学 位 論 文 の 要 旨

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学 位 論 文 名: Application of Biopsy Samples Used for *Helicobacter pylori* Urease Test to Predict Epstein–Barr Virus-Associated Cancer

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論 文 内 容 の 要 旨

INTRODUCTION

Persistent gastric mucosal damage caused by *Helicobacter pylori* infection is a substantial risk of gastric cancer (GC). The Epstein-Barr virus (EBV) is also associated with 10% of all GC cases. Patients with EBV-associated GC (EBVaGC) primarily suffer from *H. pylori* gastritis. The *H. pylori* CagA protein stimulates EBV-mediated epigenetic modifications and cell proliferation in a coinfection model. Also, several cases show that the initial infection of *H. pylori* promotes the development of EBVaGC. We found that EBVaGC is usually present close to the border between the atrophic and normal gastric mucosa. These findings indicate an association between *H. pylori* and EBV in the occurrence of GC. However, very few reports have described where and when both pathogens infect the gastric mucosa. Nevertheless, performing gastric biopsy as a clinical test solely to confirm EBV infection in gastric epithelial cells is inconvenient.

Several targeting genes have been reported to detect the viral genomic DNA (gDNA) from human samples using quantitative polymerase chain reaction (qPCR). Among them, there are the *Bam*H I fragment A leftward frame 5 (BALF5), dyad symmetry region (DSR), and *Bam*H I fragment W (*Bam*H I W). Because the affinity of each primer to the target sequence and the DNA quality from tissue samples affect the gene amplification efficiency, we evaluated each primer set to establish the most sensitive and accurate qPCR assay. Our previous study utilizing more than 10 tissue samples to detect EBV infection was less effective because the assay used

numerous valuable gastric biopsy samples. Furthermore, we failed to show where and when EBV infected the mucosal epithelia.

To overcome the obstacles, a less invasive and more sensitive assay is required. A DNA probe quantitative polymerase chain reaction (qPCR) was applied on old biopsy samples used for the rapid urease test (RUT) to count EBV genomic DNA (gDNA). Since our proposal detects both EBV and *H. pylori* from the same sample, the quantitative datas' can be compared with the degree of atrophic gastritis. This new evaluation system will enable clinicians to diagnose EBVaGC before the patients present with clinical manifestation.

MATERIALS AND METHODS

gDNAs' were extracted from the gastric biopsy samples of 58 patients with atrophic gastritis with no histories of antibiotic therapy against *H. pylori* infection. The atrophic gastritis was identified using endoscopic observation and classified according to the Kimura-Takemoto criteria. *H. pylori* infection from two gastric mucosal biopsies of the suspected lesions was evaluated using RUT. The correlations between the atrophic gastritis degree and the EBV gDNA copy number were analyzed as well.

A double-quencher DNA probe was used in our qPCR assay. AGS, AGS-EBV, MKN28, MKN28-EBV, and Raji's cell lines gDNAs' were utilized to assess the assay sensitivity and specificity. Moreover, we also confirmed it sensitivity by diluting AGS-EBV gDNA with murine stomach gDNA under various conditions. The study protocol involving humans being had approved by Kanmon Medical Center and the Research Ethics Committee of Shimane University.

The statistical analyzes between groups' were compared by the Chi-square test, Fisher exact test, and independent t-test. Samples with more than 100 EBV gDNA copy numbers per μg of DNA were selected and analyzed via box-and-whisker plots. The p-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The qPCR assay targeting several EBV genes was performed on gDNA derived from AGS, AGS-EBV, MKN28, MKN28-EBV, and Raji cell lines. We used a double quencher DNA probe to produce a more specific and sensitive result. Our experiment showed that *Bam*HI W,

DSR, and BALF5 qPCR probes did not react with the gDNA from EBV-negative cell lines. Each gene was detected in EBV-positive but not EBV-negative cells.

The genes relatives' expressions were significantly different between AGS-EBV and MKN28-EBV ($p < 0.05$). We performed several qPCR assays to estimate the condition to be tested in human samples. Accordingly, we used murine gDNA to assume the gDNA obtained from biopsy samples. As a result, *Bam*H I W primers and probe showed high specificity and sensitivity compared to BALF5 (R^2= 0.9518 and R^2= 0.9404, respectively). The primer efficiency was confirmed by creating a calibration curve from a plasmid-derived template containing *Bam*HI W EBV DNA fragment, R^2=0.9999.

Targeting *Bam*HI W, we applied the same assay into only two biopsy specimens after RUT from 58 atrophic gastritis patients. The results showed that EBVs' were detected in 44 cases (75.9 %). The viral copy numbers were ranging from 12.6 to 4754.6. Most EBV-infected patients had moderate atrophic gastritis with a copy number higher than 900 ($p < 0.05$). We believed EBV similarly transmitted to the mucosa to an experimental cell-to-cell transfer of EBV to gastric epithelial cells. Therefore, monitoring the carcinogenic progress in EBVaGC clinical cases will help to clarify the dynamics of EBV infection in human gastric mucosa.

CONCLUSION

The double-quencher DNA probe qPCR assay is highly sensitive for detecting EBV infection in RUT samples in a manner that reduces the patient burden. Implementing this EBV detection method in the gastric mucosa during the early stage of gastritis will be a helpful modality for preventing EBVaGC.