Increased gastric expression of MMP-7 in hypergastrinemia and significance for epithelial-mesenchymal signaling

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Varro A, Kenny S, Hemers E, McCaig C, Przemeck S, Wang TC, Bodger K, Pritchard DM. Increased gastric expression of MMP-7 in hypergastrinemia and significance for epithelial-mesenchymal signaling. Am J Physiol Gastrointest Liver Physiol, 292:G1133–G1140, 2007. First published January 11, 2007; doi:10.1152/ajpgi.00526.2006.—Chronic hypergastrinemia is associated with enterochromaffin-like (ECL) cell hyperplasia, which may progress to gastric carcinoid tumors. The latter consists of epithelial cells and stroma, and both compartments usually regress after normalization of hypergastrinemia. We previously showed that matrix metalloproteinase (MMP)-7 in gastric epithelial cells was upregulated by Helicobacter pylori and described MMP-7-dependent reciprocal signaling between the epithelium and a key stromal cell type, the myofibroblast. Here, we describe the regulation of gastric MMP-7 by gastrin and the potential significance for recruiting and maintaining myofibroblast populations. Biopsies of the gastric corpus and ECL cell carcinoid tumors were obtained from hypergastrinemic patients. Western blot analysis, ELISA, immunohistochemistry, and promoter-luciferase (luc) reporter assays were used to study MMP-7 expression. Gastric myofibroblasts were identified by α-smooth muscle actin (α-SMA) expression, and the effects of MMP-7 on myofibroblast proliferation were investigated. In hypergastrinemic patients, there was an increased abundance of MMP-7 and α-SMA in gastric corpus biopsies and ECL cell carcinoma tumors. In the latter, MMP-7 was localized to ECL cells but not stromal cells, which were nevertheless well represented. Gastrin stimulated MMP-7-luc expression in both AGS-Gh4c2 and primary human gastric epithelial cells. Conditioned medium from gastrin-treated human gastric glands stimulated myofibroblast proliferation, which was inhibited by neutralizing antibodies to MMP-7. MMP-7 increased the proliferation of myofibroblasts via the MAPK and phosphatidylinositol 3-kinase (PI3K) pathways. In conclusion, stimulation of gastric MMP-7 by elevated plasma gastrin may activate epithelial-mesenchymal signaling pathways regulating myofibroblast function via MAPK and PI3K pathways and contribute to stromal deposition in ECL cell carcinoma tumors.

matrix metalloproteinase-7; pernicious anemia; multiple endocrine neoplasia type 1; enterochromaffin-like cell carcinoid tumors

MUCOSAL ORGANIZATION throughout the gastrointestinal tract depends on interactions between the epithelium and subepithelial cells including fibroblasts and myofibroblasts as well as blood vessels, immune cells, and neurons. Recent work has suggested an unexpected diversity in the relevant signaling systems (13). In particular, in addition to growth factors and cytokines, it is now recognized that proteolytic enzymes may also play a role in these signaling processes (13, 20). Matrix metalloproteinases (MMPs) are involved in remodeling of the extracellular matrix and the liberation of growth factors and are frequently increased in inflammatory conditions, injury, and cancer (10, 24). Most MMPs are produced in subepithelial cells, but an exception is MMP-7 (also known as matrilysin or PUMP), which is predominantly expressed in epithelial cells both of the gut and other organs including airways, mammary glands, and the urogenital tract (9, 19, 25). Recent work has shown that in the stomach, there is increased MMP-7 with Helicobacter pylori infection (2, 4, 33). This is associated with increased myofibroblast abundance, involving both cell proliferation and migration via increased IGF-II bioavailability through cleavage of its binding protein, IGF binding protein (IGFBP)-5, which is released from myofibroblasts (13, 20).

It is well established that a prolonged elevation of plasma gastrin is found in achlorhydria, e.g., pernicious anemia (PA), as well as in gastrinoma, including on a background of multiple endocrine neoplasia (MEN) type-1, and in some patients on prolonged proton pump inhibitors (5, 7, 17, 30). Elevated plasma gastrin is associated with hyperplasia of histamine-secreting enterochromaffin-like (ECL) cells in the gastric corpus (3, 17, 18). In some hypergastrinemic patients, there is a progression via dysplastic ECL cells to ECL cell carcinoma tumors (11, 23); thus, the latter are reported to occur in patients with hypergastrinemia on a background of either gastric inflammation, as occurs in PA, or MEN-1 but are generally uncommon in patients with sporadic gastrinoma (5, 17). In the case of patients with hypergastrinemia of antral origin, resection of the antrum to lower plasma gastrin has been reported by several groups to lead to regression of ECL cell carcinoma tumors (12, 14, 15). Interestingly, both ECL cells and the stroma regress, suggesting that gastrin acts via ECL cells to trigger a stromal reaction. The present study was based on the specific hypothesis that gastrin stimulates MMP-7 expression, which, in turn, stimulates proliferation of a key stromal cell type, the myofibroblast. We therefore asked whether 1) there is increased expression of MMP-7 in gastric biopsies of hypergastrinemic patients and in a transgenic mouse model overexpressing human gastrin, 2) there is also increased MMP-7 in ECL cell carcinoid tumors, 3) gastrin stimulates expression of a MMP-7 promoter/luciferase reporter construct in cultured cells in vitro, 4) increased MMP-7 is accompanied by increased myofibroblast abundance, and 5) MMP-7 stimulates myofibroblast proliferation through mechanisms involving MAPK and phosphatidylinositol 3-kinase (PI3K) pathways.

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MATERIALS AND METHODS

Cells, plasmids, and drugs. The gastric cancer cell line AGS-Ga, permanently transfected with the CCK-2 receptor, was routinely cultured in Ham’s F-12 medium as previously described (27). Human gastric adherent glands and myofibroblasts were prepared as previously described (20, 33). A vector consisting of 2.3 kb of the human MMP-7 promoter coupled to firefly luciferase (i.e., MMP-7-luc) was kindly donated by Lynn Matrisian (Vanderbilt University, Nashville, TN). Human recombinant (r)MMP-7, MMP-7 fluorogenic substrate, AG-1478, AG-825, Ro-340432, and mouse purified EGF were obtained from Calbiochem (Nottingham, UK). Heptadecapeptide amidated gastrin (G17) was purchased from Peninsula (Merseyside, UK). All other chemicals were obtained from Sigma (Dorset, UK).

Animals. The gastric corpus was taken at 3 mo of age from sex-matched FVB/N, gastrin-overexpressing (InsGas), gastrin knock-out (GKO), and wild type (WT) mice for protein extraction and histochemistry as previously described (20). Some GKO mice fed ad libitum were treated with G17 (20 mmol ip at 09.00 and 16.00 hours on day 1 and 09.00 hours on day 2), and the gastric corpus was taken for extraction for Western blot analysis 3 h later. Procedures were approved by the Ethics Committees of the Salford and Trafford Health Authority (Manchester, UK), South Sefton, and Royal Liverpool and Broadgreen University Hospitals National Health Service Trusts. All patients gave informed consent.

Human gastric gland and myofibroblast preparations. Isolated human gastric glands were prepared from endoscopic biopsies of H. pylori-negative patients as previously described (33). Human gastric myofibroblasts were prepared as previously described (20, 34).

Animals. The gastric corpus was taken at 3 mo of age from sex-matched FVB/N, gastrin-overexpressing (InsGas), gastrin knock-out (GKO), and C57Bl/6 [wild type (WT)] mice for protein extraction and histochemistry as previously described (20). Some GKO mice fed ad libitum were treated with G17 (20 mmol ip at 09.00 and 16.00 hours on day 1 and 09.00 hours on day 2), and the gastric corpus was taken for extraction for Western blot analysis 3 h later. Procedures were approved by the Local Animal Welfare Committee and were in accordance to United Kingdom legislations.

Western blot analysis. Protein extracts of human gastric biopsies and gastric myofibroblasts were prepared, and Western blot analysis was performed as previously described (13, 20). Samples were probed with antibodies to MMP-7 (Chemicon, Chondlers Ford, UK), α-smooth muscle actin (α-SMA; RDI, Flanders, NJ), phospho-p42/44 MAPK (New England BioLabs, Hertford, UK), and phospho-Akt (Thr473) (Cell Signaling Technology), followed by horseradish peroxidase-conjugated secondary antibody and detection by an incubation with SuperSignal West Pico Chemiluminescent Substrate (Pierce) and HyperFilm (Amersham) as previously described (28). Samples were reprobed for GAPDH (Biodesign, Saco, MA) or β-actin (Santa Cruz Biotechnology) and total MAPK using anti-ERK1 (BD Transduction Laboratories, Bedford, MA) or anti-Akt antibodies (BD Transduction Laboratories) as appropriate.

MMP-7 ELISA. Plasma samples were processed for the detection of MMP-7 using a Quantikine Human MMP-7 (total) Immunoassay kit (R&D Systems, Minneapls, MN) according to the manufacturer’s instructions.

Algin RIA. Plasma samples from fasted control, MEN-1, and PA patients and from mice were assayed for total amidated gastrin concentrations using antibody L2 (which reacts with G17 and G34 but not progastrin or Gly-gastrins) as previously described (8). The upper limit of normal fasting gastrin concentrations in this assay was 30 pM (5, 26).

Fluoregenic substrate assays. Medium from gastric glands treated with G17 was incubated with specific MMP-7 fluorogenic substrate, and fluorescence was measured using excitation at 280 nm and emission at 360 nm according to the manufacturer’s instruction as previously reported (20).

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue sections were processed for the immunohistochemical detection of MMP-7 (Chemicon) and α-SMA (RDI) after antigen recovery as previously described (33).

Transfection and luciferase assays. AGS-GaR cells (2 × 10⁵) were plated in full medium. The following day, the medium was removed, and cells were cotransfected with MMP-7-luc (1.0 µg/well) and Renilla luciferase (0.1 µg/well; Promega, Madison, WI) using TransFast (Promega) as previously described (33). Medium was then replaced with 2 ml serum-free medium, and cells were incubated with G17 for 8 h. Luciferase activity was measured with Bright-Glo or Dual-Glo (Promega) using a LumiCount Plated reader (Packard BioScience) (33). In addition, to explore the control of MMP-7-luc in primary gastric epithelial cells, we developed the following new method. Primary human gastric glands (33) were transfected using Combimag (OzBiosciences, Marseille, France) on a magnetic plate according the manufacturer’s instructions, and cells were incubated with G17 for 20 h. Luciferase activity was measured by a dual-luciferase assay (Promega). Results in both cases are presented as fold increases over the unstimulated control, so 1.0 signifies no change in luciferase activity. The protein concentration was determined when appropriate using a Bio-Rad detergent compatible protein assay (Bio-Rad, Herts, UK) to monitor the plating efficiency.

Cellular targeting of MMP-7-luc in primary adherent glands. To study the cellular targeting of MMP-7-luc expression, transfected human glands were double immunostained with goat anti-luciferase antibody (Rockland Immunocchemicals, Gilbertsville, PA) together with one of the following antibodies: rabbit anti-pepsinogen (a gift from Mike Samloff, Center for Ulcer Research, Los Angeles, CA), anti H′ K′ ATPase (Calbiochem), anti-vesicle monoamine transporter type 2 (VMAT-2) (16), mouse anti-trefoil factor-1 (TFF-1; Dako, Glostrup, Denmark), and mouse anti-TFF-2 (NovoCastr, Newcastle-upon-Tyne, UK) antibodies, with the appropriate FITC- or Texas red-conjugated secondary antibodies, raised in the donkey (Jackson Immunoresearch, Soham, UK), using Vectashield mounting medium with 4′,6-diamidino-2-phenylindole (Vector Laboratories, Peterborough, UK) to counterstain nuclei. Slides were examined using a Zeiss Axioplan-2 microscope (Zeiss Vision, Welwyn Garden City, UK), and images were captured using a JVC-C3 charged-coupled device camera and KS300 software combined with deconvolution software (Image Associates, Oxfordshire, UK). Ten fields of three patients were counted in each case, and results are expressed as percentages of the total cell number as previously reported (33).

Proliferation assays. The incorporation of [³H]thymidine into human gastric myofibroblasts was studied using methods previously described (29). Cells (25,000) were cultured in six-well plates in serum-free medium for 48 h and stimulated for 18 h with conditioned medium from G17-treated glands with or without mouse monoclonal MMP-7 neutralizing antibody (4 µg/ml, Chemicon). [³H]thymidine (2 µCi/ml) was added for the last 2 h; cells were processed as previously described (29).

Statistics. Results are presented as means ± SE; comparisons were made using ANOVA or Student t-tests where appropriate and were considered significant at P < 0.05.

RESULTS

Increased gastric MMP-7 in both the human and mouse gastric corpus with hypergastrinemia. In initial studies, we found elevated circulating MMP-7, as determined by ELISA,
in patients with hypergastrinemia due to PA and MEN-1 compared with control subjects (Fig. 1A). To determine whether the increase of plasma MMP-7 was reflected in the gastric corpus, we then performed Western blot analysis of gastric biopsies from both patient groups. The abundance of MMP-7 was indeed significantly increased in the gastric corpus of both PA and MEN-1 patients compared with control subjects (Fig. 1B and D). Subsets of both PA and MEN-1 patients exhibited ECL cell carcinoid tumors, which are recognized to be gastrin dependent, and when these were separately sampled, we again found increased MMP-7 abundance compared with control subjects (Fig. 1C and D). Increased gastric MMP-7 was not, however, associated with mild hypergastrinemia, because in biopsies from subjects with fasting plasma gastrin concentrations in the range of 30–100 pM, MMP-7 abundance was not different from controls (gastrin: 8.7 ± 1.3 pM, n = 9, compared with 58.6 ± 8.4 pM, n = 8; control MMP-7 abundance: 100 ± 20.2% compared with 101.6 ± 21.9% in hypergastrinemic patients).

To explore a causal link between gastrin and MMP-7, we then examined a mouse model of hypergastrinemia (InsGas), in which there are increases in ECL cell markers at 3 mo (6). We found increased abundance of MMP-7 in the stomach of InsGas mice compared with the parental (FVB/N) strain (Fig. 2A and B). Moreover, treatment of mice null for the gastrin gene with exogenous G17 also increased MMP-7 abundance, whereas there were no significant differences in MMP-7 between GKO mice and their WT counterparts (Fig. 2, C and D).

**Gastrin stimulates MMP-7 promoter activity in primary cells.** To determine whether gastrin might regulate MMP-7 transcription, we initially studied the response of a promoter-luciferase reporter vector (MMP-7-luc) transfected into AGS-GR cells; these cells were chosen because they have been widely used in the past as an experimental model in studies (32, 37) of gene expression in ECL cells (Fig. 3A). In response to concentrations of gastrin in the range of 100 pM–10 nM, there was a progressive increase in MMP-7-luc expression; the concentration for half-maximal stimulation was 2 nM. We then asked whether the same stimulation occurs in primary human gastric gland cells. To this end, we developed a novel method for transfection of adherent glands with MMP-7-luc as described in MATERIALS AND METHODS. In primary human gastric glands, G17 (1 nM, 20 h) also significantly stimulated MMP-7-luc expression (Fig. 3B). Using these methods, 5.7 ± 1.2% of all cells expressed the construct. Importantly, expression of MMP-7-luc was targeted to subsets of TFF-1-positive surface epithelial, chief (pepsinogen), and ECL (VMAT-2) cells (Fig. 3A–I, and Table 1). There was no targeting of the construct to parietal cells (H+–K+-ATPase) (Fig. 4J) or to TFF-2-positive neck cells (Fig. 4K). The former could, however, be transfected with other constructs, for example, the promoter of the β-subunit of H+-K+-ATPase coupled with green fluorescent protein (GFP). Moreover, a control GFP-vector was expressed in all cell types in this system (not shown).
Increased abundance of the myofibroblast marker α-SMA in both the human and mouse gastric corpus with hypergastrinemia. Previous work has established that MMP-7 is localized mainly to epithelial cells but not to stromal cells in the gastric mucosa (33). In line with this, in the present study, we found strong immunostaining of MMP-7 in ECL cells in gastric carcinoid tumors (Fig. 5A). In contrast, there was little or no staining of stromal cells. Even so, myofibroblasts identified by staining with antibody to α-SMA were abundant in gastric ECL cell carcinoid tumors and were often dispersed throughout the tumor (Fig. 5B). There was also increased α-SMA abundance, as detected by Western blot analysis in the corpus of both PA and MEN-1 patients, including ECL cell nodules, compared with the mucosa from normal subjects (Fig. 6, A and B). Similarly, there was increased MMP-7 and α-SMA in mice overexpressing gastrin (Fig. 6, C and D).

MMP-7 released by gastrin from cultured gastric glands stimulates human gastric myofibroblast proliferation. The presence of abundant myofibroblasts in gastric carcinoid tumors suggested that there might be a gastrin-activated signaling pathway from the epithelium to myofibroblasts. To examine whether MMP-7 might be one such mediator, we first treated gastric gland cultures with G17 and measured MMP-7 enzyme activity. G17 (1 nM, 20 h) stimulated MMP-7 enzyme activity by 272.2 ± 36% compared with control (100 ± 19.9%, P < 0.05). Moreover, conditioned medium from cultured human gastric glands treated with G17 stimulated human gastric myofibroblast proliferation, and this was significantly reduced by MMP-7 neutralizing antibodies (Fig. 7A), whereas the proliferative effect of another growth factor, EGF (50 ng/ml), was not affected (EGF: 199.4 ± 23.7%; EGF + MMP-7 antibody: 177.6 ± 15.8%). G17 itself had no effect on myofibroblast proliferation (control: 100 ± 5.56%; 1 nM G17: 89.1 ± 10.3%). These data are, therefore, compatible with the idea that native MMP-7 released from epithelial cells by gastrin stimulates myofibroblast proliferation.

MMP-7 stimulates human gastric myofibroblast proliferation via MAPK and PI3K. To examine the cellular mechanisms that mediated the effects of MMP-7 on myofibroblast proliferation, we then considered the involvement of MAPK and PI3K pathways, as these have been previously implicated in the proliferation of colonic myofibroblasts (13). The MEK inhibitor U-0126 (10 μM) reversed the proliferative effect of recombinant MMP-7 in a concentration (2 μg/ml) that had been previously shown to be optimal for myofibroblast proliferation (20) and so too did the PI3K inhibitor LY-294002 (50 μM; Fig. 7B). Compatible with the idea that MMP-7 activates the MAPK pathway, we found increased phosphorylation of p42/44 ERK, which peaked within 10 min of the application of rMMP-7 (Fig. 7C). Similarly, there was also increased phosphorylation of Akt, which is a known target of PI3K; the time course was distinct from that of p42/44 ERK activation with a progressive increase in phosphorylation after up to 240 min of incubation in MMP-7 (Fig. 7D). The activation of ERK and Akt pathways occurred in parallel, since the MEK inhibitor U-0126 inhibited MMP-7-induced p42/44 but not Akt phosphorylation and, vice versa, the PI3K inhibitor LY-294002 inhibited MMP-7-induced Akt but not p42/44 phosphorylation. In contrast, the PKC inhibitor Ro-320432, the EGF receptor tyrosine kinase inhibitor AG-1478, and the Erb-2 receptor tyrosine kinase inhibitor AG-825 had no effect on MMP-7-stimulated proliferation, suggesting that these signaling molecules are not implicated in the actions of MMP-7.
DISCUSSION

The present study was based on the hypothesis that elevated plasma gastrin concentrations stimulate gastric MMP-7 expression, which, in turn, increases myofibroblast proliferation. The main findings of the study were that MMP-7 is increased in ECL cell carcinoid tumors and the gastric corpus of hypergastrinemic patients and that gastrin acts on gastric glands to release MMP-7 in sufficient concentrations to trigger myofibroblast proliferation. ECL tumors provide an interesting model for studies of epithelial-stromal interactions since their growth is dependent on gastrin, which, in turn, is thought to act exclusively on epithelial cells. When gastrin is removed in PA patients by antrectomy, there is tumor regression, including the stromal component, suggesting that ECL cells release factors regulating stromal cell abundance and deposition of extracellular matrix proteins (14, 15). We suggest now that gastrin activates a sequence of events that includes ECL cell proliferation and increased expression of MMP-7, which then stimu-

![Fig. 4. A 2.3-kb portion of the human MMP-7 promoter targets expression to specific cell types in primary human epithelial cells. A–C: MMP-7-luc (green) expression in trefoil factor (TFF)-1 (red)-positive cells. D–F: MMP-7-luc (green) expression in pepsinogen (red)-positive chief cells. G–I: MMP-7-luc (green) expression in vesicle monoamine transporter type 2 (VMAT-2; red)-containing enterochromaffin-like (ECL) cells. J and K: MMP-7-luc (green) expression was not colocalized with H^+ -K^+ -ATPase (J; red) or TFF-2 (K; red).]
Carcinoid tumor.

Endocrine cells but not stromal cells in the MMP-7 immunoreactivity was localized to concentrations similar to those in the plasma of hypergastrinemic expression of an MMP-7-promoter/luciferase reporter construct at also shown in an in vitro system that gastrin was able to stimulate the direct stimulation of MMP-7 expression in response to gastrin. ECL cell numbers, and, in studies of cultured cells, we observed increased ECL cell numbers (3, 18, 23) without changed expres- sion at the level of single cells. Set against this, however, it should possible that the apparent elevation of MMP-7 simply reflects most at risk of developing ECL cell carcinoid tumors (5). It is increased plasma gastrin concentrations of however, patients exhibiting increased MMP-7, i.e., with pro- duced myofibroblast proliferation, expanding the extracellular matrix around ECL cells.

Previous studies (2, 4, 33) have shown increased gastric epithelial expression of MMP-7 with H. pylori infection. Recently, we (13, 20) reported the consequences of increased epithelial MMP-7 production by H. pylori on the gastric microenvironment, namely, rapid cleavage of IGFBP-5 by secreted MMP-7, followed by increased bioavailability of IGF-II from myofibroblasts. IGF-II stimulated the proliferation and migration of myofibroblasts and increased the proliferation of epithelial cells. The present data suggest that hypergastrinemia is also associated with increased MMP-7 expression. Our data suggest that gastrin increases MMP-7 expression at concentrations only observed in frank hypergastrinemia, since it was observed in groups with plasma gastrin concentrations of >100 pM but not in subjects with fasting plasma gastrin concentrations of 30–100 pM. Moreover, in a transgenic mouse model of hypergastrinemia (31), we also found increased MMP-7. In both humans and mice, circulating gastrin concentrations above 100 pM appeared to be required for increased MMP-7. The available data do not, therefore, suggest that there is physiological control of MMP-7 by gastrin. Importantly, however, patients exhibiting increased MMP-7, i.e., with prolonged plasma gastrin concentrations of >100 pM, include those most at risk of developing ECL cell carcinoid tumors (5). It is possible that the apparent elevation of MMP-7 simply reflects increased ECL cell numbers (3, 18, 23) without changed expression at the level of single cells. Set against this, however, it should be noted that exogenous gastrin increased MMP-7 in mice null for gastrin over a time course that could not be attributed to increased ECL cell numbers, and, in studies of cultured cells, we observed direct stimulation of MMP-7 expression in response to gastrin.

Thus, in addition to describing increased MMP-7 in vivo, we have also shown in an in vitro system that gastrin was able to stimulate the expression of an MMP-7-promoter/luciferase reporter construct at concentrations similar to those in the plasma of hypergastrinemic patients. For these experiments, we first made use of AGS-GaR cells as this system has been intensively used for similar previous studies (32, 37). However, to establish whether primary cells might also express MMP-7-luc, we developed a novel methodology to transfect primary human gastric epithelial cells using cultured gland cells in which all the main cell types are preserved and in which cell-cell junctions are intact. In this system, MMP-7-luc was expressed in a cell-restricted manner. Importantly, the pattern of expression of luciferase driven from the MMP-7 promoter resembled that of the WT gene (33). In particular, subsets of TFF-1-, pepsinogen-, and VMAT-2-expressing cells all exhibited luciferase activity after transfection, whereas TFF-2- and H+K+ATPase-expressing cells did not. Further work will be necessary to define in detail the molecular basis of this expression. It is, however, important to note that luciferase expression in primary cells was increased by gastrin in at concentration of 1 nM. In the future, this method should be generally applicable to studies of other genes normally expressed in cell-restricted patterns in the gastric epithelium. In the present context, the main conclusion was that 2.3 kb of the MMP-7 promoter is sufficient to determine the physiological pattern of expression in the stomach and to support increased expression in response to gastrin.

It is increasingly clear that myofibroblasts play an important role in determining the organization of epithelia during development, in wound healing, and in cancer. The identification of MMP-7 as a novel target of gastrin in this regard therefore raises new possibilities for manipulating epithelial-stromal interactions both experimentally and therapeutically in ECL cell carcinoid tumors. Our data suggest that MMP-7 is able to stimulate MAPK and PI3K pathways in myofibroblasts, leading to an expansion in cell numbers. These pathways are often activated in inflammation, injury, and cancer due to increased growth factor production or mutations leading to accelerated cell proliferation.

Previous work has established the expression of MMP-7 in gastric cancer and an association with poor outcome (1, 21, 35). The possible cellular mechanisms are still largely unexplored. They may include remodeling of the extracellular matrix leading to fibrosis (36, 38), increased cell invasion, or suppression of apoptosis (10, 25). Our data suggest that there is also increased stimulation of stromal cells, and, because these in turn produce growth factors acting on the epithelium, an aggressive cycle of stimulation may be established. The proliferative responses of myofibroblasts to MMP-7 appear to depend on its proteolytic activity, since we have found that heat-inactivated MMP-7 has no effect (data not shown). While the available data suggest that MMP-7 works via cleavage of

![Fig. 5. Immunohistochemical localization of MMP-7 and the myofibroblast marker α-smooth muscle actin (α-SMA) in an ECL cell carcinoid tumor from a PA patient. A: MMP-7 immunoreactivity was localized to endocrine cells but not stromal cells in the carcinoid tumor. B: α-SMA was localized to myofibroblasts that surround tumor cells. Magnification: X40.](image-url)
IGFBP-5 and liberation of IGF-II, it is worth noting that at least in colonic myofibroblasts, there is some evidence that MMP-7 activates other MMPs that participate in the response (13); further work will be need to determine whether comparable protease interactions occur in the present system. Moreover, the direct evidence for MMP-7 stimulation of myofibroblast cell proliferation comes from studies in vitro, and there is now a need to extend this work to include direct studies of the effects of MMP-7 on myofibroblast cell numbers in vivo.

Taken as a whole, the present data identify MMP-7 as a previously unsuspected target of gastrin that is increased in hypergastrinemic conditions. We also show that in at least one condition, ECL cell carcinoid tumors, MMP-7 is a potential regulator of the stromal compartment via activation of MAPK and PI3K pathways. Previous studies (13, 20) have established that MMP-7 degrades IGFBP-5 released by myofibroblasts, thereby liberating IGF-II. Moreover, there is already evidence that in different cellular systems, gastrin is able to activate distinct paracrine pathways stimulating EGF receptors (29) and FGF receptors (22). However, whereas previous studies were based on a cancer cell line (AGS cells), the present work focused on primary epithelial cells and myofibroblasts. The relative importance of these different pathways in the normal stomach remains to be established; indeed, it should be noted that the gastric myofibroblasts used in our study were obtained from tissue adjacent to gastric tumors and may well not be representative of myofibroblasts in normal gastric tissue. Even so, the present study indicates that, in the future, it will be profitable to determine the precise molecular mechanisms by which MMP-7 contributes to the maintenance of the stromal compartment in epithelial tumors.

Fig. 6. Increased α-SMA abundance in both humans and mice with hypergastrinemia. A: Western blot of the gastric corpus from both PA and MEN-1 patients and an ECL cell carcinoid biopsy (N) from a PA patient showed increased α-SMA compared with the control sample. B: Quantification of Western blots showed increased α-SMA in both the gastric corpus of PA (n = 11) and MEN-1 (n = 6) patients and in the ECL cell carcinoid tumors itself (n = 6) compared with controls (n = 14) normalized to GAPDH. *P < 0.05 vs. control. C: Western blot of the gastric corpus from InsGas mice showed increased α-SMA compared with FVB/N mice. D: Quantification of Western blots showed increased α-SMA in the gastric corpus of InsGas (n = 5) compared with FVB/N (n = 7) mice normalized to GAPDH. *P < 0.05 vs. FVB/N mice.

Fig. 7. Conditioned medium (CM) from gastrin-treated human gastric glands stimulates proliferation of human gastric myofibroblasts by MMP-7 via MAPK and phosphatidylinositol 3-kinase (PI3K). A: Proliferation of human gastric myofibroblasts was stimulated by coculture with CM media from G17 (1 nM, 20 h)-treated gastric glands. Treatment of glands with neutralizing mouse monoclonal MMP-7 antibody (Ab) partially inhibited proliferation. Treatment of myofibroblasts with nonimmune mouse IgG had no effect (n = 3). B: Stimulation of [3H]thymidine incorporation by recombinant MMP-7 (3 U/ml) in primary human gastrin-treated myofibroblasts was virtually abolished by the MEK inhibitor U-0126 and the PI3K inhibitor LY-294002. C and D: Western blots showed increased abundance of phospho-p42/44Erk 10 min after the addition of recombinant MMP-7 (C) and increased abundance of phosphorylated Akt (p-Akt) 240 min after the incubation with recombinant MMP-7 (D), **, significant differences between MMP-7-treated and control samples. *Decreases in MMP-7-stimulated proliferation following application of the compound specified.
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GASTRIN-REGULATED MMP-7 EXPRESSION IN THE STOMACH


