学 位 論 文 の 要 旨

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学 位 論 文 名	Gastric Epithelial Attachment of *Helicobacter pylori* Induces EphA2 and NMHC-IIA Receptor for Epstein-Barr virus
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論 文 内 容 の 要 旨

INTRODUCTION

Epstein-Barr virus (EBV) was the first human oncogenic virus to be discovered, and its persistent infection promotes the oncogenic transformation of cells. EBV-associated gastric cancer (EBVaGC) is a representative EBV-positive epithelial cell tumor that accounts for 10% of all gastric cancer cases. EBVaGC tumor cells originate from a single cell clone infected with EBV. In contrast, it has been reported that more than 95% of patients with gastric cancer have a history of *Helicobacter pylori* (*H. pylori*) infection, and *H. pylori* is the major causative agent of gastric cancer.

Accordingly, it has long been argued that *H. pylori* infection may have some effect on the development of EBVaGC, because it is a subtype of gastric cancer. It has been reported that additional EBV infection assists the oncogenic process of gastric cancer caused by *H. pylori* infection. When gastric epithelial cells are infected with EBV, DNA methylase is induced, and the expression of tumor suppressor genes, such as adenomatous polyposis coil (APC), breast cancer susceptibility gene 1 (*BRCA1*), and phosphatase and tensin homolog deleted from chromosome 10 (*PTEN*), is decreased via promoter methylation. In addition, the expression of Src homology region 2 domain-containing phosphatase 1 (SHP1) is suppressed, resulting in the relative activation of oncogenic SHP2. Therefore, EBV infection promotes the activation of the SHP2-Ras pathway, which is initiated by the phosphorylation of *H. pylori* -derived cytotoxin-associated gene A (CagA).

In contrast, I investigated the possibility that exposure of gastric epithelial cells to *H. pylori* accelerates the tumorigenic steps induced by EBV infection. I observed an increase in

EBV copy number in epithelial cells cultured with *H. pylori*. I assumed that 1 of the virulence factors of *H. pylori* assisted the infectivity of EBV. The following pathogenic factors were investigated: CagA, which is encoded by the bacterial cag pathogenicity island (*cagPAI*) gene and promotes the tumorigenicity of gastric epithelial cells, vacuolating cytotoxin A (VacA), which induces cytokines from gastric epithelial cells, and Flagellin A (FlaA), which is a major component of flagella.

MATERIALS AND METHODS

Cell lines: AGS cells and MKN28 cells are both cancer cell lines; GES-1, an immortalized fetal gastric epithelial cell line, and AGS-EBV were used. Cells were cultured in RPMI-1640 medium supplemented with 10% Fetal bovine serum.

H. pylori strains: CPY6271, CPY3401 and TN2 along with isogenic mutant were used. Bacteria were cultured in Brucella broth or *Brucella* agar plates supplemented with 5% heat-inactivated horse serum under microaerophilic condition.

Virus: Cell-free enhanced green fluorescence protein-EBV (eGFP-EBV) was produced from AGS (+) cells following *Bam*HI Z fragment leftward open reading frame 1 (*BZLF1*) gene transfection using Lipofectamine 2000.

Gastric epithelial cells were seeded in cell culture plates and attached to the bottom of the wells for 12 h. Cells were infected with *H. pylori* at an multiplicity of infection (MOI) of 100 and incubated at 37°C and 5% CO_2 for 7 h. *H. pylori* was removed by washing and subsequently infected with eGFP-EBV at an MOI of 125 in the presence of PC/SM. After 48 h of EBV infection, the infection efficiency was assessed using fluorescence microscopy, flow cytometry, and quantitative polymerase chain reaction.

RESULTS AND DISCUSSION

The treatment of gastric epithelial cells with *H. pylori* increased the efficiency of EBV infection. An increase was also observed when CagA-, VacA-, and FlaA-deficient *H. pylori* strains were used, but not when cag pathogenicity island deficient *H. pylori* was used. The treatment of epithelial cells with *H. pylori* induced the expression of accessory EBV receptors, ephrin type-A receptor 2 (EphA2) and non-muscle myosin heavy chain IIA (NMHC-IIA), and increased the efficiency of EBV infection depending on their expression levels. Moreover, the increase in EphA2 protein in the membrane fraction is more pronounced than the increase in *EPHA2* mRNA extracted from whole cells, suggesting that *H. pylori* treatment may facilitate the intracellular relocalization of low-affinity EBV receptors. When gastric epithelial cells were treated with EPHA2 or NMHC-IIA siRNA, EBV infection via *H. pylori* attachment was decreased.

CONCLUSION

H. pylori pretreatment enhances EBV infectivity in gastric epithelial cells via cagPAI-

dependent upregulation of EphA2 and NMHC-IIA.