PO Box 2345, Beijing 100023, China
www.wjgnet.com
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World J Gastroenterol 2006 August 28; 12(32): 5219-5222
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

K-19 mRNA RT-PCR in detecting micrometastasis in regional lymph nodes of gastric cancer

Jian Suo, Quan Wang, Hong-Juan Jin, Hong Li, Hang Zhao

Jian Suo, Quan Wang, Hong-Juan Jin, Hong Li, Hang Zhao, Department of General Surgery, First Hospital, Changchun 130021, Jilin Province, China
Supported by funds from the Science and Technology Department of Jilin Province, China
Correspondence to: Dr. Quan Wang, Department of General Surgery, First Hospital, Changchun 130021, Jilin Province, China. wangquan-jlcc@hotmail.com
Telephone: +86-431-5612421
Received: 2005-04-26 Accepted: 2005-09-20

Abstract

AIM: To investigate the value and prospect of RT-PCR in detecting micrometastasis in regional lymph nodes of gastric cancer.

METHODS: Histopathology was used and K19 mRNA expression was detected by RT-PCR in tumor tissues and lymph nodes from gastric cancer patients undergoing radical resection of gastric carcinoma.

RESULTS: K19 mRNA was expressed in all tumor specimens of 30 cases; of the 126 lymph nodes, 26 were histopathologically positive (20.6%), and 42 positive (33.3%) by RT-PCR. Amplification fragments of 460 and 540 bp were shown in all the tumor tissues and metastatic lymph nodes after K19 and β-actin RT-PCR, while only a 540 bp fragment appeared in the lymph nodes of non-tumor patients.

CONCLUSION: K19 mRNA RT-PCR is sensitive and specific in testing micrometastasis in regional lymph nodes of gastric cancer, and it is superior to routine histopathology.

Key words: K-19 mRNA; RT-PCR; Micrometastasis; Gastric cancer

Suo J, Wang Q, Jin HJ, Li H, Zhao H. K-19 mRNA RT-PCR in detecting micrometastasis in regional lymph nodes of gastric cancer. *World J Gastroenterol* 2006; 12(32): 5219-5222

http://www.wjgnet.com/1007-9327/12/5219.asp

INTRODUCTION

Gastrointestinal cancer is the most common malignant tumor of the digestive tract. For histologically node-negative gastrointestinal cancer, even after curative resection of an early cancer, some patients die of metastasis and recurrence[1]. Metastasis and recurrence result from dissemination of cancer cells. Micrometastasis has been proposed by many investigators. Usually it is through blood and lymphatic vessels and no more than 2 mm in diameter[2]. Detection of occult metastatic cells is useful for prognosis, prediction of recurrence, and adjustment of therapies.

Among many prognostic factors, lymph node metastasis is one of the most useful indicators for patients with gastric and colorectal carcinoma[3-7]. Metastasis is usually detected by conventional histological examination, but many negative lymph nodes have micrometastasis on re-examination by serial sectioning and immunohistochemical assay[8-11]. Serial sectioning and immunohistochemical staining certainly increase the yield of occult metastasis, however, it seems to be time-consuming and labor intensive. These methods have not been performed routinely in most hospitals. To overcome this drawback, diagnostic procedures for the detection of micrometastasis at the genetic level have developed rapidly, such as RT-PCR[12-14]. RT-PCR can detect genes that are exclusively expressed in carcinoma cells but not in normal lymph nodes or bone marrow. It is a highly sensitive and specific method. It was reported that by RT-PCR it was possible to detect one cancer cell from among 10^4 to 10^6 normal appearing lymph node cells. Lymph node occult metastasis of gastrointestinal cancer indicated by K19 mRNA expression can be considered as confirmation of the presence of metastasis. The current study was designed to investigate the value and prospect of RT-PCR in detecting micrometastasis in regional lymph nodes of gastric cancer by examination of K19 mRNA expression.

MATERIALS AND METHODS

Tissue samples

The 30 tumor specimens and 126 lymph nodes were obtained through radical resection of gastric carcinoma of patients from the Department of General Surgery, First Hospital, Jilin city from 2001 to 2002, and tumor specimens were confirmed by pathology. The specimens

were processed immediately after the resection: tumor tissues were obtained; lymph nodes were peeled off carefully. Fat tissue and blood were wiped off, lymph nodes were cut into two halves by clean bistouries, and sterilized physiological saline was used for rinsing to prevent the contamination of tumor cells. One half of a lymph node was fixed by formaldehyde; the other half was immersed into liquid nitrogen, and then preserved in a -70℃ freezer till the next day for RNA extraction. Meanwhile, lymph nodes from 8 non-tumor patients were used as negative control.

Reagents

The reagents included TRI reagent (GIBCO), AMV, Taq enzyme, DNTPs and Rnasin (Promega), Marker and Olig (dt) (TaKaRa); the rest of the reagents were all homemade provided by local suppliers.

Methods

Primer design and synthesis: Primer design of CK19 and β-actin was based on previous methods[15] with some modifications. CK19 primer is: 5'-AGGTGGATTCCG CTCCGGGCA-3', 5'-ATCTTCCTGTCCCTCGAGC A-3'. The amplification fragment of the primer (Wubo Gene Corp., Beijing) was 460 bp. β-actin primer is: 5' -GTGGGGCCCCAGGCACCA-3', 5'-CTTCCTTAATG TCACGCACGATTTC-3'; the amplification fragment of the primer(Dinguo Bio Corp., Beijing) was 540 bp. It was used as an internal control to determine that the RNA did not decompose.

RNA extraction: TRIzol was used to extract total RNA. The absorbency (A) was tested. Then gel electrophoresis was performed to identify its components.

Reverse transcription (cRNA synthesis): Reverse transcription system was 50 μL, containing RNA 4 μL, Oligo(dt) 2 μL, 5×Buffer 10 μL, dNTPs 4 μL, Rnasin 1 μL, AMV 4 μL and DEPC-H_2O_2 5 μL incubated for 60 min at 42℃, to acquire cRNA.

PCR reaction: The reaction system was 100 μL, containing cDNA 25 μL, 10 × Buffer 10 μL, dNTPs 8 μL, K19 primer 2 μL, β-actin primer 0.5 μL, Taq enzyme 1 μL, $MgCl_2$ 6 μL, DEPC-H_2O_2 47.5 μL. The cycle parameters of the reaction system were 94℃ for 45 s, 55℃ for 45 s, 72℃ for 1 min, 35 cycles and extention for 10 min at 72℃.

PCR product analysis: PCR products were electrophoresed on 2% agarose gel, EB stained, and observed with ultraviolet light and photographed. The results were compared with pathological result.

Statistical analysis

We used t-test to compare between the histopathological results and RT-PCR results. $P < 0.05$ was taken as significant.

RESULTS

Comparison between histopathological result and K19 mRNA RT-PCR in detecting micrometastasis in regional lymph nodes

In 126 lymph nodes, 26 were positive in both routine histopathological testing and RT-PCR, K19 mRNA was expressed in 42 lymph nodes by RT-PCR amplification. It showed that there was metastasis in 16 lymph nodes which could not be found by histology examination. Of the regional lymph nodes, 20.6% were positive in histology, while 33.3% were positive in K19 mRNA by RT-PCR; and there were none that were positive for histology and negative by RT-PCR. In 30 cases of gastric tumor, 3 were positive in regional lymph nodes by RT-PCR while pathology showed no metastasis.

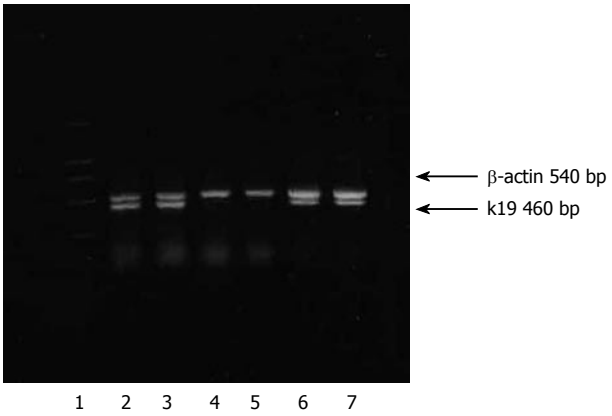

Figure 1 Representative results of RT-PCR. Lane 1: Marker; Lane 2: Tumor tissue sample; Lane 3: Negative lymph node by routine histopathology (K19 positive); Lane 4: Negative lymph node by routine histopathology (K19 negative); Lane 5: Normal lymph node of the non-tumor cases; Lanes 6, 7: Positive lymph node by routine histopathology.

Specificity of RT-PCR amplification

All the tumor tissues and metastatic lymph nodes showed amplification fragments of 460 bp and 540 bp after RT-PCR amplification of K19 mRNA and β-actin, while in the lymph nodes of 8 non-tumor cases it showed only specific amplification fragments of 540 bp, indicating no K19 mRNA amplification product was expressed. Thus this system had superior amplification specificity (Figure 1).

DISCUSSION

There has been no uniform criterion for micrometastasis. In general, a focus not larger than 2 mm is called micrometastasis, which can not be easily found by routine method, whereas RT-PCR could increase the detection rate greatly. Zheng *el al*[16] suggest selecting an ideal marker gene of the tumor as a histology specific marker. Keratin 19 is one of the histology markers, which is highly specific and only expressed in tumor tissue and tumor-originating normal tissue, but not expressed in normal mesenchymal tissues like lymph nodes[17-20].

In this study, K19 mRNA was expressed in both tumor tissue and metastatic lymph nodes, but not in non-tumor cases. It indicates that K19 mRNA is applicable to detect the micrometastasis in regional lymph nodes by RT-PCR amplification. Moreover, the results of our study suggest that RT-PCR is more sensitive than routine histopathology in detecting micrometastasis and K19 can be a sensitive index for detecting metastasis in regional lymph nodes,

which accords with Liu's report[21].

Because RT-PCR is the method that is sensitive and has great capability to amplify, sometimes there can be a pseudopositive, and the main reason could be that the lymph nodes are contaminated by tumor cells and normal epithelial cells and there is cross-contamination of byproduct during RT-PCR amplification. In the experiment, as the internal control, β-actin ensured reliability and the result showed that no K19 mRNA was expressed in any of the non-tumor cases under the same amplification condition, indicating that there was no pseudopositive in the RT-PCR amplification system. K19 mRNA RT-PCR has a high sensitivity and specificity in detecting micrometastasis in regional lymph nodes of gastric cancer and can detect the subtle metastasis which cannot be found by routine histology. This is of great clinical significance. According to the present staging criterion for gastroenteric cancer, the positive result will lead to change in the staging of tumor and alteration in therapy and prognosis judgment. Ye[22] and Yan[23] concluded that compared with lymph node-negative cases, K19 mRNA RT-PCR has obvious prognostic value on recurrence and survival time for the patients after the operation, even if there is a single metastasised tumor cell in the lymph node. Thus, the resection for early and intermediately staged patients should be as radical as possible[24-25], so as not to miss the micrometastasis in lymph nodes and to reduce the recurrence; and the adjuvant therapy and follow-up should be enhanced as well.

The development of the subtle tumor cells in the lymph nodes depends on the immunity of the human body and other factors, which is possible but does not develop into an obvious metastasis. At present, there are still some questions left unanswered about detecting the micrometastasis in regional lymph nodes: one is that pseudopositives are possible because of its great capability of amplification; the other is how to choose a more specific tumor marker. Before the advent of serial analysis of micrometastasis of tumor cells, tumor cells were separated by an immunomagnetic method and extracted, which was thought to be the most attractive technique[26]. Okadda[27] put forward that a multiple-marked RT-PCR has a better effect on detecting micrometastasis in lymph nodes, and researchers are trying to find more sensitive and specific tumor markers including combinations of multiple-markers.

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S- Editor Wang J **L- Editor** Zhu LH **E- Editor** Ma WH