Advances in Brief

Presence of Streptococcus anginosus DNA in Esophageal Cancer, Dysplasia of Esophagus, and Gastric Cancer

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Abstract

We recently reported cloning of Streptococcus anginosus (S. anginosus) DNA fragments containing the 16S ribosomal gene from DNA samples of surgical specimens of gastric cancers. To investigate the specificity of S. anginosus infection, Southern blot analysis with S. anginosus 16S ribosomal DNA probe and PCR analysis with S. anginosus-specific primers were performed in DNA samples prepared from 15 esophageal cancers, 43 gastric cancers, 16 lung cancers, 10 cervical cancers, 14 renal cell carcinomas, 10 colorectal cancers, and 19 bladder cancers. We frequently found S. anginosus DNA sequences in DNA samples from esophageal cancer and gastric cancer tissues, as well as in those from dysplasia of the esophagus of esophageal cancer patients. No S. anginosus DNA bands were detected by Southern blot analysis on DNAs from the noncancerous portions of the esophagus or the stomach. By PCR analysis with 35 cycles, only 7% of the noncancerous portion of the esophagus was shown to contain S. anginosus sequences. No S. anginosus sequences were found in DNAs from cancers in lung, cervix, and kidney, but they were found in 1 of 10 colon cancers.

Introduction

It is now well established that multiple genetic alterations and infection by some bacterial or viral agents play important roles in the development of some types of cancer (1). It is also known that inflammation occurs during the multistep carcinogenic process. It has been suggested that Helicobacter pylori (H. pylori) infection might be associated with gastric cancer (2-10). To investigate microorganisms associated with several human cancers, we searched their DNA fragments by the modified in-gel competitive DNA reassociation method, which is useful for isolation of viral DNA from cancer tissue (11). We have reported previously that 9 of 43 (20%) DNA samples from surgical specimens of gastric cancers contained sequences that were quite homologous to a portion of the 16S rRNA gene of Streptococcus anginosus (12).

In this study, the frequency of the presence of S. anginosus DNA was investigated in DNA samples that were freshly isolated from surgical specimens of various types of cancers by Southern blot analysis with a S. anginosus 16S rRNA probe and by PCR analysis with specific primers for the detection of S. anginosus.

Materials and Methods

Tumor Tissues. Primary carcinoma tissues and their adjacent normal tissues were obtained at the time of surgery at the National Cancer Center Hospital (Tokyo, Japan). All of the specimens were frozen immediately in liquid nitrogen and stored at -80°C until use.

DNA Preparation and Southern Blot Analysis. Genomic DNA was prepared from culture cells and tissues by standard method. The extracted DNA was digested with EcoRI, and 10 µg of digested DNA were fractionated on 0.8% agarose gel and transferred to Hybond-N+ (Amersham). Hybridization was carried out in 50% formamide, 5X SSC (1X SSC, 150 mM NaCl-15 mM sodium citrate), 5X Denhardt's reagent, 5 mM EDTA, 0.1% SDS, 10% dextran sulfate, and 100 µg/ml denatured salmon sperm DNA at 42°C for 14-16 h. All DNA-probes were labeled with Redivue [α-32P]dCTP (Amersham) using a Rediprobe DNA labeling system kit (Amersham). The filter was washed twice in 0.1X SSC and 0.1% SDS at room temperature and twice at 65°C and exposed to Kodak XAR film at -70°C. The hybridization intensity in each sample was quantified by Fujix BAS2000 bioimaging analyzer (Fuji Photo Film Co., Tokyo, Japan).

PCR Amplification of S. anginosus rDNA. PCR for amplification of S. anginosus rDNA fragment was performed in a total of 50 µl of PCR mixture containing 10 mM Tris-HCI (pH 8.3); 50 mM KCl; 3 mM MgCl2; 0.001% gelatin; 20 µg/ml each primer; 1 mM each dATP, dCTP, dGTP, and dTTP; and 5 units of Taq DNA polymerase. The cycling condition was 35 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C. The last cycle had an additional extension at 72°C for 10 min. The sequences of the primers are: 5'-GAACGGTGGATGAACCCGTTAGTTA-3' for St1, 5'-CTACGATTTCACCCGTACACATG-3' for St2, and 5'-AAGCATCTAACATGTGTTACGTA-3' for St3.

Results

Southern Blot Analysis for Detection of S. anginosus rDNA on Surgical Specimens of Various Types of Cancer. From a genomic DNA library constructed from DNA of a gastric cancer tissue sample, we previously cloned a 1.7-kb DNA fragment, which contained a sequence with 99% homology to a portion of the 16S rRNA gene of S. anginosus (12). The diagram of the cloned DNA fragment containing the 5' end of the S. anginosus 16S rRNA gene is shown in Fig. 1. The rDNA fragment can hybridize to bacterial rDNA but not to human rDNA. By Southern blot analysis with the 1.7-kb S. anginosus DNA fragment as a probe, 11 of 15 (73%) esophageal cancers showed more than four bands, although the sizes of the bands varied from sample to sample. Only 2 of 15 DNA samples, samples 6 and 9, from the corresponding noncancerous portion of esophageal cancer gave positive bands to the 1.7-kb S.
Detection of \textit{S. anginosus} Infection to Human Cancers by PCR.

By PCR analysis using specific primers, \textit{St1} and \textit{St3}, for detection of \textit{S. anginosus}, a 0.1-kb rDNA fragment was amplified in 14 of 15 (93\%) esophageal cancers, whereas only 1 of 15 (7\%) of the corresponding noncancerous portion of the esophagus showed positive signals. The \textit{S. anginosus} rDNA fragment could be detected from 1 DNA sample of 15 noncancerous portions of the esophagus after 35 cycles of PCR amplification, but no sample gains a positive signal after 25 cycles (data not shown). It was amplified in 18 of 43 (42\%) gastric cancers in correspondence with our previous findings that \textit{S. anginosus} DNA sequences were detected in 9 of 43 (20\%) gastric cancer tissues by Southern blot analysis (12). No \textit{S. anginosus} DNA sequences were found in 16 cases of cervical cancer, 10 cases of lung cell carcinoma, 19 cases of bladder cancer, and 14 cases of renal cell carcinoma, whereas they were found in 1 of 10 (10\%) colorectal carcinomas. Representative data of the PCR analysis are shown in Figs. 5 and 6. The results of Southern blot analysis and PCR analysis are summarized in Table 1. Taken together, it is indicated that \textit{S. anginosus} DNA sequences are frequently present in surgical specimens of esophageal cancer and gastric cancer but rarely in matched noncancerous portions and are

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Southern blot analysis of DNA samples from surgical specimens of tumor tissues (Lanes T) and matched noncancerous portions (Lanes N) of esophageal cancer. Top, case numbers. Ten \(\mu\)g of EcoRI-digested DNA were hybridized with the 1.7-kb 16S rDNA probe of \textit{S. anginosus}.}
\end{figure}
S. anginosus DNA occurs in cancer cells, PCR analysis using the 16S rDNA of various bacteria. *, identical nucleotides; N, undetermined nucleotides.

Fig. 3. Results of a computed homology search of St3 primer sequence. Top, the nucleotide sequence of St3 primer; bottom, homologous sequences of a portion of the 16S rDNA of various bacteria. *, identical nucleotides; N, undetermined nucleotides.

Discussion

The results that different sizes of DNA bands were detected depending on the samples by Southern blot analysis with the portion of 16S rDNA as a probe could be due to the presence of many copies of the rRNA gene in the bacteria or to superinfection of the different strains of Streptococcus or the other Streptococcus-related bacteria. S. anginosus had five to seven copies of the rRNA genes, which was revealed by Southern blot analysis of five strains of S. anginosus (data not shown). It should be noted that no amplification of the rDNA fragment was found by PCR analysis in DNA samples from both the cancer and noncancerous portion of esophageal cancers of patients 6 and 9, in whom positive bands of the same sizes were detected from both the cancer and noncancerous portion by Southern blot analysis (Fig. 2). In these two cases, it is possible that there are bacteria infections other than S. anginosus, and this bacteria contained rDNA with relatively high homology to that of S. anginosus. Further studies are required to identify the bacteria in noncancerous portion from patients 6 and 9, whose DNAs gave positive signals with Southern blot analysis but negative signals by PCR analysis. These results of Southern blot analysis and PCR analysis of DNA samples of various cancer tissues clearly showed that the presence of S. anginosus DNA was associated with cancers in the upper digestive tracts. The Streptococcus milleri group, including S. anginosus, S. constellatus, and S. intermedius, is known to be present frequently in the buccal cavity and cause dental periapical abscesses (14–16).

Although no report indicating the integration of bacterial DNA into mammalian cells in vivo has been published, it is also possible that DNAs of S. anginosus are integrated into the host

Southern: -+ = - = -+-++-+-++ = =-+- +

0.1 Kb

Primers: St1 and St3

Fig. 4. Specific amplification of S. anginosus rDNA from various bacterial DNA with St1 and St3. The ~0.1-kb PCR product was obtained with St1 and St3 primers, and the 0.6-kb product was obtained with St1 and St2 primers from DNA samples as follows: Lane 1, no template; Lane 2, human placenta; Lane 3, gastric cancer tissue (3261); Lane 4, Bacillus; Lane 5, E. coli; Lane 6, Pseudomonas; Lane 7, Enterofaecalis; Lane 8, S. Sanguis (O); Lane 9, S. intermedius; Lane 10, S. anginosus (GIFU8787); Lane 11, S. anginosus (GIFU8781); Lane 12, S. anginosus (GIFU10026); Lane 13, S. anginosus (GIFU100033); Lane 14, S. anginosus (GIFU10037).

Fig. 5. PCR amplification of DNA samples from surgical specimens of tumor tissues and matched noncancerous portions of esophageal cancer using the primers St1 and St3. The ~0.1-kb PCR product of DNA samples from the surgical specimens of 13 cases of esophageal cancer was electrophoresed on agarose gel. Results of Southern blot analysis described in Fig. 2 are shown (top) as follows: −, not detected; +, detected. Lanes T, tumor tissues; Lanes N, matched noncancerous portions.

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Table 1  Frequency of presence of S. anginosus DNA in various cancer tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>n</th>
<th>No. (%) of positive samples</th>
<th>By Southern blot</th>
<th>By PCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td>15</td>
<td>11 (73)</td>
<td>9 (62)</td>
<td>14 (93)</td>
</tr>
<tr>
<td>Normala</td>
<td>15</td>
<td>2 (13)b</td>
<td>1 (7)c</td>
<td>1 (7)c</td>
</tr>
<tr>
<td>Stomach</td>
<td>43</td>
<td>9 (21)d</td>
<td>18 (42)</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>10</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td></td>
</tr>
</tbody>
</table>

a  Adjacent apparently normal esophagus tissues.
b  Two samples gave positive signals, but by PCR analysis, both of these samples were shown to be negative for S. anginosus sequences.
c  The amounts of PCR products in a normal tissue are much less than those of the corresponding cancer tissue.
d  These data are from our previous report (12).

geno. We have examined a possible integration of S. anginosus DNA into the host genome, but PCR analysis in DNA samples of cancer cell lines and metastatic lymph nodes of esophageal cancer failed to demonstrate the integration. The degree of infection of S. anginosus was much more apparent in the dysplasia and cancer of the esophagus than that in the corresponding noncancerous portion. These results indicated that the S. anginosus infection occurred at an early stage of esophageal cancer. Further studies on esophageal cancer from different regions of the world and the dysplastic stage are needed. To draw any definitive conclusion on the causative role of S. anginosus, it is imperative to conduct an intervention study on high-risk groups of esophageal cancer. PCR analysis using specific primers St1 and St3 provides a quick and sensitive method for detection of S. anginosus infection in a large number of clinical samples, including paraffin-embedded tissues and biopsy samples. Our present data might suggest that S. anginosus could have a significant role in the carcinogenic process of most cases of esophageal cancer and some cases of gastric cancer by causing inflammation and by promoting the carcinogenic process.
Acknowledgments

We express our sincere appreciation to Dr. T. Ezaki (Gifu University School of Medicine), for providing DNAs of five strains of *S. anginosus*.

References

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*Cancer Res* 1998;58:2991-2995.

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