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WJG, 1999 December; 5(6):533-534
World Journal of Gastroenterology
 ISSN 1007-9327

Comparison of gene expression between normal colon mucosa and colon carcinoma by means of messenger RNA differential display

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Subject headings colonic mucosa; colonic neoplasms; RNA, mess enger; gene expression

INTRODUCTION

Messenger RNA differential display technique, developed by Dr. Liang in 1992[1], is a powerful new tool for identifying and cloning differentially expressed genes in a certain type of cell line, tissue or a special developing stage. Using this method, large amounts of molecular biological information can be obtained easily and quickly. In our study, this technique was used to compare genes expressed differentially between normal colon mucosa and colon carcinomas, in order to understand the molecular biological basis of colon cancer.

MATERIALS AND METHODS

Samples

Fifteen samples of colon carcinoma were obtained from radical colectomy, samples of normal colon mucosa were obtained 8 cm - 10 cm apart from colon carc inoma.

Reagents

Guanidine thiocyanate and β-mercaptoethanol were purchased fro m BRL (America); T_{12}MN primer and AP primer were presented by the National Laboratory for Oncogene and Related Genes, Shanghai Cancer Institute.

Experimental procedures

RNA preparation Total RNA was isolated from normal colon mucosa and colon carcinoma using single-step method of guanidine thiocyanate described by Chomczynski[2,3], some steps had been modified.

Reverse transcription DNA-free RNA was reversely transcribed using the oligo-dT primer T_{12}MN in the presence of [γ-^{32}P]dATP.

PCR amplification Using cDNA products as temples, PCR reaction s were performed in the presence of [γ-^{35}S] dATP, the primer combination was T_{12}MN and AP. Following denaturation for 30 seconds at 94 ℃, the PCR steps consisted of 30 seconds at 94 ℃, 2 minutes at 40 ℃, 30 seconds at 72 ℃ for 40 cycles, followed by 5 minutes at 72 ℃. Amplified PCR products from normal colon mucosa and colon carcinomas were separated side by side on a 7.5 M urea/6% polyacrylamide gel.

Recovery of differentially expressed bands After autoradiograp hy, the cDNA bands representing differently expressed mRNAs were excised from the gel. For each band, extracted cDNA was reamplified for 30 cycles with the same primers and the same PCR conditions used in the initial PCR, except that no radioactive dNTP was included. After PCR, the product was run on 1.5% low melt agarose gel and stained with ethidium bromide.

Northern blot analysis PCR bands of the expected size were cut from the gel, purified and used as probes. DNA probes were radio-labeled by the random prime labeling method, hybridized with RNA from pre samples and other RNA samples respectively.

RESULTS

Total RNAs from normal colon mucosa and colon carcinomas were isolated using single-step method of guanidine thiocyanate, then reversely transcribed into first string of cDNA, T_{12}MA, T_{12}MC, T_{12}MT and T_{12}MG were used as oligo-dT primer individually. The result of alkaline denatured electrophoresis indicated that cDNA molecules were in the range of 0.5kb-5kb. cDNAs were amplified

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Received 1999-04-08

by PCR reaction using the primer combination. T_{12} MN and AP_2, AP_4, PCR products were separated by 6% polyacrylamide gel electrophoresis. Fourteen bands were obtained which were differentially displayed between normal colon mucosa and colon carcinoma. Eight bands (T_1-T_8) were highly expressed in carcinomas, and the other 6 bands were expressed only in normal tissues. T_1 band was verified to be highly expressed in tumor, but had no expression in normal tissues by Northern blot. This cDNA band would be used for cloning and sequencing.

DISCUSSION

The technique of mRNA differential display, by means of combinating T_{12}MN and arbitrary primer AP, can detect all expressed genes in mammalian cells and recover their molecular biological information. These cDNA fragments can be used as probes to isolate target genes from genomic DNA or cDNA library for intensive molecular biological identification. The technique has several advantages over other methods, such as simplicity, sensitivity, reproducibility, versatility and speed, so that it has been used in researches of many diseases, especially in molecular biological study of malignant tumors. We screened 14 cDNA fragments by means of mRNA DD, one of these bands (T_1) was highly expressed in the colon carcinoma which was used for differential display. Using T_1 band as probe, we found that it was also highly expressed in many other colon carcinomas (12/15). In the further study, this DNA fragment can be used for cloning and sequencing. Checking the database of Genebank, if the sequence of this cDNA band has no homology to the sequences of other nucleic acids, it can be considered as partial cDNA fragment of a new colon carcinoma-related gene. By screening the genomic DNA or a certain cDNA library, the full-length cDNA can be cloned, which may be helpful in the study of the molecular biology of colon carcinoma.

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Edited by WANG Xian-Lin
Proofread by MA Jing-Yun