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World J Gastroenterol 2006 March 7; 12(9):1408-1411
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Detection of carcinoembryonic antigen mRNA in peritoneal washes from gastric cancer patients and its clinical significance

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Received: 2005-06-29 Accepted: 2005-10-09

Abstract

AIM: To establish a more sensitive method for detection of free cancer cells in peritoneal washes from gastric cancer patients during surgery and to evaluate its clinical significance.

METHODS: The carcinoembryonic antigen (CEA) mRNA levels in peritoneal washes from 65 cases of gastric cancer were detected by real-time RT-PCR. Peritoneal lavage cytology (PLC) was applied simultaneously to detection of free cancer cells. Negative controls included peritoneal washes from 5 cases of benign gastric disease and blood samples from 5 adult healthy volunteers.

RESULTS: There was no CEA mRNA in peritoneal washes from benign gastric disease patients and in blood of adult healthy volunteers. The positive percentage of free cancer cells detected by real-time RT-PCR was 47.7% and only 12.3% by PLC. The positive rate of CEA mRNA was significantly related with serosa invasion between peritoneal metastasis and stage of gastric cancer.

CONCLUSION: Real-time RT-PCR is a sensitive and rapid method for the detection of free cancer cells in peritoneal washes. The presence of free cancer cells in peritoneal washes is related to the pathologic stage of gastric cancer.

Key words: Real-time RT-PCR; Peritoneal washes; Gastric cancer; Carcinoembryonic antigen

Zhang YS, Xu J, Luo GH, Wang RC, Zhu J, Zhang XY, Nilsson-Ehle P, Xu N. Detection of carcinoembryonic antigen mRNA in peritoneal washes from gastric cancer patients and its clinical significance. *World J Gastroenterol* 2006; 12(9):1408-1411

http://www.wjgnet.com/1007-9327/12/1408.asp

INTRODUCTION

Gastric cancer is one of the most common causes of cancer death in China. The postoperative survival rate of patients with advanced gastric cancer remains very low[1-3]. About 50-60% of gastric cancer patients with serosal invasion after curative resection eventually develop peritoneal metastases[4, 5]. Free cancer cells in the abdominal cavity can predict the prognosis of gastric cancer patients[6-8]. Peritoneal lavage cytology (PLC) has been used to examine cancer cells in peritoneal washes for more than a decade. But it lacks sensitivity and is time consuming[9, 10]. Since CEA is a specific marker of gastric cancer cells, detecting CEA mRNA in peritoneal washings from gastric cancer patients may determine the treatment strategy[11]. To date, many methods to detect CEA mRNA of cancer cells are available, but their sensitivity remains to be improved[11-13]. Recently real-time reverse transcription polymerase chain reaction (RT-PCR) has been used to detect CEA mRNA of free cancer cells in peritoneal washes from gastric cancer patients and micro peritoneal metastasis in these patients[14-17]. In the present study, we used real-time RT-PCR to detect CEA mRNA in peritoneal washes during gastrectomy with its clinical significance evaluated.

MATERIALS AND METHODS

Patients and collection of samples

Sixty-five patients with gastric cancer (aged 35-76 years, mean age 60.6 ± 11.5 years, 50 men and 15 women) undergone curative surgery in our hospital were enrolled

Table 1 Sequences of CEA primers and hybridization probe

Primers	Sense	5′- AACTTCTCCTGGTCTCTCAGCT
	Anti-sense	5′- GCAAATGCTTTAAGGAAGA
Probes	Donor	5′- TGAAATGAAGAAACTACACCAGG-FL
		LC-5′- CTGCTATATCAGAGCAACCCCAA-P
Acceptor		

Table 2 Comparison of clinical pathological factors and CEA mRNA expression

	Positive CEA mRNA [1](%)	*P*-value
Gender:		
Male	48 (24/50)	
Female	47 (7/15)	1.000
Depth of invasion:		
pT3	61 (22/36)	0.024
pT1+pT2	31 (9/29)	
Cancer location		
Cardia	53 (10/19)	0.618
Gastric body	40 (10/25)	
Gastric antrum	52 (11/21)	
Lymph node metastasis		
yes	47 (21/45)	1.000
no	50 (10/20)	
TNM stages		
I + II	33 (10/30)	
III + IV	60 (21/35)	0.046

[1] It is considered as positive If CEA mRNA is detected.

in the present study. No patients received preoperative radiation therapy or pre-chemotherapy prior to their enrollment. Five patients with benign gastric disease undergone gastrectomy and 5 blood samples from health volunteers were included as negative controls. During the operation, peritoneal metastasis was found in 6 patients. At the beginning of operation, 50 mL of saline was poured into the Douglas cavity and aspirated after gentle stirring. After centrifugation, a certain amount of washes was cytopathologically examined. The total RNA was extracted from the rest washes or from blood cells using phenol-chloroform kit according to the manufacturer's instructions(Fermentas Company, Hanover, USA). All experiments were approved by the local ethic committee.

Real-time RT PCR

CEA specific primers and probes were synthesized by the Shengyou Company (Shanghai, China). The donor probe was labeled with fluorescence at the 3' end, while the acceptor probe was labeled with LC Red 640 at 5' end (Table I). The standard CEA mRNA sample was taken from a liver metastasis in a colon cancer patient. All procedures were carried out according to the manufacturer's protocol. In brief, real-time RT-PCR of CEA mRNA was performed in two steps. For reverse transcription, it was carried out in a 40-μL reaction mixture containing 1 μg oligo(dT)18, 2 μg total RNA, 4 μL 10 mmol/L dNTP and 400 units RevertAid M-MuLV reverse transcriptase. For real-time PCR amplification, it was carried out in a 20 μL reaction mixture containing 2 μL cDNA, 22.5 pmol of both forward and reverse primers, 5 pmol probes, 2 μL 10 mmol/L dNTP and 1 unit Tag DNA polymerase. Thermal cycling conditions included at 95 °C for 1 min to activate Taq polymerase. After that, 45 cycles of PCR amplification were performed at 95 °C for 5 s, at 60 °C for 10 s and at 72 °C for 10 s. Samples were amplified in duplicate. All PCRs were performed on LightCycler (Roche Diagnostics).

Table 3 Comparison of CEA mRNA in gastric cancer patients with or without peritoneal metastasis (mean ± SD)

Peritoneal metastasis	CEA mRNA	*P*-value
Yes	16006.2±18242.	$P<0.001$
No	40.5±158.9	

Statistical analysis

The statistical significance of differences in clinical pathological factors and positive rates of CEA mRNA was analyzed with the chi-square test. Comparisons of CEA mRNA values between groups with or without peritoneal metastasis were analyzed by the Mann-Whitney test. $P<0.05$ was considered statistically significant.

RESULTS

Positive CEA mRNA was found in 31 (47.7%) out of the 65 patients by real-time RT-PCR, whereas free cancer cells were found only in 8 patients (12.3%) by cytological method. CEA mRNA in peritoneal washes was significantly correlated to the depth of tumor invasion, peritoneal metastasis and different pathologic classifications (Tables 2 and 3).

DISCUSSION

TNM classification[18, 19] or modified TNM classification[20, 21] has been used to evaluate the prognosis of patients with gastric carcinomas for many years. However, it cannot cover all clinical situations in these patients[21]. The prognosis of gastric cancer is poor mainly due to intraperitoneal relapse. Free cancer cells (FCC) may exfoliate from cancer-invaded serosa contributing to peritoneal dissemination, which is the most frequent pattern of recurrence in patients with gastric cancers[5]. The presence of FCC in the peritoneal cavity is significantly correlated with classical prognostic factors (TNM classification)[22]. Since 1998, Japanese Gastric Cancer Association (JGCA) has suggested that the presence of free cancer cells in the peritoneal cavity should be considered as an independent prognostic marker in patients with gastric cancers[23]. Detecting micro peritoneal metastasis in gastric cancer patients during gastrectomy may determine the prognosis of gastric cancer patients. Many methods can detect free cancer cells in peritoneal washes. Peritoneal lavage cytology (PLC) is an

established routine method for detecting free cancer cells in the peritoneal washes, but the positive rate of PLC for FCC is only 11%[24]. In our study, the positive rate of PLC for FCC was 12.3% (Table 2). More sensitive and specific methods are necessary for the improvement of detecting FCC in peritoneal washes. With the development of PCR technology[25], real-time RT-PCR can detect specific markers of free cancer cells in peritoneal washes. CEA mRNA is one of the most common specific markers of FCC, although Goeminne *et al*[26]reported that mesothelial cells and infiltrating leukocytes also express CEA. This cross-reaction could be avoided by combining different markers. Real-time RT-PCR allows rapid amplification and accurate quantification of CEA mRNA and online data analysis without a post-PCR procedure, making it an applicable method for routine diagnosis[27]. In the present study, no CEA mRNA expression was detected in the control samples collected from patients with benign gastric diseases or from volunteer's blood. Moreover, the positive rate of CEA mRNA was related with membrane invasion, peritoneal metastasis, and stage of gastric cancer, suggesting that free cancer cells in peritoneal washes are closely related to tumor stages. The mean value of CEA mRNA was much higher in patients with peritoneal metastasis than in those without peritoneal metastasis (Table 3), suggesting that CEA mRNA can be used in the evaluation of peritoneal metastasis. Marutsuka *et al*[28] reported that RT-PCR can be used in routine assays. With improvement of skill the sample handling could be shortened and becomes more practicable.

In conclusion, real-time PCR is a sensitive and rapid method for the detection of free cancer cells in peritoneal washes.

ACKNOWLEDGEMENTS

The authors thank Qing-Feng Mu for providing excellent technical assistance.

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S- Editor Wang J **L- Editor** Wang XL **E- Editor** Wu M