学 位 論 文 の 要 旨

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学 位 論 文 名　In Vivo Evidence for the Role of RegI in Gastric Regeneration: Transgenic Overexpression of RegI Accelerates the Healing of Experimental Gastric Ulcers

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論 文 内 容 の 要 旨

INTRODUCTION

Gastric ulcer healing is a complicated but finely regulated process, and serves as a good model for studying the molecular mechanism of tissue regeneration. In the early stage of this process, signaling pathways, which transduce proliferation signal from the site of the injury to the gastric progenitor cells, would be engaged. At the end of the pathways would be growth factors that directly interact with the progenitor cells. These factors are particularly important because they initiate and maintain progenitor cell proliferation that is fundamental for reconstruction of the epithelial portion of gastric tissue, however, there is little information about their molecular nature. Based on its temporal and spatial pattern of expression during the healing of gastric ulcers, RegI is thought to be a key growth factor governing the proliferation of gastric progenitor cells which are fundamental for reconstruction of gastric tissue. However, at this time, there is no direct *in vivo* evidence for this hypothesis. The aim of the current study was to utilize RegI-transgenic (Tg) mice to test the role of RegI protein in the healing of experimentally induced gastric ulcers.

MATERIALS AND METHODS

Adult male and female mice (9 to 15-week-old) were used in the experiments. As a first step, we examined RegI expression and histological appearance of their stomachs before ulcer induction. 【water immersion and restraint stress ulcer】 Adult Tg and Wt littermate pairs were subjected to water immersion stress and their stomachs were examined macroscopically for erosions. The animals were placed in restraint cages and immersed in a water bath (25℃) to the level of the xiphoid process for 24 hr. After the experiments, the animals were anesthetized and sacrificed by cervical vertebral dislocation. Their stomachs were immediately removed and inflated with 2mL of 1% formalin solution for 10 min. After opening along the greater curvature, gastric injury visible to the naked eye was found in the gastric mucosa as elongated black-red lines (1-10mm long by 0.5-1.5 mm wide) parallel to the long axis of the stomach in mice. The length (in millimeters) of each erosion was measured and cumulated as the ulcer index (UI) (in millimeters). 【HCl/ethanol ulcer】 In addition, we checked the ulcer index 6 hr, 12 hr, 18 hr and 24 hr after HCl/ethanol injection to generate a time course. The animals received 0.2 mL of 0.2 M HCl/40% ethanol orally. The animals were sacrificed at each time point and their stomachs were processed as described above to obtain the ulcer index. Expression levels of *c-fos* and *c-myc* proto-oncogenes were examined over time by real-time RT-PCR to assess gastric cell proliferation.

RESULTS AND DISCUSSION

In the stomachs of the adult wild-type (Wt) mice, we were unable to detect any RegI-specific signals by immunohistochemistry, whereas, a significant level of RegI expression was seen in the tissue of RegI-transgenic (Tg) mice. In spite of the difference of RegI expression, we found no histological difference in the gastric mucosa between Wt and Tg mice in the adult stage. For all the littermate pairs tested, the ulcer index of the Tg mice was less than that of the Wt mice at the end of water immersion and restraint stress. The mean index values were 8.3 for Tg mice and 23.7 for Wt mice, respectively. Therefore, the transgene reduced the ulcer index by two-thirds. In the time course study of HCl/ethanol induced ulcer, almost all the littermate pairs showed the superiority of Tg mice compared with Wt mice in the ability of decreasing ulcer

index, except for the pairs examined after 6 hr. In the Wt mouse, *c-fos* gene expression reached a maximum at 6 hr, prior to the initiation of the recovery phase, then decreased gradually. The temporal pattern of expression of *c-fos* gene in the Tg stomach was similar, however, the expression levels were significantly higher in Tg, especially at 12 hr.

In the current study, we used two experimental systems based on different theories to induce ulcer formation (stress-induced and HCl/ethanol-induced) and employed a large battery of littermate pairs in order to reach a definitive conclusion. In both experimental systems and in almost all the littermate pairs tested (with the only exception of the 6 hr group in HCl/ethanol experiment), Tg mice showed lower ulcer indices than Wt, clearly demonstrating the strong positive influence of RegI on ulcer healing. This is the first *in vivo* evidence that RegI contributes to gastric ulcer healing. In the injury phase, the mean index value for Tg was higher than that for Wt. This was reversed in the recovery phase, when Tg showed constant superiority in healing over Wt. Generally speaking, the recovery phase corresponds to the phase of progenitor cell proliferation, when the cells divide, migrate and differentiate to reconstruct the gastric tissue architecture. As RegI was effective in the recovery phase rather than in the injury phase, it is reasonable to speculate that RegI participates in ulcer healing by stimulating or supporting progenitor cell growth. In addition, expression of *c-fos* gene, which was used as proliferation markers, was significantly higher in Tg than in Wt mice in the time course following HCl/ethanol-induced injury. Throughout the time course, the gene showed higher expression levels in Tg mice. The increase in *c-fos* gene expression (which is known to precede cell proliferation) was prominent at the beginning of the healing phase. These data demonstrate that the fraction of proliferating cells increased in HCl/ethanol-treated Tg mice in the course of ulcer healing and support the hypothesis that RegI employs a growth-promoting mechanism to contribute to ulcer healing.

CONCLUSION

This is the first *in vivo* evidence that RegI plays a role in gastric ulcer healing. We suggest that RegI exerts its effects by promoting growth and not by cytoprotection.