学 位 論 文 の 要 旨

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学　位　論　文　名　Role of Milk Fat Globule-Epidermal Growth Factor 8 in Colonic Inflammation and Carcinogenesis

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論 文 内 容 の 要 旨

INTRODUCTION

Engulfment of apoptotic cells for maintaining immune homeostasis is regulated by a variety of molecular mechanisms. Milk fat globule-epidermal growth factor 8 (MFG-E8), a secreted glycoprotein, forms a link between phosphatidylserine on apoptotic cells and $\alpha_v\beta_3$-integrin on phagocytes for enhancing clearance of those cells, and is an essential molecule for preventing abnormal immune activation under physiological conditions. MFG-E8 also directly regulates a variety of cellular functions under various disease conditions and anti-inflammatory effects in the intestinal tract were recently reported. In addition, MFG-E8 enhances cell proliferation and migration, as well as anti-apoptosis and vascularization processes, which contribute to regeneration and repair of damaged tissues in various organs. On the other hand, those functions are also closely associated with malignant cell growth and tumor progression. However, its role in the pathogenesis of colon cancer remains largely unknown. In the present study, we employed MFG-E8 knockout (KO) mice to examine the effect of MFG-E8 on colonic inflammation as well as its relationship to colon cancer development, and compared those findings to results obtained with wild type (WT) mice.

MATERIALS AND METHODS

Experimental colitis was induced in 7-week-old male C57BL/6N WT and MFG-E8 KO mice by administration of dextran sodium sulfate (DSS). Colitis activity parameters (body weight, colon length, histological score, proinflammatory cytokine profile) were determined in obtained inflamed colon tissues, with regenerating parameters (histological PCNA and Ki67 scores) also examined. A mouse colitis associated cancer (CAC) model was induced with those mice by intraperitoneal injection of azoxymethane (AOM), then they were given a single administration of DSS and examined after 18 weeks, while a mouse sporadic cancer model was induced by intraperitoneal injections of AOM 6 times each week and examined after 31 weeks. The numbers and size of colonic tumors in KO mice were determined, and compared to those in WT mice. To elucidate the role of MFG-E8 in CAC development, we established DSS-induced colitis in WT and KO mice, and investigated the time course changes of MFG-E8 expression in colonic tissues. Furthermore, to confirm the role of MFG-E8 in tumor promotion *in vitro*, Colon-26 cells, a mouse colonic epithelial cell line, were pretreated with or without MFG-E8, then proliferation was evaluated using a BrdU assay. To evaluate MFG-E8 expression in inflamed colonic mucosa and tumor tissues, anti-MFG-E8 antibody immunostaining was performed with endoscopic biopsy specimens obtained both active and inactive mucosa of ulcerative colitis patients, as well as surgically or endoscopically resected tumor tissues. The animals were cared for and handled in accordance with guidelines from the National Institutes of Health and Institute for Animal Experimentation of Shimane University. The human study protocols were approved by the ethics committee of Shimane University Faculty of Medicine.

RESULTS AND DISCUSSION

We initially examined age-related changes in BW (5~30 weeks), and colon length and histology (6 and 30 weeks) in both KO and WT mice without inflammatory induction, and did not detect any differences for these parameters between those groups. In the DSS colitis model, MFG-E8 deficiency exacerbated several colitis parameters including body weight loss, histological score, and colonic expression of inflammatory cytokines. Since MFG-E8 KO mice show severe colitis, we speculated that they would be more susceptible to development of CAC than WT mice. Contrary to our speculation, the average number of colon tumors and average tumor size per mouse in the KO mice were significantly lower than in the WT mice. These findings indicate that a lack of MFG-E8 reduces inflammation-associated tumor development as well as tumor growth even in the presence of severe colitis.

To confirm the role of MFG-E8 in CAC development, we examined its expression in

colonic tissues in DSS colitis model mice. Colonic expression of MFG-E8 was significantly increased in WT mice during the regeneration phase of DSS-induced colitis. We also found that the prevalence of PCNA- and Ki67-positive epithelial cells was significantly greater in WT as compared to KO mice. We previously reported that MFG-E8 expression is upregulated in mononuclear cells infiltrating the lamina propria during the regeneration phase of DSS-induced colitis. A similar expression pattern has also been found in human inflammatory colonic mucosa of UC patients. These findings suggest that MFG-E8 secreted by infiltrating inflammatory cells stimulates epithelial proliferation in a paracrine manner during colitis, which may enhance turnover of epithelial cells and initiate CAC development.

Next, we examined whether lack of MFG-E8 has an influence on tumor incidence and growth in a sporadic colon cancer model. Although we did not find a significant difference for the number of tumors between KO and WT mice, average tumor size per mouse was significantly lower in the former. These results suggest that MFG-E8 promotes tumor growth regardless of the presence of colonic inflammation.

The role of MFG-E8 in colonic epithelial cell proliferation *in vitro* was also examined. Treatment with recombinant MFG-E8 significantly stimulated proliferation of Colon-26 cells, whereas treatment with a neutralizing antibody or siRNA targeting α_v-integrin significantly reduced proliferation of those cells stimulated with rMFG-E8, indicating that the cell proliferation effect of MFG-E8 is dependent on integrin-mediated signaling.

We also evaluated MFG-E8 expression in 17 advanced colon cancer specimens, with positive findings noted in 73.0%. Notably, abundant expression was observed in the deeper invasive parts of the cancer tissues. In addition, we performed immunostaining for detection of MFG-E8 in tumor tissues from adenoma (n=26) and early cancer (n=23) cases, and noted expression rates of 18.2% and 57.0%, respectively. Thus, MFG-E8 expression in human colon adenomas was shown to gradually increase from early to advanced cancer.

CONCLUSION

We investigated the role of MFG-E8 in intestinal inflammation and its relationship with tumor development in a murine CAC model. MFG-E8 expression was up-regulated in inflamed colonic tissues and initiated CAC development, which may be dependent on increased epithelial cell proliferation via $\alpha_v\beta_3$-integrin. Furthermore, MFG-E8 promoted tumor growth in both CAC and sporadic colon cancer models. These results are the first to show the role of MFG-E8 in the pathogenesis of colon cancer. For development of a novel therapy targeting MFG-E8, additional findings regarding various physiological, immunological, and clinical aspects are necessary.