学 位 論 文 の 要 旨

氏名 吉 野 生 季 三

学 位 論 文 名 **INTERLEUKIN-8 REGULATES EXPRESSION OF REG PROTEIN IN *HELICOBACTER PYLORI*-INFECTED GASTRIC MUCOSA**

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著 者 名 Nagisa Yoshino, Shunji Ishihara, MAK Rumi, CF Ortega-Cava, Takafumi Yuki, Hideaki Kazumori, Shin Takazawa, Hiroshi Okamoto, Yasunori Kadowaki, Yoshikazu Kinoshita

論 文 内 容 の 要 旨

INTRODUCTION

Helicobacter pylori infection is generally accepted as a crucial event in the development of peptic ulcer disease and atrophic gastritis, and has been implicated in the development of gastric carcinomas. Chronic inflammation induced by *H. pylori* infection is closely associated with epithelial cell proliferation and apoptosis, which are related to cellular turnover in gastric mucosa. The regenerating gene, *Reg,* was originally isolated as a growth factor from a cDNA library developed from rat regenerating pancreatic islets. We previously reported that Reg protein was mainly expressed in gastric fundic enterochromaffin-like (ECL) cells in rat stomachs and its production was stimulated during gastric mucosal regeneration. Although cell growth factors have been suggested to be key molecules in the pathogenesis of *H. pylori*-related diseases, little is known regarding the expression of Reg protein and its regulation in human gastric mucosa. Therefore, we investigated the *in vivo* expression of Reg protein in human gastric mucosa, with and without *H. pylori* infection. Further, we examined the effect of interleukin (IL)-8, a major pro-inflammatory cytokine produced in *H. pylori*-infected gastric mucosa, on the expression of Reg protein.

MATERIALS AND METHODS

Patients and biopsy specimens

Twenty-four patients with *H. pylori*-induced chronic gastritis and 27 with *H. pylori*-negative

non-ulcer dyspepsia were studied. Multiple endoscopic biopsy specimens were taken from the gastric corpus and antrum of each for the following examinations.

Histopathology and immunohistochemistry

Histopathology was evaluated according to the updated Sydney System using hematoxylin and eosin sections. Immunohistochemistry was performed for semi-quantitative detection of the Reg protein in tissue sections. An index of positive staining was constructed using 50 gastric glands in each section and expressed as the percentage of Reg-positive glands. To evaluate the histological localization of Reg-producing cells in gastric mucosa, immunohistochemical double staining for Reg protein and chromogranin A was also performed using the sections.

Western blotting

Protein was extracted from the biopsy specimens and transferred to polyvinylidene fluoride membranes, with samples of 50 μg per lane subjected to SDS-PAGE. After blocking, the membranes were incubated with mouse anti-human Reg monoclonal antibody (mAb) at 4°C for 12 hours, then reacted with goat anti-mouse IgG at room temperature for 2 hours. The signals were detected by chemiluminescence, with the expression of β-actin also detected as an internal control.

Detection of IL-8 protein in gastric mucosa

IL-8 contents in protein extracted from the biopsy samples were evaluated by an enzyme-linked immunosorbent assay (ELISA).

Effects of IL-8 on Reg protein expression in ECC10 cells

Approximately 2×10^6 ECC10 cells were seeded onto 100-mm culture dishes for 24 hours, then stimulated with human recombinant IL-8 in various concentrations. Total protein was extracted from the cells at 24 hours after IL-8 treatment, after which the expression of Reg protein was examined by Western blotting.

Reporter gene assay with Reg promoter

The effect of IL-8 on the transcriptional activation of *Reg* was evaluated using a reporter gene luciferase assay. To construct the Reg-promoter, a 1135-bp promoter fragment of the *Reg1A* gene was amplified by PCR and cloned into the *Bgl* II site of a pGL3-Basic vector. ECC10 cells were transfected with the reporter gene vector along with the internal control and stimulated

with various concentrations of human recombinant IL-8 for 12 hours. Cell lysates were used for the measurement of luciferase activity with a PicaGene Dual luciferase kit.

RESULTS AND DISCUSSION

H. pylori infection showed a significant correlation with both polymorphonuclear neutrophil activity and chronic inflammation in human gastric mucosa. The immunoreactive signals for Reg were detected in both *H. pylori*-infected and uninfected subjects, and were located in the deeper part of the gastric fundic and pyloric glands. Semi-quantitative immunohistochemistry and Western blotting results showed that Reg expression was significantly increased in *H. pylori*-infected gastric mucosa, which was associated with both increased inflammatory scores in histological sections and IL-8 content in gastric mucosa. The histological localization of Reg-producing cells was found to be quite similar to that found previously in rat stomach samples, while Reg protein expression was detected in chromogranin A-positive endocrine cells in human gastric mucosa. However, unlike our rat stomach findings, a distinct expression of Reg protein was also detected in chromogranin A-negative cells, identified by the basal localization of their nuclei and their basophilic cytoplasm, which are morphological characteristics of chief cells.

IL-8 is an important pro-inflammatory cytokine that has a relationship with the infiltration of polymorphonuclear leukocytes in *H. pylori*-associated gastritis. In the present study, IL-8 dose-dependently stimulated Reg protein expression in ECC10 cells and, as a result of that finding, we also examined the transcriptional activation of *Reg* expression in ECC10 cells stimulated with IL-8 using a luciferase assay. Stimulation with IL-8 led to a significantly higher transcriptional activation by the *Reg* promoter in ECC10 cells, which supported the Western blotting results for the detection of Reg protein expression in those cells.

CONCLUSION

The present results showed for the first time that *H. pylori* infection significantly induces Reg protein expression in human stomach gastric epithelial cells, which is stimulated by IL-8. Our findings provide evidence of a novel link between Reg protein and *H. pylori* infection, and may help to explain the molecular mechanisms underlying *H. pylori*-associated diseases.