学 位 論 文 の 要 旨

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学　位　論　文　名　　Increased Expression of Midkine in the Rat Colon During Healing of Experimental Colitis

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論 文 内 容 の 要 旨

INTRODUCTION

A variety of cytokines and growth factors are well-known mediators that maintain the physiological and immunological functions of the intestinal tract. Inflammatory bowel disease (IBD) is characterized by chronic intestinal immune-mediated disorders of unknown etiology, though several kinds of growth factors have been shown to be increased in intestinal inflammatory sites, suggesting a possible link between those growth factors and mucosal regeneration by epithelial cell proliferation and migration. Midkine (MK), a member of a newly described family of heparin-binding growth and differentiation factors, has been identified as the product of a retinoic acid-responsive gene and demonstrated to bind to receptor-like protein-tyrosine phosphatase (RPTP)-β. We previously reported that the MK-RPTP-β system was upregulated in rat stomachs with experimental mucosal lesions induced by indomethacin and acetic acid, suggesting that MK may stimulate mucosal regeneration during the healing process following gastric mucosal damage. Although MK has been suggested to be a key molecule in the repair of gastrointestinal mucosal injury, nothing is known regarding its role in the pathogenesis of IBD. In the present study, we investigated the relationship between the MK-RPTP-β system and mucosal regeneration during experimental colitis.

MATERIALS AND METHODS

Animals and experimental colitis

Seven-week-old male Sprague-Dawley rats were used. Experimental colitis was induced by administering a 5% dextran sulfate sodium (DSS) solution in drinking water for 7 days. Rats were euthanized at 1, 3, 5, and 7 days after the end of DSS administration, after which a segment of each distal colon was dissected to investigate mRNA expression or for histological examinations.

Detection of tissue expression of MK, RPTP-β and pro-inflammatory cytokines

The gene expression of MK, RPTP-β, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and cytokine-induced neutrophil chemoattractant (CINC)-2β were investigated in the rat distal colons using Northern blot analysis and a reverse transcription-polymerase chain reaction (RT-PCR) method. Lase-rcapture microdissection (LCM) coupled with RT-PCR was used for detection of RPTP-β expression in colonic epithelial cells. To determine localization of the MK protein in the histological sections, two-color immunofluorescence staining for MK and vimentin was performed.

Effects of inflammatory cytokines on MK expression in 3Y1 cells

In order to study the influence of pro-inflammatory cytokines toward MK expression *in vitro*, the rat fibroblast cell line 3Y1 was used. Cells were incubated with IL-1β, TNF-α, and CINC-2β for 3, 6, 12, 24, or 48 hours, after which time-course changes of MK gene expression were examined by Northern blot analyses.

Assays for cell migration and proliferation of IEC-6 cells after stimulation with MK

The rat small intestine epithelial cell line IEC-6 was used for the assays. The effects of MK on the restitution of IEC-6 cells were evaluated using an intestinal wound repair model developed with cultured epithelial monolayer sheets. To quantify the migration effects of MK on cultured IEC-6 cells, a different migration assay was used with 24-well Boyden chambers. Further, the MK-induced growth effect was investigated using an assay of cellular DNA synthesis, which was assessed by [^{3}H]-thymidine incorporation.

Analysis for MK-induced ERK1/ERK2 activation in IEC-6 cells

IEC cells were pre-treated with MK for 15, 30, or 60 minutes, then total cellular proteins were extracted. Next, MK-induced MAP kinase ERK1/ERK2 activation in IEC-6 cells was

investigated using Western blot analysis of the phosphorylated ERK1/ERK2 protein.

RESULTS AND DISCUSSION

The gene expression of MK was significantly increased during DSS-induced colitis and peaked at 3 days after the end of DSS administration. In the histological sections, double immunoreactive signals for MK and vimentin were detected in cells present in the mucosal and submucosal layers of the colon, showing that MK is expressed in vimentin-positive mesenchymal cells including fibroblasts in damaged colonic tissues. Results of LCM coupled with RT-PCR clearly indicated RPTP-β expression in colonic epithelial cells. Further, the expression of IL-1β, TNF-α, and CINC-2β in rat distal colons increased with and peaked at 1 day after the end of DSS administration, which preceded the augmentation of MK expression.

Since the increased expression of MK was preceded by that of pro-inflammatory cytokines in the distal colon during DSS-induced colitis, the direct effects of those pro-inflammatory cytokines on MK gene expression were investigated *in vitro*. Stimulation by IL1-β and TNF-α induced MK expression in the 3Y1 cells in a time-dependent manner. Although our *in vivo* results suggested that MK has an influence on epithelial functions during the healing process of colitis, its precise role remains unknown. To clarify this point, we investigated the effects of MK on the migration of IEC cells using an intestinal wound repair model. The migration assay results clearly showed that MK-treatment accelerated wound repair dose-dependently. However, in contrast to those findings, no significant effect on epithelial cell proliferation was observed. Since we did not detect MK-dependent ERK1/ERK2 activation, a major intracellular signaling pathway in MK-dependent cell proliferation, we concluded that the proliferating effect of MK toward intestinal epithelial cells (IEC-6) was lower than that on other cells with a different tissue origin. Further studies are necessary to evaluate the essential role of MK in intestinal epithelial cell migration.

CONCLUSION

The present study is the first to show that intestinal inflammation up-regulates the MK-RPTP-β system, which may stimulate mucosal regeneration during the process of healing of colitis. Additional investigations regarding the role of MK may contribute to the development of new options for treatment of IBD.