removed [1]. Polyps appear at an average age of 16 years and cancer at an average age of 39 years. Mutations in the APC gene are found in about 90% of cases, and approximately 80% of the APC mutations result in a truncated APC protein [2]. Mutations causing the classic phenotype of FAP are found from codons 168 to 1,580. Within this region, a severe form of FAP is associated with mutations in codons 1,250–1,464 and individuals harboring these mutations have profuse polyps with greater than 5,000 polyps. An attenuated form of FAP (AFAP), resulting from mutations in the 5′(5′ of codon 158) or 3′(3′ to codon 1,596) ends of the gene and in select areas of exon 9 [3–11], is clinically characterized by less than 100 adenomatous polyps. When contrasted with FAP, AFAP has a 10–15 year later onset of adenomas and cancer, and a predominance of proximal colonic adenomas, a decreased lifetime cancer risk (estimated at 50–80%), and dramatic phenotypic variability within families [12–14]. Extracolonic features are sometimes observed in FAP and AFAP patients and include adenomatous polyps of the duodenum and small bowl, fundic gland polyps of the stomach, osteomas, dental abnormalities, epidermoid and sebaceous cysts,

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fibromas, lipomas, desmoid tumors, and congenital hypertrophy of the retinal pigment epithelium (CHRPE), the latter two features demonstrating genotype–phenotype correlations [1]. Although FAP and AFAP combined account for less than 2% of colon cancer diagnoses, approximately 80% of all colon adenomas and cancers harbor somatic mutations in the APC gene [15].

Mutations in the APC gene appear to be an early step in the progression of normal colon epithelia to adenomas and cancer [16]. The APC protein is a key regulator in the control of cell growth through the Wnt signaling pathway. Disruption of the APC gene function results in increased β-catenin levels and transcriptional up regulation of genes involved in proliferation including the oncogenes c-Myc and cyclin D1. Beyond this commonly recognized role, recent work has suggested that the APC protein product may have a function in maintaining chromosomal integrity during cell division implicating it in the chromosomal instability observed in APC deficient cancers [17]. Additionally, the APC protein associates with microtubules at the leading edge of migrating astrocytes, suggesting that the APC protein may play a role in cell polarity [18].

We describe an individual with an attenuated FAP phenotype and an APC intron 4 mutation. Analysis of monoallelic hybrid cells from this individual revealed that a transcript is generated from this allele, but the mutation causes aberrant mRNA splicing and predicts a truncated protein consistent with the attenuated FAP phenotype. This observation not only demonstrates that this intronic mutation is disease causing, but also may help to clarify how APC mutations result in dysfunction of the APC gene, a long debated issue.

**Materials and methods**

All aspects of this study were reviewed and approved by the Institutional Review Board of the University of Utah.

**Clinical evaluation**

Medical history and endoscopic procedure reports were obtained, and physical examination was performed on the patient with colonic adenomatous polyposis, with particular attention to signs or symptoms pertinent to inherited colon cancer conditions or other gastrointestinal and medical issues.

**Generation of hybrid cells**

Conversion technology by GMP Genetics, Inc. (Waltham, Massachusetts) was used to generate monoallelic cells [19]. Mouse E2-cells were fused with EBV-transformed patient lymphoblasts and hybrid cells were selected to contain a single copy of human chromosome 5 in a mouse background as determined by genotyping with D5S2488 and D5S1456. Hybrid cells were maintained in DMEM (high glucose) supplemented with 10% fetal bovine serum, 0.5 mg/ml Gentecin (G418), HAT supplement, penicillin (100 U/ml), and streptomycin (100 μg/ml).

**cDNA synthesis**

Total RNA was extracted from cells using Trizol (Gibco BRL, Rockville, Maryland). To synthesize cDNA, the random hexamer priming method with superscript first-strand synthesis for RT-PCR was used (Gibco BRL).

**PCR**

PCR amplification of APC cDNA was accomplished using human specific primers with M13 forward and reverse tags (underlined). The primers are: APC exon 3 forward, 5’ TGTAAAACGACGGCCAGTGGTT CATTTCAGAGGAAGGG 3’ and APC exon 5 reverse, 5’ CAGGAAACAGCTATGACCCCATCGCAAACGATTACCTAGG TACT 3’. Standard PCR conditions with 1.5 mM MgCl₂ and an annealing temperature of 64.5 °C (cDNA) or 59 °C (genomic) were used.

**Sequencing of genomic DNA and RNA**

The sequence reaction was performed on cDNA and genomic DNA PCR products in both directions using the M13 forward (TGTAAAACGACGGCCAGT) and reverse (CAGGAAACAGCTATGACCGT) primers and reaction products were run on Applied Biosystems 3700 capillary sequencer (Foster City, California).

**Results**

**Clinical history**

The patient first presented to his primary care physician with rectal outlet bleeding at age 24. He was found to have an adenomatous polyp that was removed by endoscopy. He was referred to a gastroenterologist at age 36 for evaluation of unexplained weight loss. The colonoscopy revealed multiple diminutive polyps in the cecum to the sigmoid colon that were all 4 mm or less in diameter, some of which were biopsied. A single 8-mm polyp was removed in the ascending colon. Histopathological evaluation showed the presence of adenomatous polyps but no cancer. Due to a suspicion of FAP, both upper and lower endoscopy were performed at age 37. Again, approximately 50 diminutive polyps (3–6 mm in diameter) were found from the cecum through the descending colon. Random biopsy sampling showed that nine of 11 polyps were tubular adenomas and 2