Hepatocyte growth factor activates several transduction pathways in rat pancreatic acini


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Abstract

The receptor of hepatocyte growth factor (HGF), c-met induces different physiological responses in several cell types. Little is known about the role of HGF in exocrine pancreas. However, abnormal HGF signaling has been strongly implicated in pancreatic tumorigenesis and association of HGF with pancreatitis has been demonstrated. We have studied the presence of c-met and activation of their intracellular pathways associated in rat pancreatic acini in comparison with cholecystokinin (CCK) and epidermal growth factor (EGF). C-met expression in rat exocrine pancreas was identified by immunohistochemistry and immunoprecipitation followed by Western analysis. Tyrosine phosphorylation of c-met is strongly stimulated as well as kinase pathways leading to ERK1/2 cascade. HGF, but not CCK or EGF, selectively caused a consistent increase in the amount of p85 regulatory subunit of PI3-K present in anti-phosphotyrosine immunoprecipitates. Downstream of PI3-K, HGF increased Ser473 phosphorylation of Akt selectively, as CCK or EGF did not affect it. HGF selectively stimulated tyrosine phosphorylation of phosphatase PTP1D. HGF failed to promote the well-known CCK effects in pancreatic acini such as amylase secretion and intracellular calcium mobilization. Although HGF shares activation of ERK1/2 with CCK, we demonstrate that it promotes the selective activation of intracellular pathways not regulated by CCK or EGF. Our results suggest that HGF is an in vivo stimulus of pancreatic acini and provide novel insight into the transduction pathways and effects of c-met/HGF in normal pancreatic acinar cells.

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1. Introduction

Hepatocyte growth factor (HGF) was identified independently as both a growth factor for hepatocytes and a fibroblast-derived cell motility factor, scatter factor (SF). It binds to its receptor tyrosine kinase (c-met), which was discovered as an activated oncogene [1]. In vivo, the expression of c-met is predominately found in cells of epithelial origin while HGF/SF expression is usually restricted to fibroblasts and stromal cells in the surrounding mesenchyme [2]. Activation of signal transduction pathways in response to HGF stimulation is mediated in part by autophosphorylation of specific tyrosine residues within the intracellular region of c-met. Phosphorylation of Tyr1234 and Tyr1235 located within the activation loop of the tyrosine kinase domain activates the intrinsic kinase activity of the receptor, while phosphorylation of Tyr1349 and Tyr1356 in the C-terminus activates a multisubstrate docking site conserved among Met family members [2]. This multisubstrate docking site mediates the binding of several adapter proteins such as Grb2, Shc, Crk/CRKL and Gab1 which in turn recruit several signal transducing proteins to form signaling complexes [2].

HGF/SF induces different cellular responses depending on the cellular context: proliferation, cell dissociation and motility, cell movement through the extracellular matrix, angiogenesis, tissue regeneration, cell differentiation and
morphogenesis [2]. Whereas normal HGF signaling is involved in many aspects of embryogenesis, abnormal HGF signaling has been strongly implicated in tumorigenesis, particularly in the development of invasive and metastatic phenotypes [2]. Recently, c-met mutations have been identified in patients with several types of carcinoma [2] and cell lines generated that express mutated Met receptors that mimic those found in human cancers from tumors that are both highly invasive and metastatic [3,4]. Additionally, several groups have reported that c-met and/or HGF expression is increased in a variety of human tumors (reviewed by Jeffers et al. [5]).

Although the physiological role of HGF has been well studied in epithelial cell types, to date, little is known about the biological responses evoked by HGF in pancreas. Previous data in the rat demonstrated that the period of maximal expression of HGF and its receptor matches the period of rapid proliferation of the rat gland, including endocrine and exocrine pancreas [6]. It has been proposed that HGF and c-met could be implicated in normal rat pancreas development and maturation [6]. Additionally Vila et al. [7,8] reported HGF as a potent mitogen for normal human exocrine pancreatic cells in vitro, and suggested that HGF and c-met may play a role in growth regulation of normal human pancreas.

It has been demonstrated that a concomitant HGF [9] and c-met [9–12] overexpression in human pancreatic cancers, which suggests that there is excessive activation of c-met-dependent signaling pathways, may have an important pathogenic role during the early stages of neoplastic promotion and also contribute to pancreatic cancer growth in vivo.

On the other hand, HGF and c-met mRNA expression were dramatically increased during chronic [13] and cerulein-induced acute pancreatitis [14] in the rat, which suggests an essential role of this growth factor in the morphological changes during the development of these diseases of the exocrine pancreas. The regenerative process after acute inflammation of the pancreas also appears to involve HGF action [15].

It is proposed that c-met likely activates similar signal transduction pathways to promote invasive growth during both embryonic development and tumor progression, and additional effects during and after pancreatitis. Therefore, insights into the signaling requirements to promote its physiological role in normal pancreatic tissue will likely be applicable to its associated events in both tumorigenesis and pancreatitis. Thus the aim of this study was to analyze intracellular signaling pathways activated by HGF and its receptor c-met in rat primary pancreatic acini. C-met expression in rat pancreatic acini was studied and HGF-induced signal transduction cascades were compared with those activated by another growth factor, epidermal growth factor (EGF), and by the main gastrointestinal hormone that regulates pancreatic acinar cell function, cholecystokinin (CCK).

2. Materials and methods

2.1. Materials

Male Wistar rats (150–200 g) were obtained from the animal farm, Faculty of Veterinary, UEX, Spain; purified collagenase (CLSFA) from Worthington Biochemicals, Freehold, NJ; COOH-terminal octapeptide of CCK-8 and recombinant Hepatocyte Growth Factor (human) from Bachem AG, Bubendorf, Switzerland; EGF from Calbiochem, La Jolla, CA, anti-phosphotyrosine monoclonal antibody, (PY20) and anti-PTP1D monoclonal antibody from Transduction Laboratories, Lexington, KY; anti-phosphotyrosine monoclonal antibody (4G10) and anti-rat PI3-Kinase antibody (rabbit whole antiserum) from Upstate Biotechnology Inc., Lake Placid, NY; anti-phospho-Akt polyclonal antibody, anti-Akt polyclonal antibody, anti-phospho ERK1/2 polyclonal antibody and anti-ERK1/2 polyclonal antibody from Cell Signaling Technology, Beverly, MA; anti-c-met polyclonal antibody from Santa Cruz Biotechnology, Inc., Santa Cruz, CA; Tris/Glycine/SDS buffer (concentrated 10×) and Tris/Glycine buffer (concentrated 10×) from BIO-RAD, Richmond, CA; Hyperfilm ECL from Amersham, Arlington Heights, IL; enhanced chemiluminescence detection reagents and anti-mouse IgG-horseradish peroxidase conjugate from Pierce, Rockford, IL, and nitrocellulose membranes from Schleicher & Schuell, Keene, NH.

2.2. Methods

2.2.1. Rat pancreatic acini isolation

Dispersed rat pancreatic acini were prepared according to modifications [16] of the procedure previously published [17]. Unless otherwise stated, the standard incubation solution contained (mM): HEPES (25.5) [pH 7.4]; NaCl (98); KCl (6); NaH2PO4 (2.5); sodium pyruvate (5); sodium fumarate (5); sodium glutamate (5); glucose (11.5); CaCl2 (0.5); MgCl2 (1); glutamine (2); albumin 1% (w/v); trypsin inhibitor 1% (w/v); vitamin mixture 1% (v/v); and amino acid mixture 1% (w/v) performed with 100% O2 as the gas phase.

2.2.2. Immunoprecipitation

Pancreatic acini isolated from one rat were preincubated with standard incubation solution for 3 h at 37 °C. Acini were then incubated with agonists at concentrations and times indicated, washed with phosphate buffered saline (PBS) with 0.2 mM Na3VO4, and sonicated for 5 s at 4 °C in lysis buffer. Lysates were centrifuged at 10,000 × g for 15 min. Protein concentration in supernatant was standardized to 500 μg/ml and 1 ml was incubated overnight at 4 °C with anti-phosphotyrosine (PY20) mAb (4 μg) or anti-c-Met pAb (4 μg), bridging antibody (4 μg) and 25 μl of protein A-agarose. Immunoprecipitates
were washed three times with PBS and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting.

2.3. Western blotting

Proteins in total cellular lysates, immunoprecipitates or pancreatic homogenates were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Western blotting was realized as previously described [18,19] using the following primary antibodies concentrations: 0.2 µg/ml anti-c-met pAb or 0.25 µg/ml anti-phosphotyrosine mAb (4G10) or 1:1000 anti-phospho-ERK1/2 pAb or 1:500 anti-ERK1/2 pAb or 1:1000 anti-phospho-Akt pAb or 1:500 anti-Akt pAb or 1:750 anti-rat-PI3-Kinase whole antiserum or 0.25 µg/ml anti-PTP1D pAb.

2.3.1. Immunohistochemistry

 Fragments of surgically resected rat pancreas were fixed in 3% paraformaldehyde in PBS (0.1 M, 7.4 pH) for 15 min and in 70 °C ethanol for 15 min, dehydrated in ethanol and paraffin embedded. Transverse sections were cut at 9 µm. Immunohistochemistry was carried out using the peroxidase–antiperoxidase (PAP) method. Briefly, after treatment with normal goat serum (1:30 diluted), sections were incubated with anti-c-met primary antibody (1:250). The goat anti-rabbit PAP-conjugated secondary antibody was diluted 1:500 and a solution of diaminobenzidine (DAB) (0.025% DAB and 0.015% H2O2) was used as chromogen to visualize peroxidase. Finally, sections were washed, dehydrated in ethanol and mounted in Depex. Control procedures included a second-level control and the absorption control. In the first procedure, the primary antibody was omitted in some sections. In the second control procedure, sections were immunostained using the primary antibody previously incubated for 2 h with the antigen used as immunogen (1:5 antibody/antigen). These control sections showed no immunoreactive product (Fig. 1c).

2.3.2. Intracellular Ca2+ measurements

After isolation, pancreatic acini were resuspended in Na-HEPES medium containing: NaCl 130 mmol/l, KCl 5 mmol/l, HEPES 20 mmol/l, KH2PO4 1.2 mmol/l, d-glucose 10 mmol/l, CaCl2 1 mmol/l, trypsin inhibitor 0.1 mg/ml, and loaded with 2 µM fura 2/AM for 30–40 min at 25 °C. After loading, the cells were washed and used within the next 3 h. For measurements of [Ca2+], 2 ml of acinar cells were placed in quartz cuvettes in a spectrofluorophotometer RF-5001PC (Shimadzu Europe GmbH, Duisburg, Denmark). Fluorescence was measured at 500 nm after excitation at 340 nm (F340) and 380 nm (F380). Stimuli were added directly to cell suspensions in the cuvette to the final concentration indicated. At the end of each experiment, maximum and minimum fluorescence ratio values were obtained by the respective addition of Triton X-100 (20 µl of a 10% stock solution) and EGTA (150 µl of a 0.5 mol/l stock solution in Tris–HCl 3 M, pH 8.9). Values for [Ca2+] were calculated, after subtraction of background auto fluorescence, as described previously [20].

2.3.3. Amylase release

Amylase release was measured using the procedure published previously [17,21]. Briefly, acini from one pancreas were suspended in 100 ml of standard incubation solution. Acini were incubated with agonists at 37 °C for 30 min. Amylase activity was determined using the Phadebas reagent (Pharmacia) and was expressed as the percentage of cellular amylase released into the extracellular medium during incubation.
2.3.4. Statistics

All data provided are reported as mean ± standard error (S.E.).

3. Results

3.1. Identification of c-met in rat pancreatic acini and its regulation by HGF stimulation

The immunohistochemical study showed an overall and clear anti-c-met immunoreactivity in the pancreatic tissue (Fig. 1a). No clear differences on anti-c-met immunoreactivity were observed when compared both fixative procedures. Acinar cells and islet cells showed anti-c-met immunoreactivity, although the endocrine islet cells appeared a little less immunostained. The epithelial cells of interlobular ducts also showed anti-c-met immunoreactivity. The stronger immunoreactivity was localized in the periphery of acinar cells (Fig. 1b); on the contrary, the nucleus always appeared as an immunonegative structure (Fig. 1a).

To study the expression of c-met in rat pancreatic acini, immunoprecipitates from the whole acinar lysates using a c-met antibody were separated in SDS-PAGE and analyzed by Western blotting using anti-c-met-specific antibody. Results shown in Fig. 2 (left panel) demonstrated a unique and specific band at the suitable molecular weight of the c-met (145 kDa).

To investigate whether c-met was regulated by tyrosine phosphorylation after HGF stimulation, we first immunoprecipitated and analyzed by Western blotting the whole cell lysates using the anti-c-met antibody and anti-phosphotyrosine antibody. Results shown in Fig. 2 (middle panel) demonstrated a unique and specific band at the suitable molecular weight of the c-met (145 kDa).

Fig. 3. c-Met is tyrosine phosphorylated following HGF stimulation. Rat pancreatic acini were treated with vehicle or HGF at concentration and time indicated, and then lysed. Whole cell lysates were immunoprecipitated with anti-phosphotyrosine antibody and Western blotting analysis was performed using anti-c-met antibody. In panel A, the upper panel shows a representative experiment with HGF at the indicated times. Values shown in the bottom graph are mean ± S.E. of four independent experiments. Quantification of bands was performed by scanning densitometry. Panel B, results are shown from one experiment where acini were incubated with different concentrations of HGF, which is representative of three others. Panel C, effect of HGF, CCK-8 or EGF on c-met tyrosine phosphorylation: acini were incubated with HGF (1 nM, 5 min), or CCK (10 nM, 2.5 min) or EGF (10 nM, 1 min) and the results shown are representative of four independent experiments.
precipitated the receptor from the acinar lysates and then analyzed by Western blotting using a specific anti-phosphotyrosine antibody 4G10 (Fig. 2, middle panel). In unstimulated conditions, there is no visible tyrosine phosphorylation of the c-met in rat pancreatic acini (lane 1), while 1 nM HGF treatment promoted a marked increase in tyrosine phosphorylation of its own receptor (lane 2). Similar results were observed when antibodies are used in the reverse order, we first immunoprecipitated phosphotyrosine-containing proteins from the acinar lysates using PY20 antibody. Immunopurified proteins were subsequently analyzed by Western blotting using the specific anti-c-met antibody (Fig. 2, right panel).

### 3.2. Tyrosine phosphorylation of c-met induced by HGF is time- and concentration-dependent

In rat pancreatic acini c-met tyrosine phosphorylation induced by 1 nM HGF was time-dependent (Fig. 3, panel A) with an increase detected within 1 min and a maximum reached within 5 min. The extent of phosphorylation remained elevated for at least until 30 min after the addition of HGF. The tyrosine phosphorylation state of the receptor induced by HGF in pancreatic acini was dose-dependent (Fig. 3, panel B) with a half-maximal effect at about 0.1 nM. A detectable increase was seen after incubation with 0.03 nM HGF for 5 min (lane 2) and the maximum effect was observed at 1 nM HGF (lane 7).

Tyrosine phosphorylation of c-met specifically occurred as a consequence of the addition of its ligand HGF, because with other well-known stimuli of pancreatic acinar cells such as CCK or EGF there was no appreciable effect (Fig. 3, panel C).

### 3.3. HGF and intracellular calcium mobilization in rat pancreatic acini

As a secretor epithelial type, pancreatic acinar cells possess a physiological function that is critically dependent on the intracellular calcium concentration [22]. We investigated whether HGF treatment affects this intracellular messenger by determining the intracellular calcium concentration [19,20]. As seen in Fig. 4, HGF stimulation of pancreatic acini did not cause any effect on the intracellular calcium levels, whereas CCK mobilized intracellular calcium in the same cells under the same experimental conditions.

### 3.4. HGF does not promote enzyme secretion in pancreatic acinar cells

To investigate whether c-met activation in pancreatic acinar cells is involved in enzyme secretion, we studied its effects on amylase release. Pancreatic acinar cells responded to CCK stimulation by releasing amylase into the medium in a concentration-dependent manner, whereas HGF did not have any effect on the levels of amylase released at any concentration tested (Fig. 4).

### 3.5. Activation of intracellular pathways by HGF stimulation in rat pancreatic acinar cells

HGF stimulation promoted the rapid phosphorylation of the p44 and p42 ERK1/2 in pancreatic acini (Fig. 5, panel A). Phosphorylation of ERK1/2 was time- and concentration-dependent after HGF stimulation (Fig. 5, panels A and B). A detectable increase was observed at 1 min, whereas the maximal phosphorylation degree was obtained after 2.5 min incubation with HGF (Fig. 5, panel A). A visible effect was observed with 0.06 nM HGF and the maximal phosphorylation was achieved with 0.3–0.6 nM HGF (Fig. 5, panel B). The extent of phosphorylation of ERK1/2 promoted by HGF was similar to that obtained with a maximally effective concentration of CCK in these cells and higher than that seen with EGF (Fig. 5, panel A).
Stimulation of pancreatic acinar cells with HGF led to a rapid and marked increase in the amount of p85 regulatory subunit of the PI3-K present in anti-phosphotyrosine immunoprecipitates (Fig. 6). The level of p85 subunit associated with the phosphotyrosine immunoprecipitates was dependent of the incubation time, being detectable at 1 min (4-fold increase) and remains elevated above control levels (3-fold increase) until at least 30 min (Fig. 6, panel A). This effect of HGF on the regulatory subunit of the PI3-K was concentration-dependent (Fig. 6, panel B) and was exclusively linked to HGF stimulation as maximal concentrations of CCK and EGF did not promote any effect in the regulatory subunit associated with the anti-phosphotyrosine immunoprecipitates (Fig. 6, panel C).

Fig. 6. HGF stimulation increases the amount of p85 subunit (PI3K) in anti-phosphotyrosine immunoprecipitates. Rat pancreatic acini were treated for indicated times with no addition or with indicated HGF concentration and lysed. Lysates were immunoprecipitated with anti-phosphotyrosine PY20 mAb. Resulting immunocomplexes were analyzed by Western blotting with anti-p85 subunit antibody. Panel A, time course of association of p85 with tyrosine phosphorylated proteins. The upper panel is a representative experiment showing a time-dependent effect. Lower panel: quantification of bands was performed by scanning densitometry. Values shown at the bottom graph are mean ± S.E. of four independent experiments. Panel B, HGF concentration-dependence of p85 association. Acini were incubated 2.5 min with indicated HGF concentrations and the results shown are representative of four independent experiments. Panel C, ability of HGF, CCK-8 or EGF to cause p85 association. Acini were incubated with HGF (1 nM, 2.5 min), or CCK (10 nM, 2.5 min) or EGF (10 nM, 1 min) and the results shown are representative of four independent experiments.
HGF treatment of pancreatic acinar cells promoted a rapid increase in the phosphorylation of Serine 473 of the protein serine/threonine kinase B or Akt (Fig. 7, panel A). This effect was time-dependent (Fig. 7, panel A) and concentration-dependent (Fig. 7, panel B) and was specific for HGF treatment, as CCK or EGF did not cause any effect on the phosphorylation of Akt (Fig. 7, panel C). In these conditions, the level of Akt protein remained unaffected (Fig. 7, panel A).

The tyrosine phosphatase PTPD1 was itself tyrosine phosphorylated in pancreatic acini following HGF treatment in a time- and dose-dependent manner (Fig. 8). The increase...
in PTPD1 tyrosine phosphorylation was rapid (detected within 1 min) as seen in Fig. 8 (panel A). Detectable phosphorylation was seen with 0.06 and 0.3 nM HGF and caused a maximal 4-fold increase (Fig. 8, panel B). This effect was exclusive to HGF as maximal concentrations of CCK and EGF did not cause any effect on its tyrosine phosphorylation levels (Fig. 8, panel C).

4. Discussion

The expression of c-met has been predominately found in cells of epithelial origin [2]. C-met expression in rat pancreas was originally described by RT-PCR and follows the rate of pancreatic cell proliferation with the maximal matching a period of fetal rapid proliferation of the pancreatic gland [6]. The study was performed using total RNA isolated not only from exocrine but also endocrine pancreas. In the present study, we have also demonstrated the presence of c-met (Met) in adult rat pancreas by immunohistochemistry. This agrees with previous results in the literature. Anti-c-met immunoreactivity has been demonstrated in fetal [23] and adult [13] pancreatic epithelial cells. Moreover, we have isolated rat pancreatic acinar cells and confirmed the expression of c-met by immunoprecipitation followed by Western blot with a specific antibody.

C-met is a prototypic member of a small subfamily of receptor tyrosine kinase (RTKs) that can induce different physiological responses in several cell types. Activation of signal transduction pathways in response to HGF stimulation is mediated in part by autophosphorylation of specific tyrosine residues within the intracellular region of c-met [2]. We have demonstrated in this study that HGF treatment of rat pancreatic acinar cells promoted the tyrosine phosphorylation of c-met in a time- and concentration-dependent manner. Transactivation of growth factor receptors such as EGF has been described in response to stimulation of a different receptor in the same cells [24]. Our results show that tyrosine phosphorylation of c-met was only achieved after HGF stimulation but not after another growth factor, EGF or the main physiological regulator of these cells, CCK. These results show that there is no transactivation of c-met promoted by CCKA receptor or EGF receptor activation in rat pancreatic acini. Additionally, our results support the idea that c-met mediates intracellular signaling in pancreatic acinar cells at least in part by autophosphorylation of specific tyrosine residues. It is known that tyrosine phosphorylation of Tyr1234 and Tyr1235, located within the kinase domain, activates the intrinsic kinase activity of c-met receptor, while phosphorylation of Tyr1349 and Tyr1356 in the C-terminus activates a multisubstrate docking site that mediates the binding of several adapter proteins. One of these known adapter proteins is Grb2, which in turn recruits the signal transducing protein SOS to activated Met to induce Ras-ERK1/2 signaling [2]. In this regard, results from this study show that HGF stimulation of rat pancreatic acinar cells rapidly leads to phosphorylation and in turn activation of p44 and p42 ERK1/2. HGF is able to induce this intracellular signal cascade in a comparable degree to that caused by the pancreatic secretagogue CCK. However, HGF stimulation of ERK1/2 is stronger than the stimulation caused by another growth factor, EGF.

PI3-K, a heterodimer with an 85-kDa regulatory subunit and a 110-kDa catalytic subunit, is activated by a variety of stimuli and is involved in the triggering of many different cell activities. Our results demonstrated that the amount of the p85 regulatory subunit of the PI3-K is clearly increased in the anti-phosphotyrosine immunoprecipitates following HGF stimulation. This data can be explained at least by three different hypotheses. (i) The p85 regulatory subunit can be phosphorylated at tyrosine residues after HGF stimulation [25]. In this regard, there are discrepancies in the literature regarding the tyrosine phosphorylation of p85 following HGF stimulation [26,27]. (ii) A second explanation implies that the p85 regulatory subunit of PI3-K can be associated directly with c-met via a phosphotyrosine binding domain (PTB) located within the multisubstrate binding site originated after HGF stimulation at the C-terminus [28]. According to this explanation, in our study, HGF stimulation in pancreatic acinar cells promotes that autophosphorylated c-met is immunoprecipitated with the anti-phosphotyrosine antibody, as demonstrated in Fig. 3, and so is the p85 bound to it via its PTB domain. (iii) A third plausible explanation involves Gab1 (Grb2-associated binding protein-1), which is an adapter protein recruited by Grb2 after HGF stimulation. Gab1 is a protein containing a N-terminal pleckstrin homology (PH) domain that binds the membrane lipid phosphatidylinositol 3,4,5-trisphosphate (PIP3), product of the PI3-K. Following HGF treatment Gab1 is tyrosine phosphorylated which induces its direct association with several transducers including PI3-K [29]. Then, in our model, following HGF treatment PI3-K would co-immunoprecipitate with HGF-induced tyrosine phosphorylated proteins including Gab1.

Independently of how the p85 subunit becomes a signaling molecule, our results clearly show that PI3-K is involved in HGF-induced intracellular signaling in rat pancreatic acini. It is well demonstrated that downstream of PI3-K, Akt (or PKB) is a key molecule. Activation of Akt is dependent on the sequential phosphorylation of the molecule at two specific sites, Thr 308 and Ser 473. Full activation is achieved when Ser 476 is phosphorylated. In our study, we have found that HGF activates intracellular signaling leading to Akt phosphorylation at the Ser 473.

One of the SH2-containing proteins recruited by c-met is the tyrosine phosphatase PTP1D (also called SHPTP2, PTPT2C, SHPTP3, Syp, or SHP2) and upon c-met activation, PTP1D becomes phosphorylated on tyrosines and serves as an adapter protein (reviewed by Furge et al. [2]). Moreover, downstream from c-met receptor, through the association of PTP1D with Gab1 [2,30] previous studies have identified this phosphatase as both positive modulator of ERK1/2.
activity and selective activator of PI3-Kinase [30]. In our study in rat pancreatic acini, we have found that c-met activation, but not EGF-receptor activation, selectively leads to tyrosine phosphorylation of the protein phosphatase PTP1D in a time- and dose-regulated manner.

Our data demonstrated that HGF/c-met signaling is not involved in one of the main pancreatic acinar cell function, secretion of digestive enzymes. The biological role of HGF in normal exocrine pancreas is unclear. Previous data suggested that, according to their expression levels, HGF and its receptor could be implicated in normal pancreas development and maturation because their period of maximal expression matches the period of rapid proliferation of the endocrine and exocrine pancreas [6]. In pancreatic AR42J cells derived from rat pancreatic acinar tumors, HGF induced differentiation via ERK1/2 pathway [31]. It is interesting to note that in our study the comparison of those intracellular signal cascades activated by either growth factors HGF and EGF or the pancreatic secretagogue CCK, points at HGF as a stimulus comparable to CCK in activating the ERK1/2 cascade in rat pancreatic acini. Additionally, our study reveals that HGF is a more potent stimulus for pancreatic acinar cells than EGF, which has been previously used as the typical growth factor in these cells [32,33]. It is well known that PI3-K and Akt signaling molecules are involved in several biological functions, including cell survival and proliferation. These biological functions have been demonstrated for HGF only in fetal and early phases of postnatal pancreas development [6]. In our study, HGF was an exclusive stimulus in rat pancreatic acinar cells regulating the PI3-K/Akt cascade because it was not regulated by CCK or EGF. Therefore, the suggested role in pancreatic acinar cell survival and proliferation during development [6] could be potentially extended to adult age, considering the demonstrated ability of HGF to induce PI3-K/Akt and possibly other involved pathways. Further work is necessary to demonstrate this suggested role of HGF in pancreatic acinar cells.

In summary, the data presented in this study demonstrate the expression of the c-met in pancreatic acinar cells and describe a variety of intracellular signaling pathways activated by HGF in vivo. Our results demonstrate that HGF intracellular signaling in pancreatic acinar cells involves autophosphorylation of c-met, ERK1/2 activation, tyrosine phosphorylation of the phosphatase PTP1D, modulation of the p85 regulatory subunit of PI3-K and subsequent activation of its downstream target Akt. Comparing the degree of activation of the different signaling pathways, our results reveal that HGF is a stronger stimulus than EGF in pancreatic acini and its level of stimulation is comparable to the degree of stimulation described for CCK on ERK1/2 activation. The role of HGF has already been extensively investigated in pancreatic carcinoma cells. However, there is no data in the literature to date regarding the function and signaling pathways of HGF in normal pancreatic acinar cells. Our study provides novel insight into the signaling requirements coupled to c-met to promote HGF’s role in vivo in normal pancreatic acinar cells.

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