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LOSS OF HETEROZYGOSITY INVOLVING THE APC TUMOR SUPPRESSOR GENE IN HUMAN COLORECTAL CARCINOMA

Xu Wenhuai 徐文怀 Yang Dingcheng 杨定成

Department of Surgery, The First Clinical School, Beijing Medical University, Beijing 100034

To investigate whether aberration of the APC gene play any role in the development of Chinese sporadic colorectal carcinomas, we used a polymorphic site located in exon 11 which created a new restriction site for RsaI to analyze LOH for the APC gene . We found that 20/29 (68.9%) patients with colorectal cancer were informative for the APC exon 11 site and loss of one allele was detected in 40% of 20 informative cases. These data suggested that loss of heterozygosity of APC gene (APC-LOH) play a role in the pathogenesis of Chinese colorectal cancer. APC-LOH is a earlier event of colorectal tumorigenesis and may be of diagnostic value in patient suffering colorectal cancer.

Key words: Heterozygosity, APC gene, Human colorectal carcinoma.

The APC gene was isolated by positional cloning from human chromosome 5q21 and has been implicated in FAP.[1-4] The APC gene contains an 8528 bp open reading frame and is predicated to encode a 2843-aa polypeptide with few homologies to other proteins. Whether or not aberration of APC play a role in the proliferation of cells in colorectal mucosae is still an open question. In this study, we investigated possible gene aberration in Chinese sporadic colorectal carcinoma, the mechanism of APC gene inactivation and whether there is any evidence for a correlation between APC-LOH and clinical behavior of the cancer.

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MATERIALS AND METHODS

Tissues and Preparation of DNA

Surgical specimens were obtained from 29 patients with colorectal carcinoma at the first clinical school of Beijing medical university. The patients ranged from 30 to 89 years of age. There were 19 male and 10 female patients. A total of 29 primary tumor tissues, consisting of 6 well differential adenocarcinomas, 14 medium differential adenocarcinomas, 9 poorly differential adenocarcinomas, 9 Dukes A stages, 7 Dukes B stages, 10 Dukes C stages and 2 Dukes D stages. All tissues were obtained during surgery and were frozen immediately in liquid nitrogen and stored at -70°C until use. High molecular weight DNAs from the tumors and from matched normal mucosal tissues were purified by proteinase K digestion, extracted with phenol/chloroform.

PCR-RFLPs

Genomic DNA (500 ng) were used as template in a reaction volume of 50 μl containing 1 μmol of each

primer, 20 μmol concentration of each deoxynucleotide triphosphate, and 2 μnits of Taq DNA polymerase. Based on the nucleotide sequence of the APC/DP 2.5 gene reported by calson (Figure 1).[3] Primer used were PCA (sense): 5'-GGA CTA CAG GCC ATT GCA GAA -3', and PCB (antisense): 5'- TTG ACT TTT GGA GAT GTA GCC -3'. The priming regions were located within APC exon 11 at a polymorphic sequence and covered entire coding region (from codon 471 to codon 514). Reaction mixture were denatured at 95°C for 10 min. Thirty-five cycles of the reaction at 95°C, 55°C and 72°C for 1, 0.5 and 0.5 min respectively, and extension at 72°C for 10 min, were performed in a thermal cycle (Perkin-Elmer Cetus). PCR products were digested with RsaI enzymes for RFLPs and electrophoresed on polyacrylamide gels, which were stained with ethidium bromide and photographed under UV light.

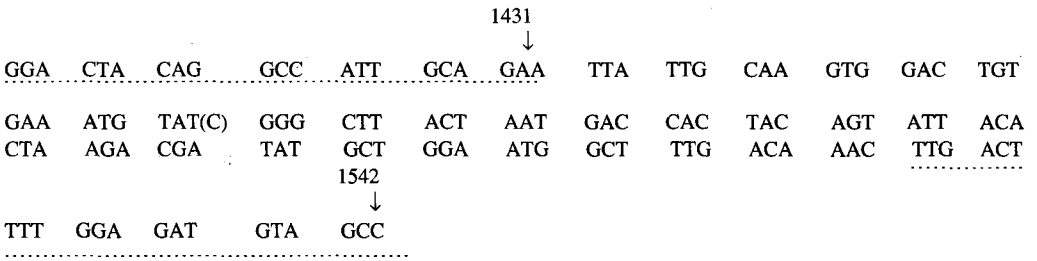

Fig. 1. The partial DNA sequence of APC exon 11

RESULTS

In this study, amplification fragment of APC gene using primer A and B was 132 bp (Figure 2). Allelic deletion of the APC gene was determined by PCR-LOH in human colorectal carcinomas. Normal DNA from heterozygous patients showed three bands at the APC exon 11 locus: a 132 bp "uncut" bands (86 bp and 46 bp) representing the allele that contained the site (Figure 3). APC-LOH was demonstrated when the tumor DNA showed loss of either the single uncut band or the cut bands. Individual cases of APC-LOH are displayed in Figure 4, 5, 6, 7 and 8. At the APC exon 11 locus, we found that 20/29 (68.9%) patients with colorectal cancer were informative or heterozygous. Loss of the heterozygous in APC was detected in 40% of 20 informative cases. No correlation was found between tumor grade and presence of APC-LOH. The highest incidence of LOH on APC locus was observed in earlier stages (Dukes A and B) of colorectal cancer (Table 1).

Table 1. Positive for APC exon 11–LOH in human colorectal tumors

Case No.	Sex	Age	Differentiation	Dukes stage
8	Female	51	Medium	A2
11	Male	65	Higher	C
12	Female	63	Medium	A2
13	Male	61	Medium	A2
14	Male	67	Poorly	B
16	Male	43	Poorly	A2
18	Male	58	Medium	A2
27	Male	61	Medium	B

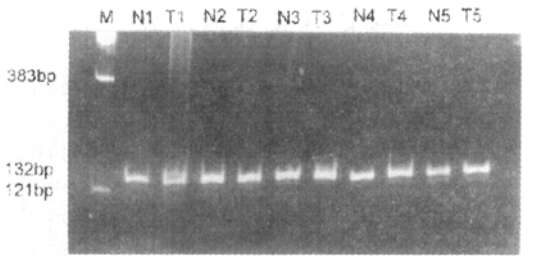

Fig. 2. PCR product of APC exon 11 using priner A and B. Amplification fragment was 132 bp.

M: marker-pBR322/pstN1

N: normal colorectal tissue

T: tumor tissue

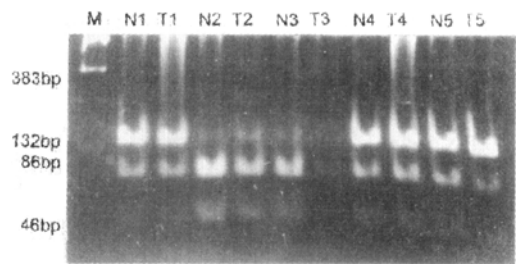

Fig. 4. PCR-RFLP of APC exon 11 with RsaI enzyme. There were no difference in tumor and normal tissue DNAs.

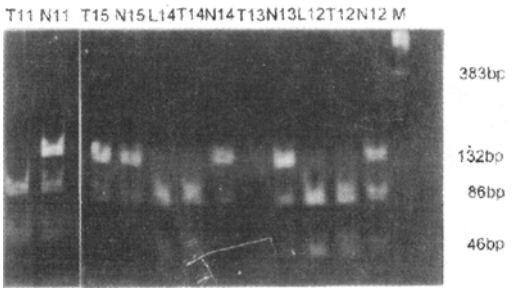

Fig. 6. LOH at APC exon 11 detected with PCR-RFLP. There were APC-LOH in tumor DNAs from the 11th, 12th, 13th and 14th patients.

L: lymph node tissues

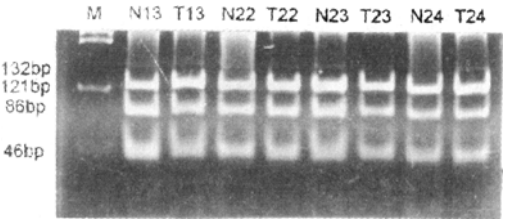

Fig. 3. All four patients in this figure are constitutionally heterozygosity or informative. There were not APC-LOH.

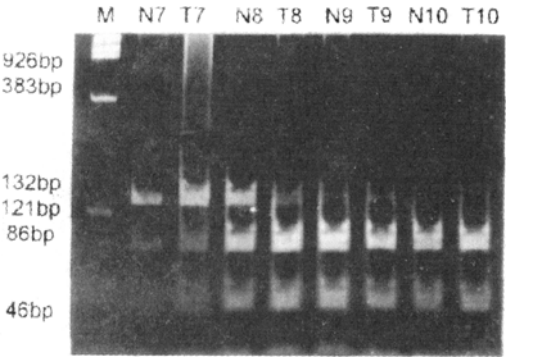

Fig. 5. LOH at APC exon 11 detected with PCR-RFLP. In the 8th, there was loss of 132 bp allele (lacking RsaI restriction site) in tumor tissue. The 9th and 10th cases were homozygous or uninformative.

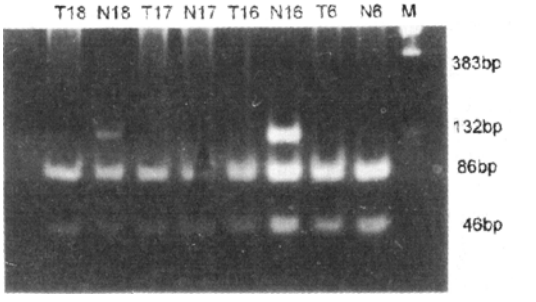

Fig. 7. LOH at APC exon 11 detected with PCR-RFLP The 6th and 7th cases were uninformative patients (homozygous for the cut allele of APC). Positive for loss of the uncut allele of APC in the 16th tumor tissue.

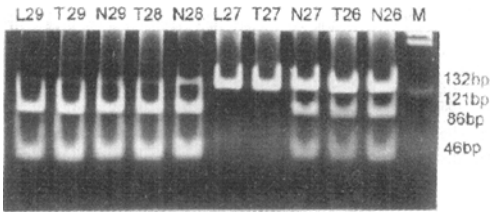

Fig. 8. LOH at APC exon 11 detected with PCR-RFLP. Positive for loss of bands (86 bp and 46 bp) of APC in the 27th tumor and metastatic lymph node tissue.

DISCUSSION

Colorectal cancer is highly virulent tumor with an specially poor prognosis. The survival of patients with colorectal cancer has remained unchanged last decade. It is considered that cancer is the result of an accumulation of various genetic changes in the cell genome occurring over an extented period of time. Many carcinogenes have the potential to generate the mutation in protooncogenes and tumor suppressor genes. The identification of molecular marks specific for colorectal cancer or a clinical stage of colorectal cancer is of great potential interest in the early detection of a neoplastic condition and as a prognostic parameter. To seek the marks for patients with colorectal tumor aggressiveness may assist in planning appropriate treatment strategies. APC gene was isolated from chromosome 5q21 and has been implicated in FAP. Carcinoma in FAP account for <1% of all colorectal cancers. Most colorectal cancers do not have a well recognized inherited basis and therefore are classified as sporadic. In this study, allele loss at APC 5q21 locus was detected in 40% of 20 informative Chinese colorectal cancer, suggesting the presence of a tumor suppressor gene in this region that is involving in the pathogenesis of colorectal cancer and the possibility that APC gene is target for DNA damage by mutagenic carcinogenes implicated in the etiology of this disease. Frequent involvement of 5q has been previously described in gastric cancer, esophageal cancer and in lung cancer. The results presented here indicated that LOH of the APC locus on chromosome 5q21 is a common event for Chinese colorectal cancer as well. We observed that almost all of these colorectal tumors occurring APC-LOH were in earlier stage (Dukes A and B). These finding implicate LOH involving APC as a key event in Chinese colorectal cancer, LOH of APC gene in colorectal tissues may act as a new mark for assessing the formation of colorectal cancer. The results of this study also support the notion that the APC gene is a recessive tumor suppressor gene. LOH in the APC gene may lead to progressive disorder of controlling growth. We think it is more likely that APC point mutations and/or other genes may be responsible Chinese colorectal cancer progression. Further study will be required to address that question.

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