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Short communication

Hepatocyte growth factor region specifically activates mucin synthesis in rat stomach

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Abstract

We investigated the effects of hepatocyte growth factor (HGF) on mucin biosynthesis and the expression of its receptor in distinct sites and layers of rat gastric mucosa. HGF stimulated the mucin biosynthesis in the surface and gland mucus cells of corpus, but not in the antrum, without its trophic effects. The HGF-receptor mRNA expression was high in the surface and deep corpus mucosa, but low in the antrum. These results demonstrate that HGF has distinct effects on mucin biosynthesis in a specific region of rat stomach, suggesting different regulatory mechanisms underlying the mucus metabolism of distinct mucus-producing cells.

Keywords: HGF (hepatocyte growth factor); Gastric mucin biosynthesis; HGF receptor; (Rat)

1. Introduction

Nakamura et al. (1984) elucidated the hepatocyte growth factor (HGF) to be a growth factor for rat hepatocytes in primary culture. HGF binds to epithelial cells via the high-affinity receptor Met (HGF receptor), a transmembrane tyrosine kinase (Bottaro et al., 1991). Considerable evidence indicates that the HGF receptor is expressed through the gastrointestinal epithelia of rat and mouse (Fukamachi et al., 1994; Nusrat et al., 1994; Yang and Park, 1995; Schmassmann et al., 1997). Although extensive research has been devoted to the role of HGF during the recovery from mucosal injury of the gastrointestinal tract (Nusrat et al., 1994; Watanabe et al., 1994; Schmassmann et al., 1997), information about its effects on the mucus cell function, particularly with regard to whether or not this peptide modulates the mucin metabolism, is lacking.

Mucus cells lining the gastric epithelium contain several cell types in distinct regions (corpus and antrum) or particular layers (surface mucosa and deep mucosa) of the mammalian stomach (Eisenberg et al., 1995). We proved that the surface mucus and the gland mucus cells of rat corpus and the pyloric gland cells of the antrum produced distinct mucins bearing a particular carbohydrate structure, using original monoclonal antibodies (Ishihara et al., 1996). In our recent studies, gastrin accelerated the mucin biosynthesis in the surface mucus cells, but not in the gland mucus cells of the rat gastric oxyntic mucosa (Ichikawa et al., 1998), indicating a distinct regulatory mechanism during the mucin biosynthesis in a specific region and layer of the gastric mucosa.

The aim of this study was to investigate the effects of HGF on the mucin biosynthesis in distinct sites and layers of the rat gastric mucosa using a scraping method to separate the surface mucus cell layer from the remaining deep mucosa of the rat stomach (Ichikawa et al., 1998, 1999). Furthermore, we examined the expression of the HGF receptor in each region of the rat gastric mucosa.

2. Materials and methods

2.1. Reagents

Recombinant human HGF was kindly supplied by Research Center, Mitsubishi Chemical (Tokyo, Japan). The

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reagents for the rat gastric mucosal culture included modified Eagle's Minimal Essential Medium (MEM) deficient in L-threonine (Thr) (GIBCO, Grand Island, NY), D-[1,6-^{3}H(N)]glucosamine hydrochloride (1787 GBq/mmol, New England Nuclear, Boston, MA), and [^{14}C]Thr (7.9 GBq/mmol, New England Nuclear). The HGF was dissolved in phosphate-buffered saline.

2.2. Tissue preparation of different sites and layers

Seven-week-old male Wistar rats (SLC, Shizuoka, Japan) were deprived of food but allowed free access to water for 24 h before the experiments. Their stomachs were immediately excised and then cut along the greater curvature. To obtain the full-thickness layer samples, the glandular mucosa was separated into the corpus and antrum, and then cut into small 2×2 mm pieces. Alternatively, after the stomach was opened, the corpus was selected and cut into 10×10 mm specimens. The surface layer of these specimens was removed from the remaining deep mucosa according to our previously described method (Ichikawa et al., 1998). The deep mucosal layer of the corpus was sliced into small pieces of approximately 4 mm^2, similar to the full-thickness layer specimens.

2.3. Tissue culture and measurement of synthesized mucin

For cultures of the full-thickness and deep-layer samples, eight tissue fragments were randomly picked out from five different stomachs and then placed, with the mucosal surface facing up, on a stainless steel grid in the central well of a plastic culture dish (60×15 mm, Falcon, Lincoln Park, NJ) and then treated with 0.75 ml of medium and 0.05 ml of the test substance. The tissue culture process was detailed in a previous report (Ichikawa et al., 1998). The medium consisted of 90% modified Eagle's MEM whose L-Thr concentration was reduced to 40 mmol/l with 370 kBq/ml [^{3}H]glucosamine and 37 kBq/ml [^{14}C]Thr. All the dishes were maintained at 37°C for 5 h in 5% CO_2 and 95% air. For culturing of the surface layer, a sheet of the surface mucosa of the corpus, with the mucosal surface facing up, was placed on a Falcon cell insert in the center of the well of a plate dish with the previously described medium and HGF solution. All these dishes were maintained as above using 95% O_2 instead of air. Upon completion of the culture period, the tissues were homogenized in Tris–HCl buffer containing Triton X-100. The homogenate was centrifuged and the obtained supernatant was applied onto a Bio-Gel A1.5m column. The fractions eluted with first and second peaks were collected and the radioactivity measured as synthesized mucin and non-mucin protein (Azuumi et al., 1980; Ichikawa et al., 1998). To compare the synthesis of the mucin and non-mucin protein, incorporation of the radiolabeled precursor into these fractions was determined and divided by the tissue protein content. The protein content was determined by the bicinchoninic acid method with a Pierce protein assay kit (Pierce, Rockford, IL), using bovine serum albumin as the standard.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR) for HGF receptor

Total RNA was extracted from each tissue of different sites and layers using the acid guanidium thiocyanate/phenol chloroform method with a kit from Nippon Gene, Toyama, Japan. The presence of mRNA was analyzed by reverse transcription of the total RNA (2 μg) followed by polymerase chain reaction (PCR) amplification as previously described (Fukamachi et al., 1994). Primers specific for the amplification of the rat HGF receptor gene (sense primer, 5′-TGTGCGTTCCCCATCAAATA-3′; antisense primer, 5′-TCGACAATAACTTCCGGAGA-3′; size of the amplified fragment, 597 bp) and those for the β-actin gene (sense primer, 5′-GTGGGTGTAGGTACTAACAAT-3′; antisense primer, 5′-CTCTTTGATGTCACGCACGATTTC-3′; size of the amplified fragment, 513 bp) were synthesized by Hokkaido Biosystem, Sapporo, Japan. For each reaction, 2 μg of the RNAs from respective tissues was reverse transcribed to cDNAs using the cDNA synthesis kit (Takara, Shiga, Japan), and the resulting cDNAs were subjected to 35 cycles of PCR using Taq DNA polymerase (Takara). The PCR products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide.

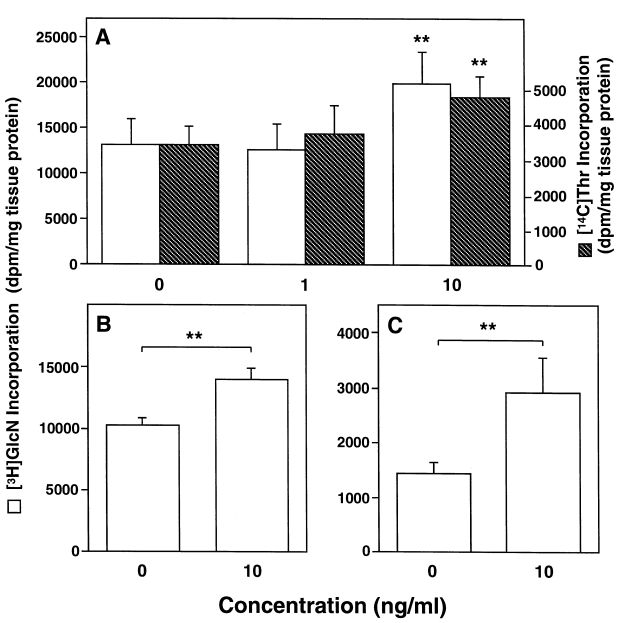

Fig. 1. Effects of HGF on [^{3}H]glucosamine (open bars) and [^{14}C]threonine (solid bars) incorporation into mucins in the full-thickness (A), deep (B), and surface (C) layers of the rat corpus mucosa. Values are expressed as disintegrations per minute (dpm) per milligram of tissue protein and are means ± S.D. from five different samples derived from five different rats. ** $P < 0.01$ as compared with the control value (0 ng/ml).

2.5. Statistical analysis

Values presented in Fig. 1 are given as means ± S.D. A one-way analysis of variance (ANOVA) with Dunnett's test was used for the statistical analysis with $P < 0.05$ taken as significant.

3. Results

3.1. Effects of HGF on mucin synthesis in distinct sites and layers of rat gastric mucosa

Fig. 1 shows the biosynthetic activity of mucin in distinct sites and layers of the rat gastric mucosa, as measured by the incorporation of radiolabeled precursors with or without HGF. In the full-thickness corpus tissue, HGF at a concentration of 1 ng/ml had no effect for 5 h of tissue culture. However, 10 ng/ml HGF enhanced the incorporation of [^{3}H]glucosamine and [^{14}C]Thr into the mucin by 52% and 40% of the control levels, respectively ($P < 0.01$, Fig. 1A). In contrast, no significant change could be detected in the non-mucin protein synthesis after the addition of HGF at either 1 or 10 ng/ml (relative radioactivity: 105 ± 24 and 97 ± 15, respectively. $n = 5$). HGF at these concentrations also had no significant effect on the ^{3}H- and ^{14}C-labeled mucins of the antral mucosa (data not shown).

To examine whether the stimulative effect of HGF on corpus mucin biosynthesis is limited to specific mucus cells of the rat corpus mucosa, the deep-layer tissues of the corpus, including the gland mucus cells but lacking a large portion of the surface mucus cells, were cultured in the presence of [^{3}H]glucosamine. Fig. 1B shows the biosynthetic activity of the mucin in the deep corpus mucosa with or without 10 ng/ml of HGF. The addition of HGF enhanced [^{3}H]glucosamine incorporation into the mucin in the deep-layer as well as in the full-thickness corpus region. Similarly, the addition of HGF significantly stimulated the mucin biosynthesis of the surface mucus cell-rich layer of the corpus mucosa (Fig. 1C).

3.2. Expression of HGF receptor in distinct sites and layers of rat gastric mucosa

Fig. 2 shows the expression of the HGF receptor mRNA measured using the RT-PCR technique in distinct sites and

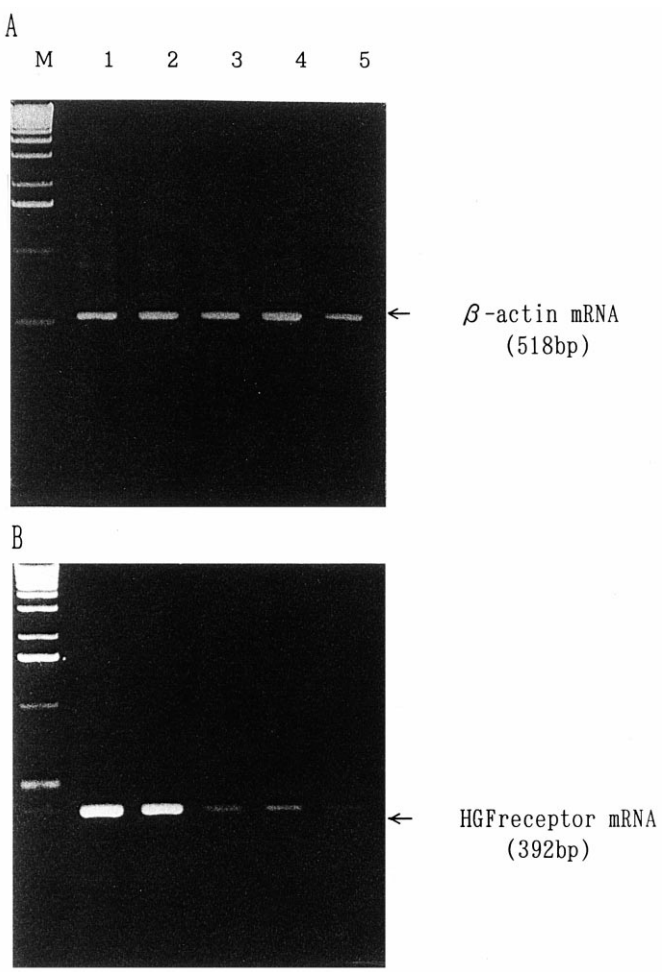

Fig. 2. Determination of β-actin mRNA (A) and HGF-receptor mRNA (B) by RT-PCR in the distinct sites and layers of the rat gastric mucosa. The PCR amplification consisted of 35 cycles. Lane 1, surface mucosal layer of the corpus; 2, deep mucosal layer of the corpus; 3, muscle layer of the corpus; 4, mucosal layer of the antrum; 5, muscle layer of the antrum; M, DNA size marker.

layers of the rat gastric mucosa. The remarkable expression of this mRNA was detected in both the surface and deep corpus mucosa of the rat stomach. In contrast, the level of expression of the HGF receptor mRNA in the antral mucosal layer was low compared with those in the corpus mucosal layer (Fig. 2B). As a positive control, the rat β-actin was assayed in all specimens to verify the efficiency of the cDNA synthesis from extracted RNA (Fig. 2A).

4. Discussion

HGF exerts several important actions, such as the stimulative effects of mitogenesis and the motility of epithelial cells in the gastrointestinal tract (Weidner et al., 1993; Nusrat et al., 1994; Watanabe et al., 1994; Schmassmann et al., 1997). Fukamachi et al. (1994) reported that HGF at concentrations of 3 and 10 ng/ml stimulated the growth of rat gastric epithelial cells in primary culture. Our most notable finding was that 10 ng/ml HGF accelerated the biosynthesis of mucin, but not non-mucin protein, in rat gastric corpus mucosa. The HGF-induced stimulation of mucin synthesis was independent of its trophic effects, because the influence of cell proliferation was generally ignored in our experimental model. Our data suggest that HGF is a significant factor controlling the mucin synthesis, as well as promoting growth and movement, in gastric epithelial cells.

In this study, the antral tissue separated from the corpus was also cultured. HGF had no significant effect on the mucin biosynthesis in this region of the rat stomach. Similarly, gastrin having a function as a growth factor in the gastrointestinal tract-significantly accelerated the mucin biosynthesis only in the oxyntic region, but yielded no significant effect on the mucin metabolism in the antral region of the rat stomach (Ichikawa et al., 1998). These findings support the notion that mucin production is regulated in different ways in the corpus and antrum of the gastric mucosa.

Mucus cells of the mammalian gastric corpus mucosa have been mainly classified into the surface mucus and gland mucus cells. Recent evidence demonstrates that mucus from these two types of human cells has distinct roles against the colonization of *Helicobacter pylori*, implicated in the etiology of gastric disease (Shimizu et al., 1996). Elucidation of the regulatory mechanism for these different mucus cells may help clarify the complicated defense mechanism of gastric mucosa. Recently, we devised a scraping method to separate the surface mucosal layer from the remaining deep mucosa of the rat stomach (Ichikawa et al., 1998). Immunohistochemical staining with RGM21, an original monoclonal antibody that strongly reacts with the surface mucus cell-derived mucin (Ishihara et al., 1996), has clearly shown that the deep mucosa remaining after scraping with forceps lacks the surface mucus cells but shows no observable damage to the underlying gland mucus cells (Ichikawa et al., 1999). Using this scraping method, we proved that the mucin biosynthesis stimulated by gastrin occurred in the surface mucus cells, and not in the gland mucus cells, of the gastric oxyntic mucosa (Ichikawa et al., 1998). In the present study, we separately surveyed the effect of HGF on the mucin biosynthesis in the deep layer, rich in gland mucus cells, and the surface mucus cell-rich layer of the corpus mucosa. In contrast to gastrin, HGF accelerated the mucin synthesis in both the surface and deep layer of the corpus mucosa, indicating the stimulatory effect of HGF on the surface and gland mucus cells.

Stimulation of the HGF receptor is sufficient to mediate the pleiotropic activities of HGF in the epithelial cell line in culture (Weidner et al., 1993) and indicates that this receptor would mediate the various physiologic responses to HGF in the animal. Yang and Park (1995) have elucidated by Northern analyses that the HGF receptor is expressed in many adult mouse normal tissues, suggesting numerous physiological roles for this receptor. Here we show that HGF receptor mRNA is detected in both the surface and deep corpus mucosa of the rat stomach. Similar results for the gland mucus cell region were obtained by Schmassmann et al. (1997) using the entire corpus mucosa of the adult rat which was not deprived of food. Further studies are needed to clarify whether the HGF receptor actually exists on both the surface and gland mucus cells of the rat gastric oxyntic mucosa. However, these findings, together with our present result of the low expression of the HGF receptor mRNA in the antral mucosa, support evidence that HGF modulates the function of two distinct types of mucus cell in the gastric corpus mucosa.

Our previous study demonstrates that three acid secretagogues, namely gastrin, histamine, and acetylcholine, have distinct effects on the mucin biosynthesis in a specific region and layer of the rat gastric mucosa (Ichikawa et al., 1998). Both gastrin- and histamine-activated mucin biosynthesis occur in the surface mucus cells, and not in the gland mucus cells of the gastric oxyntic mucosa. Acetylcholine promotes the mucin production throughout the glandular stomach. In this study, HGF modulated the mucin biosynthesis differently from these three agents, indicating this growth factor may prove useful as an experimental tool for studying the regulatory mechanism of the gastric mucin synthesis.

In summary, the present findings demonstrate that HGF has distinct effects on mucin biosynthesis in a specific region and layer of the rat gastric mucosa, suggesting different regulatory mechanisms underlying the mucus metabolism of distinct mucus-producing cells. The HGF-induced stimulation of the mucin biosynthesis occurs in the corpus mucus cells, and not in the antral mucus cells, of the rat gastric mucosa.

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