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Original contribution

Use of 18S ribosomal DNA polymerase chain reaction–denaturing gradient gel electrophoresis to study composition of fungal community in 2 patients with intestinal transplants

Qiurong Li MD[a,b], Chenyang Wang MD[a,b], Qiang Zhang MD[a,b], Chun Tang MS[a], Ning Li MD[a,b], Bing Ruan MD[c], Jieshou Li MD, PhD[a,b,*]

[a]*Research Institute of General Surgery, Jinling Hospital, Nanjing 210002, China*
[b]*Department of Surgery, School of Medicine, Nanjing University, Nanjing 210002, China*
[c]*National Key Laboratory for Diagnosis and Treatment of Infectious Diseases in China and The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China*

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Summary Fungi form a diverse microbial community in the human intestine. Little is known about the succession of species after intestinal transplantation. We investigated the alterations of the gut fungal population in 2 patients with intestinal allografts. The ileal effluent and feces were fingerprinted using denaturing gradient gel electrophoresis, with confirmation by DNA sequencing. Analysis of 18S ribosomal DNA indicated that the phylogenetic diversity of the fungal communities was higher soon after transplantation; less diversity was observed at the later time points in patient 1. The shifts in the denaturing gradient gel electrophoresis banding patterns over time were similar in the effluent and feces in this patient. Similar changes in the fungi in the effluent and feces also were observed in patient 2. Sequence analysis of denaturing gradient gel electrophoresis bands showed that *Saccharomyces cerevisiae* and *Kluyveromyces waltii* dominated the fungal microbiota in both patients. Some species, including *Candida* spp, *Cryptococcus neoformans*, *Fusarium oxysporum*, *Aspergillus clavatus*, and *Trichophyton verrucosum*, were present early. We report for the first time the temporal alterations in fungal communities in patients with an intestinal allograft. This information may provide novel insight into the roles of the fungal microbiota in the pathophysiology of the transplanted intestine.

1. Introduction

The human intestine harbors a large and complex community of microorganisms. The bacterial flora is critical to intestinal function and plays a key role in disease [1-6]. The intestine also has a diverse fungal community [7-9], and the total number of fungi is estimated to be as high as 10^6 CFU/mL in the feces according to cultivation studies [7,10]. The fungal community is important in maintaining microbial homeostasis in the gut [11]. The imbalance of fungal microbiota encumbers the mucosal barrier and leads to translocation of gram-negative bacteria [12]. However, the

* Corresponding author. Research Institute of General Surgery, Jinling Hospital, Nanjing 210002, China.
E-mail address: liqiurong@yahoo.com (J. Li).

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diversity of the fungal community and its role in intestinal pathophysiology are poorly defined.

In the recipients of intestinal transplants, a new microbial ecosystem may develop as a result of decontamination and the introduction of the donor's microbiota. The microbial community may be reestablished by complex interactions between the 2 hosts (donor and recipient) [13]. In the transplanted intestine, the specific composition and temporal patterns of the fungal communities remain unclear. The fungal colonization may differ in complexity secondary to acuity of transplant recipients, antifungal prophylaxis, immunosuppressive regimens, and surgical technique. Better knowledge of the diversity and structure of fungal communities may lead to a better understanding of their roles in intestinal transplantation.

In this study, we used denaturing gradient gel electrophoresis (DGGE) to survey the fungal diversity in the effluent and feces of 2 patients with intestinal allografts. The predominant bands were then cloned and sequenced. Sequence analysis of individual bands enabled us to identify the transitions in fungal community composition. We characterized the population structure of the fungal microbiota and showed a temporal alteration. These data provide a preliminary characterization of fungal communities after intestinal transplantation.

2. Materials and methods

2.1. Patients

Two male patients aged 27 and 18 years underwent extensive small bowel resection and isolated small bowel transplantation in December 2008 or April 2009 at Jinling Hospital in Nanjing. Approximately 300 cm of small bowel excluded from the duodenum was transplanted into the recipients. At the end of transplant, an ileostomy was created through the abdominal wall to be used for the surveillance of the transplanted tissue for possible rejection. The recipients received alemtuzumab (0.6 mg/kg of body weight) intravenously before revascularization. After intestinal transplantation, the immunosuppressive regimen was monotherapy with tacrolimus. Enteric feedings were initiated on postoperative day (POD) 8, and parenteral nutrition was discontinued on POD 40 and 31 in the 2 patients. The key clinical events of the subjects, including immunosuppressive agents, antibiotics, and diets, are summarized in Supplementary Table S1. The patients survived more than 1 year after transplant. The study was approved by the Administrative Panel for Medical Research on Human Subjects of Jinling Hospital, and informed consent was obtained from the patients. The ileal effluent was harvested via the ileostomy once a week after the first 2 months posttransplant and then once a month, and feces were collected simultaneously 12 days after transplant.

2.2. Smear of the ileal effluent and fecal samples

The ileal effluent and fecal samples were immediately diluted 1:1 with 0.85% sterile saline and homogenized. An aliquot of each sample was smeared on a sterile glass slide. The slide was hot fixed and Gram stained as described previously [14,15].

2.3. Histopathology review

The endoscopy-guided biopsies were obtained from the intestinal allograft for histologic evaluation. The samples were routinely fixed in formalin and embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

2.4. DNA extraction and polymerase chain reaction amplification of 18S ribosomal DNA

Total DNA was extracted from the ileal effluent or feces using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Fungal 18S ribosomal RNA gene fragments were amplified from isolated DNA using a nested polymerase chain reaction (PCR) approach. The primer sets NS1/FR1 were used in the initial PCR [16], and the inner PCR was with primers EF390/GC-FR1 [17]. The first PCR mixtures (50 μL) contained each primer at 0.3 μmol/L, each deoxyribonucleotide triphosphate (dNTP) at 200 μmol/L, 1.8 mmol/L $MgCl_2$, 5 μL of 10× PCR buffer, 2.5 U of *Taq* DNA polymerase, 25 ng of template DNA, and nuclease-free water. The cycling was as follows: initial denaturation at 95°C for 5 minutes, then 30 cycles of denaturation at 95°C for 15 seconds, annealing at 45°C for 30 seconds, and extension at 68°C for 1.5 minute. In the second PCR, the mixtures contained the same constituents as in the first PCR, except for replacement of the primers. A 1-μL sample of the first PCR product was used as a template in the second step. The second PCR program was the same as the first one, except that the time of extension was 30 seconds. The PCR was conducted using an ABI 2720 thermocycler (Applied Biosystems, Foster City, CA), and successful amplification was verified by electrophoresis on a 1% (wt/vol) agarose gel.

2.5. DGGE analysis of fungal 18S ribosomal DNA amplicons

DGGE was carried out using the D-Code universal mutation detection system (Bio-Rad, Hercules, CA). The PCR products were loaded at 20 μL per lane on 8% (wt/vol) polyacrylamide gels (37.5:1, acrylamide:bisacrylamide) in 1×Tris acetate EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM disodium EDTA). Gels had a denaturing gradient ranging from 25% to 52.5%, where 100% denaturant contained 7 mol/L urea and 40% deionized formamide.