Analysis of gene expression profiles in pancreatic carcinoma by using cDNA microarray

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OBJECTIVES: To survey the gene expression profiles in pancreatic carcinoma by using cDNA microarray and detect target genes for further study.
METHODS: Three mixed samples from 2 cases of normal pancreatic tissue and 4 cases of moderate-differentiated pancreatic carcinoma were studied by means of cDNA microarray consisting of 18 000 genes.
RESULTS: 1484 and 1353 different expressed genes were observed in two cancer samples respectively. We identified 455 genes altered with the same tendency in both samples, including 102 up-regulated and 353 down-regulated genes. There were 274 known genes and 181 unknown genes; 27.8% and 52.0% genes respectively had an expression level in cancer that was 2-fold higher or lower than that in normal samples. Tumor suppressor genes, growth factors and receptor genes, signal conduction genes, transcription factor genes were identified.
CONCLUSIONS: cDNA microarray is an efficient and high-throughout method to investigate gene expression profiles in pancreatic carcinoma. MBD1, EDG1 and gene hypermethylation mechanism would play an important role in the pathogenesis of pancreatic carcinoma.

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Key words: pancreatic carcinoma; cDNA microarray; gene expression profiles

Introduction

The occurrence and progress of tumor may be caused by complicated polygene abnormalities. cDNA microarray does filter and analyze correlated tumor genes in a large scale and flux by using the principle of concentration and parallel disposal.[1] In this study, samples from the resected pancreatic carcinoma of moderate differentiation were studied by means of cDNA microarray consisting of 18 000 genes. In this paper we discuss the molecular basis of the occurrence and progress of pancreatic carcinoma.

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Methods

Establishment of microarray

18 000 cDNA clones in the microarray were supplied by the Cell Biology Institution, Chinese Academy of Sciences, Beijing, China. Among them, 8951 genes represented humans or EST; both of them could be found from the genetic database. The quality of the cDNA clones was measured by electrophoresis (EP), and their average length was 1000bp. The clones were pointed into the Nylon membrane (8 cm × 12 cm) by Point Meter after amplification and purification. The volume of every

point was 100 nl and the diameter was 0.4 mm. Each of the genetic clones was double-pointed. Negative control group consisted of λ phage and DNA with pUC18 carrier.

Hybridization quality control in arrays

There were eight house-keeping genes in the Nylon membrane and each of them encoded respectively as ribosomal protein S9, actin β, 3-phosphoric acid-glycerol dehydrogenase, hypoxanthine phosphoric acid ribose transferase, 23kd protein, ubiquitin protein C, phospholipase A2, and ubiquitin sulf-esterase I. They were distributed evenly to twelve sites as the quality control point of hybridization.

Clinical samples

Normal pancreatic tissue samples were obtained from a man and a woman who had been billed by traffic accident (informed consent was obtained from their relatives) and 4 pancreatic carcinoma tissue samples from the resected tumors provided by the Pancreatic Carcinoma Diagnosis and Treatment Center at Huashan Hospital of Fudan University, Shanghai, China. Pathologic diagnosis revealed that the tumors were adenocarcinomas of grade II. Fresh samples were preserved by liquid nitrogen, and part of pathologically confirmed lesion was used for experimental study.

RNA extraction and probe definition

After RNA was extracted from the samples by using the standard Trizol RNA detachment technology, its quality and quantity were measured by electrophoresis ultraviolet respectively. Polythymine resin was also used to dissociate mRNA from total RNA. The mRNA extracted from the six samples was put into three shares of sample product, and that from two normal samples into one share of N sample product. Every two tumor samples were placed into samples S1 and S2 according to male and female matching and quantified by ultraviolet. P^{33} was added in reverse transcription, and about 1–2 μg mRNA got isotope marks acquired under the action of reverse transcriptase.

Hybridization and image processing

Nylon membrane after point production was hybridized previously with 20 ml hybridization liquid for three hours. Then the hybridized nylon membrane and the P^{33} marked cDNA probe were put into the hybridization tube. They were hybridized in a hybridization oven for 20 hours before the membrane being taken out. The membrane was soaked and rinsed for one hour, and placed again in the oven for drying. Scanning was performed immediately after it was sliced for 5 – 6 hours. FLA-3000A Scan Machine was used for image analysis, and software Array Gauge for data processing. Cutting the background signal, we defined the positive signal standard that the radiointensity was above 7 at each point.

Results

Establishment of cDNA microarray system

A microarray was established with 18 000 cDNA clones chosen randomly from different cDNA databases and the sequence was similar to that of the databases. The probe was made by the same share of hepatocarcinoma cells mRNA and hybridized with microarray independently and repeatedly. Pearson's product-moment correlation coefficient test showed that R^2 was 0.97 and confirmed that experimental repetition of the microarray was powerful.

Gene's discrepancy expressive pattern of pancreatic cancer

Samples of normal pancreatic tissues and 2 samples of pancreatic cancer were scanned after hybridization. The results showed that the maximum scan brightness difference was 1.45, which was between random two in twelve points representing the same house-keeping gene, and the brightness difference between two points in the same clones was less than 1.5, indicating that hybridization is believable. Comparison of scan results between cancer and normal pancreatic tissues showed that the 2 samples of cancer tissue had 1484 and 1353 pieces of differential expression gene respectively. Of them, 455 pieces had the same expression tendency: 102 pieces of up-regulation and 353

of down-regulation. In the 274 pieces of the known genes and 181 pieces of EST with unknown function, the difference which was more than two times occupied 27.8% and 52.0% in the two shares of the samples. In the known genes with the same expression tendency, the following were included: (1) anti-oncogene: Rb, RPS6; (2) growth factor and its receptor: IGF1, IGFBP7, LTBP4, TGFBI; (3) signal transmission related gene: EDG1, COX6A1, RBBP7; (4) transcription regulation factors: MBD1, GTF3C3, GTF2B, EEF1B1, E2F5; (5) cell proliferation cycle factors: TOK-1, MAPK14; and (6) pancreas related gene: AMY2B, pNBC.

Discussion

In this study, we used cDNA microarray consisting 18 000 genes to analyze the gene expression spectrum in pancreatic carcinoma. The microarray of cDNA clones covers a wide range of genes and is suitable for gene screening in a large scale. The house-keeping gene in the array may control the equitability of hybridization very well, and the reliability of verification can clarify the confidence level of the array. Compared with the strain of tumor cells, no-tumor cells in clinical samples are likely to confuse the result. In this study, we increased the number of samples and mixed samples for Northern blot test to decrease the false-positive rate. Gene screening showed significant expression differences in anti-oncogene, cyclin gene, growth factor and its receptor, signal transmission related gene, and transcription regulation factors. These indicated to some extent that the occurrence and progress of pancreatic cancer is a very complicated-process.

Our study revealed 102 pieces of gene up-regulation expression in the two shares of tumor samples. Among them, methyl-cytidine phosphoric acid guanosine conjugated protein 1 (MBD1) showed utmost difference, which was 74.62 times and 5.02 times the values of the normal control group respectively. MBD1 as an important transcription regulation factor located at 18q21 could affect the normal transcription process via its conjugation with the point of methylated cytidine phosphoric acid guanosine (CpG).[2] Promoter excessive methylation was one of the important mechanisms for anti-oncogene inactivation. In this study, inactive anti-oncogene such as $p16^{(INK4a)}$, $p14^{(ARF)}$ underwent promoter excessive methylation in many malignant tumors like breast cancer, liver cancer, lung cancer and kidney cancer. In the pancreatic cancer samples, the abnormally increased expression of MBD1 and significantly decreased expression of E-cadherin (CDH1), retinoblastoma (Rb) gene and E2F transcription 5 (E2F5) were noted. All of them showed anti-cancer activity: CDH1, Rb participated in the negative adjustment of cell proliferation cycle and anti-cell proliferation. The family of the E2F were important adjusting factors for cell proliferation cycle so that lots of genes related to cell proliferation must conjugate with the E2F,[4] where promoter or excessive methylation also occurred.[3-5] We conclude that promoter excessive methylation adjusts the transcription of anti-oncogene negatively, and MBD1 plays a vital role in this process.

In the up-regulation genes we found that there were insulin-like growth factor 1 (IGF 1), transforming growth factor βI (TGFβI), potential transforming growth factor β conjugated protein 4 (LTBP 4), transcription factor ⅢC (GTF3C3), transcription factor ⅡB (GTF2B), and mitoses activation protein kinase 14 (MAPK14 P38). They were related to cell growth, differentiation and apoptosis. In the down-regulation genes, there was also a pair of significant synergy expression genes such as sphingosine kinase (SPHK 1) and endothelium differentiation gene-1 (EDG-1). 1-phosphoric-sphingosine (SPP) as the sphingolipid metabolite of sphingosine under the action of SPHK 1 was active in evoking many biological reaction through its combination with the receptors of the EDG family clan, and was related to mitoses and cell differentiation.[6] The down-regulation of pancreatic amylase 2B (AMY2B) and the pancreas co-transport receptor gene (pNBC) showed that the tumor tissue lost the normal physiological function of the pancreas.

In a large screening of the pancreatic cancer related gene, the cDNA microarray could detect

valuable signs in the complex molecule network. Further study of the genes like MBD1, EDG1 as well as excessive methylation expression is also helpful to clarify the pathogenesis of pancreas carcinoma and its biological behavior.

Competing interest

The author or authors do not choose to response to the statements listed in Instructions for Authors.

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