

Expression of the bacterial gene in gallbladder carcinoma tissue and bile

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BACKGROUND: The major causive factors of gallbladder carcinoma are very complex. Cholecystitis with gallstone was reported one of the most important factors. Many research revealed that cholecystitis or gallstone can give rise to epithelial hyperplasia of gallbladder mucosa or canceration secondarily. In this study, 46 patients were detected in order to find the relationship between infection of different bacteria and formation of gallbladder carcinoma.

METHODS: Using the common gene primer of bacteria 16S ribosomal RNA (rRNA), we detected bacterial gene fragments of gallbladder carcinoma tissues in 46 patients by polymerase chain reaction (PCR). Relative bile was also detected by PCR in 18 patients who underwent operations, including U-tube drainage (1), right or left biliary tube drainage (4), radical cholecystectomy (9), and cholecystorrhaphy (4). The tissue fragments of gallbladder carcinoma from the remaining 28 patients were paraffin slices.

RESULTS: The positive rate of bacterial DNA in gallbladder carcinoma tissue was 78.3% (36/46). The sequence of 16S ribosomal RNA gene fragments amplified by PCR was approximately 371 base pairs (bp). Multiple kinds of standard bacterial gene fragments obtained from 36 patients included *Colibacillus*, *B. fragilis*, *Klebsiella*, *C. perfringens* and *Clostridium*, with a positive rate of 78.3% (36/46). Among the 36 patients, 14 patients with gallbladder carcinoma received operation and their relative bile at operation was detected bacterial gene fragments with a positive rate of 77.8% (14/18). This result was close to that in gallbladder carcinoma tissues.

CONCLUSIONS: Our results suggested that there might be a relationship between occurrence of gallbladder carcinoma and infection of different kinds of bacteria, especially anaerobic bacteria—*C. perfringens*. This reminds us that the gallbladder mucosa stimulated by anaerobic and aerobic bacteria might be the principal cause for the development of car-

cinoma.

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KEY WORDS: bacteria; gene; expression; gallbladder carcinoma; tissue; relative bile

Introduction

The development of polymerase chain reaction (PCR) technique enables us to investigate the relationship between bacteria, especially the anaerobic, and tumor occurrence.^[1-5] Since the relationship between bacterial infection and gallbladder carcinoma is obscure, we amplified the bacterial gene fragments in the gallbladder tissues from 46 patients with gallbladder carcinoma, and in 18 of them bile was also detected by PCR. The aim of this study was to assess the role of bacteria in the development of gallbladder carcinoma.^[6]

Methods

Patients

Seven male and 11 female patients, aged 18–69 years (average 41.1 years) were subjected to operations, including U-tube drainage (1), right or left biliary tube drainage (4), radical cholecystectomy (9), and cholecystorrhaphy (4). Their bile underwent PCR amplification. The tissue fragments of gallbladder carcinoma from the remaining 28 patients were taken from formalin-fixed, paraffin-embedded tissues. All samples obtained from the Affiliated Hospital of Medical College, Qingdao University, Qingdao and the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China were pathologically confirmed.

Methods

According to the literature,^[3] the sequences of bacterial primers were designed for amplifying bacterial 16S rRNA: P1 5'-TGCGGTTGGATCACCTCCT-3', P2 5'-TCCCCACCTTCCTCCAGTT-3' (Institute of Cellular Biology, Shanghai, China). Specimens of fresh gallbladder carcinoma tissues of 18 patients were obtained at operation, and stored immediately at -72 °C after washing with brine. At the beginning of the

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test, 0.2 g iced tissue was added with 10 mmol/L Tris (trishydroxymethylaminomethane)-EDTA (ethylenediamine tetraacetic acid) at pH8.0 and 1% sodium dodecyl sulfate (SDS), digested by 100 µg/µl proteinase K. DNA was precipitated for one night at 37 °C. The total incubation volume was 50 µl, containing 29 µl sterilized water, 5 µl primers (P1, P2), 4 µl 4 × dNTP (200 mmol/L), 2 units Taq DNA, 5 µl amplified DNA, re-suspended in 50 µl TE buffer (Tris/EDTA). DNA was extracted by the traditional purification method. The PCR cycle took place at 95 °C for 45 s, 58 °C for 45 s, and 72 °C for 1 min. Thirty-four cycles were carried out for each reaction. PCR-amplified products (10 µl) were applied to agarose gel containing ethidium bromide, and after electrophoresis (60 V) for 3 hours, DNA was visualized under ultraviolet light, recorded and photographed. Blank and standard contrast was provided in each group.

Results

The positive rate of bacterial DNA in the gallbladder carcinoma tissue was 78.3% (36/46). The sequence of 16S ribosomal RNA gene fragments amplified by PCR were approximately 371 base pairs (bp). Multiple kinds of standard bacterial gene fragments obtained from 36 patients included *Colibacillus*, *B. fragilis*, *Klebsiella*, *C. perfringens* and *Clostridium* with a positive rate of 78.3% (36/46). Among these patients, 14 patients with gallbladder carcinoma received operation. Their relative bile at operation was detected bacterial gene fragments, with a positive rate of 77.8% (14/18). This result was quite close to that in gallbladder carcinoma tissues.

Discussion

Bacterial rRNA is divided into 5S, 16S and 23S rRNA according to their descending coefficients. The 16S rRNA bacterial gene is the comparative DNA sequence in serial number of bacterial chromosome, which is known to exist in all kinds of bacterial chromosomes.^[7-11] Its internal structure can be divided into changeable and unchangeable areas; but no significant difference exists in the unchangeable area. The changeable area is formed in the process of bacterial evolution, which makes the difference of bacteria. Theoretically, different primers can be designed to detect all kinds of bacteria by PCR amplification.^[12-18] In this experiment a pair of common primers was designed in the unchangeable area of bacteria; five kinds of different bacteria standard strains were amplified by PCR, and all produced 371bp of DNA fragments.^[19-27] The result was similar to that of gallbladder carcinoma tissues. We conclude that bacterial fragments exist in the core center of gallbladder carcinoma

tissues.^[128-34]

The major causative factors of gallbladder carcinoma are very complex. Cholecystitis with gallstone was reported one of the most important factors. Many research revealed that cholecystitis or gallstone can give rise to epithelial hyperplasia of gallbladder mucosa or canceration secondarily. In this study, 78.3% patients (36/46) were subjected to amplification of bacteria gene fragments, most of which was *C. perfringens*. This indicates that relationship exists between gallbladder carcinoma and infection of anaerobic bacteria, especially *C. perfringens*. The gallbladder mucosa stimulated by anaerobic and aerobic bacteria might be the major cause for the development of carcinoma.^[35-43]

Competing interest

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

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