Original Article
Distribution and gene mutation of enteric flora carrying β-glucuronidase among patients with colorectal cancer

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Abstract: Objective: To explore the difference of distribution in intestinal flora among colorectal cancer patients and healthy controls and investigate characteristics and changes of sequences in beta-glucuronidase (β-glucuronidase, β-G). Methods: Bacterial genomic DNA and E. coli DNA in feces were extracted from colorectal cancer patients and healthy controls respectively. Specific primers for β-G gene were designed and amplified by PCR as templates of fecal bacteria genomic DNA and E. coli DNA respectively. Results: Compared with normal control, the amount of E. coli in cancer group increased significantly, Lactobacillus and Bifidobacterium probiotics reduced significantly, and proportional quantity of anaerobic bacteria and aerobic bacteria reversed. The intestinal flora carry β-G in both groups, and homologies with uidA gene sequences encoding the β-G were 99% and 98% respectively. In colorectal cancer group the 1141th and 1148th A base were deleted. The 1149th A base mutated into T base, and the 1158th A base mutated into G base; however, in healthy control group the 1141th and 1148th position A base was deleted, and the 1149th A base mutated into T base. Conclusion: There are differences of intestinal flora distribution between cancer group and healthy control group. The gene mutation and deletion of intestinal flora of β-G gene appear at the same time at 1141th, 1148th and 1149th in both cancer group and healthy control group, and 1158th genetic mutation appears only in colon cancer group.

Keywords: Colon cancer, intestinal bacteria, E. coli, β-glucuronidase

Introduction
Colorectal cancer is a common malignant tumor and its morbidity is increasing [1, 2]. It has been reported that the pathogenesis and development of this disease were associated with intestinal flora [3, 4]. Human intestinal florases are important factors of intestinal environment. Host’s heredity and external environment in which the host exists would affect the balance of human intestinal microbiology. The host’s healthy condition is also closely related to the balance of human intestinal microecology [5].

β-glucuronidase (β-G) is an acid hydrolase, its positive rate in E. coli is up to 97% and it has a high specificity [6]. β-G-mediated glucuronidation is the main pathway of detoxification in human body, while the activity of β-G carried by intestinal flora in colorectal cancer patients is obviously lower than that in health population [7, 8].

This paper aims to reflect changes of intestinal flora in colorectal cancer patients by analyzing the difference in intestinal microecology of stool samples between colorectal cancer patients and health population, to explore the intestinal flora in colorectal cancer patients, to investigate the relationship between the characteristics of β-G produced by the intestinal flora and colorectal cancer, and to compare sequence for β-G which is produced by E. Coli and can lead to formation of colorectal cancer between two groups, which may provide new ideas for the prevention and treatment of gastrointestinal cancer.

Materials and methods

Samples
Stool samples in colorectal cancer group were provided by Jilin City Cancer Hospital (30 samples). All the patients in colorectal cancer group did not receive antibiotics, hypertonic prepara-
Flora in colorectal cancer patients

Table 1. Culture conditions of enteric flora

<table>
<thead>
<tr>
<th>Medium</th>
<th>Bacterium</th>
<th>Dilution</th>
<th>Culture environment</th>
<th>Culture time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sb medium</td>
<td>Yeast</td>
<td>10^4, 10^5, 10^6</td>
<td>Aerobic</td>
<td>24</td>
</tr>
<tr>
<td>Staphylococcus aureus selective Agar 110</td>
<td>Staphylococcus</td>
<td>10^4, 10^2</td>
<td>Aerobic</td>
<td>24</td>
</tr>
<tr>
<td>EMB medium</td>
<td>Escherichia coli</td>
<td>10^3, 10^4</td>
<td>Aerobic</td>
<td>24</td>
</tr>
<tr>
<td>Bile esculin agar</td>
<td>Enterococcus</td>
<td>10^4, 10^5</td>
<td>Aerobic</td>
<td>48</td>
</tr>
<tr>
<td>BS medium</td>
<td>Bifidobacterium</td>
<td>10^4, 10^6, 10^8</td>
<td>Anaerobic</td>
<td>24</td>
</tr>
<tr>
<td>LBS medium</td>
<td>Lactobacillus</td>
<td>10^4, 10^5, 10^6</td>
<td>Anaerobic</td>
<td>48</td>
</tr>
<tr>
<td>PS medium</td>
<td>Peptococcus</td>
<td>10^5, 10^6</td>
<td>Anaerobic</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 2. Comparison of enteric flora in both groups (Log10)

<table>
<thead>
<tr>
<th>Enteric flora</th>
<th>Colorectal cancer group (n = 30)</th>
<th>Control group (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>7.64 ± 0.23</td>
<td>6.09 ± 0.10</td>
</tr>
<tr>
<td>Yeast</td>
<td>3.85 ± 0.91</td>
<td>3.48 ± 0.76</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>3.84 ± 1.38</td>
<td>3.77 ± 1.03</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>6.42 ± 1.76</td>
<td>6.53 ± 0.92</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>7.58 ± 0.93</td>
<td>9.19 ± 1.04</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>6.44 ± 1.13</td>
<td>7.79 ± 1.34</td>
</tr>
</tbody>
</table>

Genomic DNA of intestinal flora was extracted in accordance with the extraction kit instruction for microbial genome in stool, and E. coli DNA was extracted by water-boiling method. The above-mentioned extracts were preserved at -20°C for usage.

PCR application

30 μL PCR reaction system was used and was prepared as follows: 3 μL 10× buffer solution, 1 μL each of 12.5 μmol/L upstream primer and downstream primer, 1 μL dNTPs (2.5 mmol/L), 2.4 μL MgCl₂, 2 μL Tag DNA polymerase (1 U/μL) and 2 μL DNA template were added, and the solution was diluted with sterile triple-distilled water to 30 μL. Standard strains were taken as the positive control, and sterile triple-distilled water was adopted as the negative control. Reaction conditions: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, renaturation at 55°C for 45 s, extension at 72°C for 30 s, 30 cycles, and extension at 72°C for 30 s. 6 μL PCR products and 1 μL buffer solution above were mixed, and 2.0% agarose gel was used for electrophoresis. Finally, Gel Documentation and Analysis System was adopted to photograph [4].
Flora in colorectal cancer patients

**Purification of PCR products**

Under a UV lamp, the target fragments of PCR products which were from the colorectal cancer and healthy control group and had received AGE (agarose gel electrophoresis) were excised using a sterile scalpel. DNA agarose gel extraction kit was used to purify and extract PCR products.

**Sequencing**

20 µl PCR products of the samples to be sequenced and 5 µl each corresponding genotype primer were submitted for test. The sequencing company was Sangon Biotech (Shanghai) Co., Ltd. The sequencing results would be compared by entering http://www.ncbi.nlm.nih.gov/BLAST and be compared to GENBANK database.

**Statistical analysis**

SPSS16.0 was used to analyze data. Two-sample t test for mean comparison in completely randomized design for all the results of the colorectal cancer and healthy control group.
groups showed that the differences were all statistically significant ($P < 0.05$).

**Results**

*Intestinal flora distribution in the stool of colorectal cancer and healthy control groups*

30 colorectal cancer patients and 10 healthy persons were selected and included in the colorectal cancer and healthy control group, respectively. Anaerobic and aerobic cultures of intestinal bacteria were performed by using a variety of selective media. Results of two-sample $t$ test for comparison intestinal flora of colorectal cancer and healthy control groups showed that the differences were all statistically significant ($P < 0.05$) ([Table 2](#)).

*Agarose gel electrophoresis of genomic DNA of intestinal flora in stool*

The genomic DNA was extracted from the samples of both groups and given agarose gel electrophoresis. Then a clear and bright band was observed at about 23130 bp, and the bands in both colorectal cancer and healthy control groups were the same in length ([Figure 1](#)).

*Agarose gel electrophoresis of PCR products from colorectal cancer and healthy control groups*

The genomic DNA of intestinal flora extracted from the stool of both groups and *E. coli* DNA were amplified. The results of agarose gel electrophoresis method of genomic DNA of intesti-
Flora in colorectal cancer patients

The tested PCR products from both groups were sent to Sangon Biotech (Shanghai) Co., Ltd for sequencing. Sequence maps of the obtained fragments from the PCR products of colorectal cancer and healthy control groups are shown in Figure 3.

**BLAST results of PCR products sequences**

The BLAST analysis of GenBank showed Score =385 bits (208), Expect =4e-111, Identities =215/218 (99%) and Gaps =2/218 (0%) in uidA fragment sequences of *E. coli* isolated from the stool of healthy controls (Figure 5).

**Discussions**

The incidence of colorectal cancer is gradually increasing with diet structure changes in China [9]. It has been proved that the changes in the intestinal environment were caused by poor eating habits, and the incidence of colorectal cancer was also closely related to the changes in the intestinal environment [10, 11]. An important factor in affecting the intestinal environment is intestinal flora. The bacteria in stool include aerobe, anaerobe, facultative anaerobe, fungi, a small amount of yeast, proteusbacillus vulgaris, Aerobacter aerogenes, Gemmatimonadaceae and others, among which anaer-
obe accounts for 99%. Most of bacteria in human body have a beneficial role to health while they also cause some opposite effects. Generally, they mainly play the beneficial role in human health under normal circumstances; however, they would cause a pathogenic effect when the human body environment changes, because balanced steady-state system may be destroyed to lead to the micro dysbiosis [12, 13].

In this study, qualitative and quantitative analyses were performed for the intestinal flora from both groups. Two-sample t test for mean comparison of all the results between both groups showed that the differences were all statistically significant. Compared with the control group, the quantity of E. coli in the colorectal cancer group increased significantly; moreover, Lactobacillus, Bifidobacterium and other probiotics reduced significantly; and proportional quantity of anaerobic bacteria and aerobic bacteria reversed. The possible reasons are as follows: the intestinal lesions in colorectal cancer patients would cause a decrease in peristalsis and absorption function so that the intestinal environment changes and result in an increase of aerobe quantity. The significant increase in the quantity of E. coli of the colorectal cancer group and the obvious decrease in the quantity of Bifidobacterium were related to decrease of the intestinal immune function [14, 15]. The proportion of lactobacillus that can produce lactic acid in healthy control group was higher than that in colorectal cancer group. Thereby, lower risk of colorectal cancer in healthy population may be associated with inhibition effect of colorectal cancer from some beneficial bacteria in human intestinal tract. When the intestinal microecology is in a steady state, the short-chain fatty acids produced by the fermentation of intestinal bacteria in cecum and right colon can reduce pH value of stool to inhibit oncogenic cell growth, thus promoting apoptosis of cancer cells [6, 16].

β-G from intestinal flora can convert methylazoxymethanol glucoside in it to cancerogenic substance. If methylazoxymethanol glucoside is added into general diet, colorectal cancer may occur. However, it would not have oncogenic potential if it is taken by germ-free mice [6, 8, 17]. It is indicated that β-G from stool can catalyze procarcinogen to convert to cancerogenic substance, and β-G-mediated glucuronidation in human intestinal flora is the main pathway in detoxification of human body. The activity of β-G in intestinal flora in colorectal cancer patients was obviously lower than that in healthy population. Thereby, the detoxification in colorectal cancer patients was reduced to cause colorectal cancer. At present, no study has proved whether there was any difference in β-G gene sequences of intestinal flora between colorectal cancer patients and healthy population.

In this experiment, genomic DNA in intestinal flora from both groups and E. coli DNA were amplified by PCR technique, and PCR products were sequenced. β-G was carried by intestinal flora in both groups. The sequences in both groups were compared to uidA sequence encoding β-G in GenBank (Accession No. S69414.1), and the results showed that the homology was 99% and 98%, respectively. In colorectal cancer group, the base A at the 1141st and 1148th site were deleted, the base A at the 1149th site mutated into T, and the base A at the 1158th site mutated into G; however, in healthy control group, the base A at the 1141st and 1148th site were deleted, and the base A at the 1149th site mutated into T. It was found that colorectal cancer patients had the same base deletion and mutation with healthy controls at 1141st, 1148th and 1149th site, but it was observed that the base A mutated into G at the 1158th site only in colorectal cancer group. Therefore, differences in uidA sequences between β-G carried by human intestinal flora encoded in GenBank may cause decrease of β-G activity due to evolution and mutation of E. coli in human intestine. Methylazoxymethanol is formed by hydrolysis of β-G-dimethylhydrazine carried by intestinal flora, and methylazoxymethanol would continue to be degraded into methylidiazonium ion so as to decrease detoxification. Thereby, carcinogenic substances are easily produced to greatly increase the risk of gastrointestinal cancer. The 1158th site mutation would cause colorectal cancer. The aforementioned conclusion still needs to be verified by further experiments. In the present experiment, β-G of intestinal flora is not quantified, and expression of mRNA is not studied. Therefore, further verification is still needed, which will be of great importance in studies of intestinal microecology among colorectal cancer patients.
Flora in colorectal cancer patients

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Disclosure of conflict of interest

None.

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