CEA-mRNA Expression in Peripheral Blood of Patients with Gastrointestinal Carcinoma and its Clinical Significance*

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Abstract **Objective** To investigate the hematogenous micrometastasis status of the patients with gastrointestinal carcinoma, and the significance of CEA-mRNA expression in peripheral blood, as a predictive marker for metastasis and recurrence. **Methods** Peripheral blood were collected before and two weeks after operation from 62 patients and 22 controls (12 cases with benign gastrointestinal disease, 10 normal volunteers).CEA-mRNA expression in the blood was detected by nested reverse transcriptase (RT) polymerase chain reaction(PCR). Pathologic examination was done by conventional method. **Results** In all of 62 cancer patients, 27(43.5%) cases were positive CEA-mRNA expression before operation, and so did 26 (41.9%) postoperatively, but none in controls. In stage Ⅰ-Ⅳ, CEA-mRNA positive rate was 37.5%, 27.3%, 51.9%, 80.0% respectively. There was no significant difference between them (P>0.05). But between stage Ⅰ+Ⅱ and Ⅲ+Ⅳ, the difference was significant (P<0.05). In 35 patients with local lymph node metastasis, 62.9% (22/35) were positive for CEA-mRNA; in the 27 patients without lymph node metastasis, 18.5%(5/27) were positive expression of CEA-mRNA(P<0.01). The CEA-mRNA expression was not correlated with the differentiation of tumour cells and cancer embolus. There was no significant difference in CEA-mRNA expression before and after operation(P>0.05). **Conclusion** CEA-mRNA may be a sensitive marker for detecting subclinical metastasis, recurrence and prognosis for gastrointestinal carcinoma.

Key Words Gastrointestinal carcinoma; RT-PCR; CEA-mRNA

Gastrointestinal carcinoma is the common malignant tumor, but its subclinical metastasis and recurrence after operation can be the most important factors that affect the clinical prognosis of the patients with gastrointestinal carcinoma. If the tumor cell could be found in the lymph node, marrow or blood of the patients, as soon as possible, the doctor could conclude if there were metastasis and recurrence in the postoperation patients with gastrointestinal carcinoma. With the help of Nest-RT-PCR, it is possible that the few recurring and/or macrometastatic tumor cell can be found in peripheral blood, lymph node or marrow in the early stage of the patients [1,2]. RT-PCR is the best method for examining the micro-metastasis in the present[3]. CEA-mRNA can express in the epithelial tissue cells (including the tumor cells), especially in the carcinoma cells from the digestive tract epithelia, but cannot express in the blood of the normal[4,5]. So we examined the CEA-mRNA expression in the blood of patients with gastrointestinal carcinoma by RT-PCR, in order to conclude whether there are the circling or recurring tumor cells, and supplying the diagnosis and therapeutic basis for the patients in peroperation period.

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MATERIALS AND METHODS

Clinical materials

Selecting 62 gastrointestinal carcinoma patients treated in our hospital from June 2000 to June. 2001, they have no any anti-tumor treatment before admission. Among these 37 cases are gastric carcinoma, 25 cases are rectal carcinoma. The rate between male and female are 2.3:1, the range of ages is from 38 to 74 years old, averagely 53.4. All the cases were taken fresh blood samples with heparin for anticoagulation two weeks before and two weeks after the operation, and the expression of CEA-mRNA were examined with RT-PCR. The operation tissue samples were processed with the method of ordinary pathology method, and classifications of the clinical pathology phase were done according to UICC [6]. 10 healthy normal and 12 no-malignant disease patients (including: the normal volunteers, gastric ulcer, colitis, chronic gastritis, ulcercolitis, colon polypus etc) act as control.

The main reagent

RNA extraction liquid, proteinase K, MLV reverse transcriptase, RNAsin, dNTP, reaction buffer, TaqDNA polymerase adding sample buffer, three primers, 2% glucose agar gel(including EB) were purchased from Shanghai fuxin company which supplied positive and negative controls.

The main instrument

TL-16R high speed freezing centrifugal machine (shanghai centrifugal machine researching institution) PCR dilator (perkin, Elmer 2400, UN), gel electrophoresis instrument (FX-DY-252 electrophoresis instrument, Shanghai Fuxing high technology Ltd.). Ultraviolet transmission analytical instrument (Beijing Yicheng technology company), macropipets and so on.

RT-PCR examination

Collecting the sample and separating the seed cell from 5ml fresh blood with heparin for anticoagulation with the separating liquid of lymphocyte within 20min. Taking the total RNA of cells with Trizol according to AGPC.

Synthesizing cDNA with RT-PCR

Primers was synthesized by Shanghai Fuxing Biology Company. The sequences of primers as following:

Primers A 5′-TCTGGAACTTCTCCTGGTCTCTCA-GCTGG -3′ Primers B 5′ -TGTAGCTGTTG-CAAAT-GCTTTAAGGAAGAAGC-3′; Primers C 5′-GGGCCACTGTCGGCATCATGATTGG-3′.

Taking 1-2μl cell RNA for retrotranscription with Primers B (1.0μmol/L, the alignment and source can be seen behind). The reaction condition: 50℃ water bath 30min, retrotranscript the mRNA into cDNA.

Nest-RT-PCR Taking 2ul the production of retrotranscription, and conducting the first PCR. In this reaction system, the primers are primer A and primer B (each taking 0.2μmol/L). The reaction condition: 94℃ denaturation 30s, then raising temperature to 68℃ and sustained 5 min 20s, then circling 20 times, finally taking 2ul reaction production of the first PCR and conducting the second PCR after contrifugating. The primers are primer B and primer C. (each take 0.2μmol/L). The reaction condition is the same as before, circling 20 times, elongating 10 min in 72℃ and then end the reaction.

The Result Analysis

The end products of PCR for electrophoresis analysis. First, adding 4ul of sample buffer into the ending production of PCR, and taking 15ul lower blue liquid after mixing enough, then electrophoresizing 20-30 min (5v/cm) in 2% glucose agar gel, and the electrophoresis can be ended until the blue indicator is away the start point 2cm.Putting the glue over the ultraviolet examination analysis instrument, if the purplish red strip can be seen in 131 bp (the positive control will be at the same point), and the CEA-mRNA will be considered as positive expression. Both the positive and the negative control are supplied by the reagent box.

Statistics analysis

The precise probability method and the x^2 examination were used for all statistical data, $P<0.05$ was consided to be significant.

RESULTS

Clinical Result

37 cases of gastric carcinoma and 25 cases of colorectal carcinomas have no marked difference in histology type, differentiation degree , invading level, lymph node metastasis, and pathology stage. According to UICC, the stage can be divided as following: 8 cases were stage Ⅰ, 22 cases were stage Ⅱ, 27 cases were stage Ⅲ, 5 cases were stage Ⅳ; 30 cases were well differentiated types (including papillary adenpcarcinoma, tubular adenocarcinoma, adenoma canceration), 32 cases were poorly-differentiated types (including low diffetentiated adenocarcinoma, undifferentiated adenocarcinoma, mucinous adenocarcinoma and as well as signet-ying cell); 35 cases with local lymph node metastasis; 8 cases with tumor cell embolus in lymphatic vessels or blood vessels.

CEA-mRNA expression

Among 62 cancer patients, 27 (43.5%) were positive CEA-mRNA expression before operation, and 26 (41.9%) were positive postoperatively, but none was positive in controls. The expression of CEA-mRNA have no significant difference between before and after operation ($p>0.05$). The results of electrophoresis analysis: CEA-mRNA positive patient and the positive control were found a purplish red strip in 131bp, but none was found in controls (Fig.1).

Table 1. The relationship between CEA-mRNA positive expression and the clinical pathology element

Clinical pathology feature	n	CEA-mRNA expression		P
		Positive	(%)	
Clinical pathology stage				
Ⅰ	8	3	(37.5)	
Ⅱ	22	6	(27.3)	
Ⅲ	27	14	(51.9)	<0.05
Ⅳ	5	4	(80.0)	
lymph node metastases				
no metastasis	27	5	(18.5)	
with metastased	35	22	(62.9)	<0.01

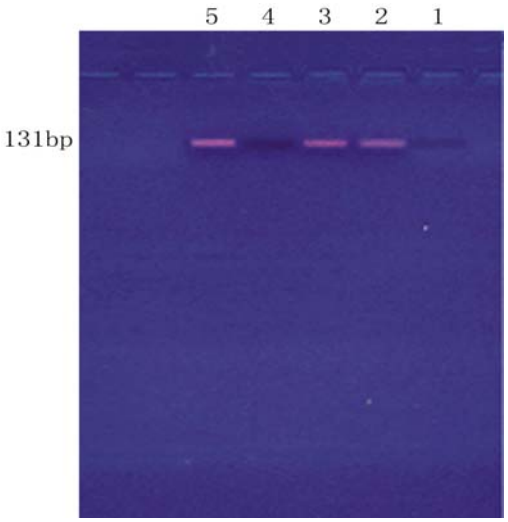

Fig.1 CEA-mRNA electrophoresis analysis
1. The product of PCR of gastric adenocarcinoma whose type is pooly differentiated(—).
2. The product of PCR of colon adenocarcinoma whose type is middle differentiated(+).
3. The product of PCR of gastric adenocarcinoma whose type is middle tubular adenocarcinoma(+).
4. Standard negative control.
5. Standard positive control (131bp).

The relationship between CEA-mRNA expression and the clinical pathology elements (see table 1). In stage I–IV, CEA-mRNA expression has no significant difference ($p>0.05$). But between stage I+II and III+IV, the difference was significant ($p<0.05$). The CEA-mRNA expression was correlated with metastases in local lymph nodes ($p<0.01$); There was no significant difference between CEA-mRNA and the differentiation of tumor cells, cancer embolus.

Among 27 cases positive expression of CEA-mRNA before operation, 19 cases remain positive after operation. So, the operation can alleviate the tumor load, and the tumor cell in peripheral blood reduced, immunity function will effect at this time. Among 35 negative expression before operation, 7 cases were positive expression after operation, so the operation will make convenience for blood spread.

DISCUSSION

In the tumor treatment, subclinical metastases and recurrence can affect prognosis. If one and many molecular biological markers which indicate tumor subclinical metastasis or recurrence can be found, these will have significant meaning for clinical treatment. Gastrointestinal carcinoma is belong to malignant carcinoma from epithelia. Examining the distinctive epithelial tissue marker such as: CK19, CK20, MUC, and CEA-mRNA expression in peripheral blood and lymph node is one of main methods for diagnosing the micrometastasis of gastrointestinal carcinoma in the earlier period. This research took RT-PCR to exam CEA-mRNA expression in peripheral blood of 62 gastrointestinal carcinoma patients, and found 43.5% gastrointestinal carcinoma patients´ CEA-mRNA expression in peripheral blood were positive before operation, and no expression in 10 normal people and 12 patients with no-maligant gastrointestional diseases. The results suggested that CEA- mRNA-RT-PCR can check out sensitively gastrointestinal carcinoma cell which was spread in circulation. with RT-PCR method, Mori[7] found among 41 gastric carcinoma and breast cancer patients who received the radical operation, about 27% were positive of CEA-mRNA, which was regarded as sensitive marker for detecting recurrence. In recent years, Chin et al

found 13 HPV-16-E16 mRNA positive of transforming gene in the peripheral blood of 15 cervical cancer with clinical metastasis. This result can confirmed that using RT-PCR to examine mRNA in peripheral blood can be a sensitive important method for clinical micrometastasis[8].

The research indicated that CEA-mRNA positive cancer cell was found in peripheral blood before operation in 27% alimentary tract tumor patient, in which 25% were stage Ⅱ gastric carcinoma, 30% were stage Ⅰ esophagus cancer and Dukes´ A colon cancer. which this test also shows the positive rate of CEA-mRNA in peripheral blood of I stage patient is 37.5%(3/8). So we can conclude that tumor cell have spread and metastasis in the early stage of gastrointestinal carcinoma.

This test showed that the expression of CEA-mRNA in stage Ⅰ-Ⅳ have no significant difference, but there was significant between Ⅰ+Ⅱ and Ⅲ+Ⅳ, The positive rate of CEA-mRNA expression raised with the development of disease. The CEA-mRNA expression rate (62.9%) in peripheral blood before operation of the patients with local lymph nodes metastasis was higher than that of the patients without lymph node metastasis ($p<0.01$). This shows that the existence of micrometastasis in gastrointestinal carcinoma peripheral blood was closely related with lymph node metastasis. For stage I patient in this team, lymph node metastasis have not been found by the examination of ordinary pathology, but the CEA-mRA examination told us the metastatic cancer cell wore found in the blood of 37.5% patient, So the blood metastasis is earlier than the metastasis in lymph node. So there may be limitations for the prognosis of gastrointestinal carcinoma just by the examination of the lymph node metastasis.

Among 35 patients who were CEA-mRNA negative in peripheral blood before operation, 7 patients became positive after operation, which suggested that even though the radical operation couldn´t abolish all the cancer cells, and meanwhile the tumor cell spread may be caused. The result of this research pointed out the gastrointestinal carcinoma patients should receive the general chemotherapy and or immunization treatment in order to reduce the subclinical metastasis and recurrence, and raise the survival rate.

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