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Specific clones of *Staphylococcus lugdunensis* may be associated with colon carcinoma

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ABSTRACT

Staphylococcus lugdunensis produces a tannase with activity that may be associated with the onset of colon carcinoma. To clarify this feature of colon carcinoma-associated *S. lugdunensis*, we obtained isolates from healthy subjects and patients with colon adenomas and carcinomas and analyzed their genetic backgrounds. In total, 40 *S. lugdunensis* isolates from 288 rectal swabs collected between 2002 and 2008 were used. These isolates were classified into four groups according to the diseases of the subjects: healthy (n = 13), colon carcinoma (n = 13), colon adenoma (n = 9), and unknown (n = 5). The isolates were also classified by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing. In addition, an antimicrobial susceptibility test and detection of resistance genes were performed for all isolates. According to the PFGE analysis, 40 isolates could be classified into five groups. Among the groups, carcinoma and colon adenoma patients were significantly more frequently (40.9%) classified into group D ($p < 0.05$), whereas healthy subjects were more frequently (38.5%) classified into group A. All isolates in group D were typed as ST27, which was clearly different than isolates in the other groups. All isolates were susceptible to the antimicrobial agents tested, including β-lactams, although seven strains produced β-lactamase. Our data suggest that a specific clone of *S. lugdunensis* might be associated with colon carcinoma and colon adenoma. This clone showed high susceptibility to many antimicrobial agents. Therefore, eradication therapy may lead to a decreased risk of colon carcinoma.

Introduction

Streptococcus bovis biotype I, subsequently renamed as *Streptococcus gallolyticus* subspecies *gallolyticus*, produces a tannase and may be associated with the onset of colon carcinoma [1,2]. Tannase hydrolyzes tannin into gallic acid. *In vivo*, gallic acid is converted to pyrogallic acid by the action of a decarboxylase [3]. Active oxygen is released at this step and excessive amounts result in oxidative stress to intestinal cells and malignant transformation [4]. We have previously isolated a tannase-producing bacterium from the feces of patients with colon carcinoma and identified this bacterium as *Staphylococcus lugdunensis* [5].

S. lugdunensis is an example of coagulase-negative staphylococci and could be a causative pathogen for sepsis and soft tissue infections [6,7]. Because the biochemical features and pathogenicity of *S. lugdunensis* are related to those of *Staphylococcus aureus* rather than other coagulase-negative staphylococci, *S. lugdunensis* has recently attracted attention in the clinical field [8].

The presence of *S. lugdunensis* might be a risk factor for colon carcinoma [5]. However, it has also been isolated from healthy subjects and from patients without colon carcinoma. This indicates the possibility that there exist carcinoma-associated and non-associated clones of *S. lugdunensis*. Identification of carcinoma-associated clones could help establish an eradication therapy that might decrease the risk of carcinoma, similar to therapy for gastric ulcers with *Helicobacter pylori*.

Therefore, to identify a carcinoma-associated clone, *S. lugdunensis* isolates were obtained from rectal swabs and genetically characterized. Moreover, the antimicrobial susceptibility of these clones was tested to establish an eradication therapy.

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Materials and methods

Bacterial strains

A total of 288 rectal swabs at the Tokyo Medical University Hospital between 2002 and 2008 were screened. Forty *S. lugdunensis* clones isolated from these swabs were used in this study. Multiple isolates from the same patient were not included. Subjects were classified into 4 groups: healthy subjects, patients with colon carcinoma, patients with colon adenoma, and unknown. *S. aureus* ATCC29213 was used as the control strain for susceptibility testing according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. *S. lugdunensis* (ATCC43809) and methicillin-resistant *S. aureus* (N315) were used as quality control strains in our laboratory. These strains were suspended in 15% glycerol and stored at −80 °C until needed.

Isolation of S. lugdunensis *from rectal swabs*

S. lugdunensis clones were isolated and identified using their tannase production activity according to procedure described in a previous report [5]. Briefly, brain-heart infusion agar (Oxoid, Hambshire, England) was pretreated with tannin. Rectal bacteria were cultured on this medium at 37 °C for 4 days under anaerobic conditions. Colonies surrounded by a clear zone were identified as tannase-producing bacteria. Among those bacteria, *S. lugdunensis* was identified by the detection of *tanA*, which is a specific gene of *S. lugdunensis*, using the polymerase chain reaction (PCR) [10].

Pulsed-field gel electrophoresis (PFGE)

Genome extraction, restriction enzyme digestion, plug preparation, and electrophoresis were performed according to the manufacturer's protocol. *Sma*I (TaKaRa Bio, Shiga, Japan) was used as the restriction enzyme. The resulting PFGE pattern was analyzed using BioNumerics Ver. 6.01 (Applied Maths, Sint-Martens-Latem, Belgium). An un-weighted pair group method with an arithmetic mean dendrogram was generated using Dice coefficients (band tolerance 1.0%; optimization 1.0%). The cut-off value of similarity was set to 80% as described previously [11].

Multilocus sequence typing (MLST)

MLST was performed according to the method described by Chassain et al. [12]. Briefly, seven housekeeping genes (*aroE*, *dat*, *ddl*, *gmk*, *ldh*, *recA*, and *yqiL)* were amplified by PCR and the resulting fragments were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). The sequence type (ST) was identified via the *S. lugdunensis* sequence typing web site (http://bigsdb.web.pasteur.fr/staphlugdunensis/).

Antimicrobial susceptibility test

An antimicrobial susceptibility test was performed using the agar dilution method according to the CLSI guidelines [9]. Oxacillin, ampicillin, amoxicillin, cefaclor, cefdinir, cefmetazole, and fosfomycin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cephalexin, ceftriaxone, minocycline, clarithromycin, and clindamycin were purchased from Tokyo Chemical Industry (Tokyo, Japan). Ciprofloxacin, rifampicin, gentamicin, and vancomycin were purchased from Wako Chemicals (Osaka, Japan). Imipenem, levofloxacin, and metronidazole were purchased from LKT Laboratory (St. Paul, MN, USA). The CLSI criteria for *Staphylococcus* spp. specified the following resistance breakpoints: clarithromycin ≥ 8 μg/mL, clindamycin ≥ 4 μg/mL, gentamicin ≥ 16 μg/mL, minocycline ≥ 16 μg/mL, rifampicin ≥ 4 μg/mL, vancomycin ≥ 32 μg/mL, ciprofloxacin ≥ 4 μg/mL, and levofloxacin ≥ 4 μg/mL. The resistance breakpoint of oxacillin was used as the criterion for *S. lugdunensis* (≥4 μg/mL).

Detection of β-lactamase production and antimicrobial resistance genes

β-lactamase activity was confirmed using the Cefinase disk method (BD Biosciences, Franklin Lakes, NJ, USA). The resistance genes *mecA*, which confers β-lactam resistance, *ermA*, *ermC*, and *mphC*, which confer macrolide resistance, and *aacA-aphD*, which confers aminoglycoside resistance, were detected by PCR as described previously [13].

Statistical analysis

Statistical differences were assessed by the chi-square and Fisher's exact tests using JMP software (SAS Institute, Cary, NC, USA). p values of <0.05 were considered to be statistically significant.

Results

Isolates from S. lugdunensis *patients*

We screened 288 rectal swabs and obtained 40 *S. lugdunensis* isolates. The distribution of subjects according to disease was as follows: 32.5% healthy (n = 13), 32.5% colon carcinoma (n = 13), 22.5% colon adenoma (n = 9), and 12.5% unknown (n = 5).

Genotyping according to both PFGE and MLST

PFGE analysis was performed to compare the genetic background of the *S. lugdunensis* clones isolated from the rectal swabs (Fig. 1). These isolates were classified into 18 types. PFGE patterns with more than 80% identity were grouped together. When comparing the PFGE group, isolates from both colon adenoma and carcinoma patients were significantly more frequently classified into group D than were isolates from other patients ($p = 0.013$, Table 1). To further analyze the genetic background, we performed MLST for representative strains that showed different PFGE patterns. Each group was closely linked to one or two STs; notably, all strains in group D were typed as ST27.

Antimicrobial susceptibility and occurrence of resistance genes

The antimicrobial susceptibilities of all *S. lugdunensis* isolates are listed in Table 2. All isolates were susceptible to agents at resis-

Table 1
Correlation between diseases and PFGE group.

Group	Total (n)	No. of patients (%)				p Value[a]
		Cancer	Adenoma	Healthy	Unknown	
A	8	1 (7.7)	2 (22.2)	5 (38.5)	0 (0)	0.430
B	9	4 (30.8)	2 (22.2)	2 (15.4)	1 (20.0)	0.476
C	9	3 (23.1)	0 (0)	3 (23.1)	3 (60.0)	0.253
D	10	5 (38.5)	4 (44.4)	1 (7.7)	0 (0)	0.013
E	2	0 (0)	0 (0)	1 (7.7)	1 (20.0)	–
NC[b]	2	0 (0)	1 (11.1)	1 (7.7)	0 (0)	–
		13 (100)	9 (100)	13 (100)	5 (100)	

[a] p Value calculated by Fisher's exact test (Cancer and Adenoma vs Healthy and unknown).
[b] NC, not classified.

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