学 位 論 文 の 要 旨

氏名　大 瀬 貴 之

学 位 論 文 名　Reg I-Knockout Mice Reveal Its Role in Regulation of Cell Growth That Is Required in Generation and Maintenance of the Villous Structure of Small Intestine.

発 表 雑 誌 名（巻，初頁～終頁，年）　Oncogene (26: 349-359, 2006)

著 者 名　Takayuki Ose, Yasunori Kadowaki, Hiroyuki Fukuhara, Hideaki Kazumori, Shunji Ishihara, Jun Udagawa, Hiroki Otani, Shin Takasawa, Hiroshi Okamoto, and Yoshikazu Kinoshita

論 文 内 容 の 要 旨

INTRODUCTION

Reg I (regenerating gene product I) was originally identified as a growth factor involved in the pancreatic regeneration. Based on its temporal and spatial pattern of expression around the gastric ulcers, Reg I was also suggested to be a controller of the gastric mucosal regeneration. Recently, we created *Reg I*-transgenic mice to demonstrate that Reg I had an activity to induce the differentiation of the gastric stem cells into the chief cell and the parietal cell lineages. This suggests that Reg I may be a key molecule in maintenance of the gastric mucosal architecture. In wild-type mice, the *Reg I* mRNA is expressed at the highest level in the small intestine among the gastrointestinal tissues, suggesting its critical role in this tissue. In the current study to clarify the role of the Reg I protein, the spatial and temporal expression pattern of Reg I in the normal mouse small intestine was determined. In addition, the *Reg I* knockout mice were examined

morphologically to show that Reg I was involved in the generation and maintenance of the crypt-villus growth axis of the small intestinal mucosa.

MATERIALS AND METHODS

The *Reg I* knockout mice (*Reg-/-*)was originally produced in the Department of Biochemistry, Tohoku University Graduate School of Medicine. The *Reg-/-* mice were mated with the ICR wild type mice, generating the *Reg+/-* mice. The male and female *Reg+/-* mice were then mated overnight and the next day was counted as day (E) 0. Pregnant mice were sacrificed at E13 and E17 of the pregnancy. The embryos were removed from the uterus and fixed in 10% formalin after the head was collected for genotyping. The bodies of *Reg+/+* and *Reg-/-* were vertically cut into small pieces, dehydrated, embedded in paraffin blocks, and sliced for immunohistochemistry. The entire small intestine was collected from the fetal mice and rapidly frozen in liquid nitrogen for the reverse transferase-polymerase chain reaction (RT-PCR). The adult mice aged 3-12 weeks were sacrificed, and the small intestine was excised and the proximal jejunum (defined as one-third of the small intestine) was immersion-fixed in 10% formalin, and embedded in paraffin blocks. Sections were stained with hematoxylin and eosin to visualize the general morphology. For immunohistochemistry, sections were incubated with a primary antibody (Ki-67, proliferating-cell nuclear antigen or musashi-1). Some *Reg-/-* and *Reg+/+* littermates were injected with 50 mg/kg body weight of 5-bromodeoxyuridine (BrdU) intraperitoneally 48 h before sacrifice. Mice were sacrificed and the proximal jejunum was fixed with formalin, embedded in paraffin, and sectioned at 6 μm. After permeabilizing with trypsin and HCl, the sections were incubated with anti-BrdU antibody.

RESULTS AND DISCUSSION

In the wild-type mice, immunohistochemistry localized the Reg I protein expression in the epithelial cells in the lower half of the intestinal villi. The Reg I expression was undetectable untill E13, when the fetal intestine still lacked the villous structure. By contrast, it dramatically increased at E17 along with the formation and maturation of the fetal intestinal villi. In the small intestine of the adult *Reg I*-knockout mice, less densely packed, round-shaped aberrant morphology of the absorptive epithelial cells were observed with the light microscopy. The electron microscopic examination further revealed a strikingly loose connection of these cells to the basement membrane. Anti- proliferating-cell nuclear antigen staining and anti-Ki67 staining demonstrated the marked decrease in the number of proliferating cells in the small intestinal mucosa of the knockout mice. The cell migration speed visualized by the pulse labeling of BrdU was significantly lower in the knockout mice. These phenotypes of the *Reg I*-knockout mice emerged, in accordance with the temporal pattern of the Reg I expression described above, from embryonic day 17. These data suggest that Reg I interacts with the stem cells to stimulate their growth and promotes the proliferation of the intestinal stem cells. Accordingly, both the cell growth rate and the cell migration speed in the small intestine of the *Reg I*-knockout mice decreased significantly. In contrast to those observations, however, there was no difference in the height of the villi between the wild-type and the *Reg I*-knockout mice. This result implies that there may be another morphogen that determines the length of the villi independent of the stem cell replication rate.

CONCLUSION

We suggest that Reg I is a cell growth regulator that is required to generate and maintain the villous structure of the small intestine. Reg I may act as a growth factor throughout the whole gastrointestinal tract, which is essential for maintaining the proper mucosal architecture.