Stimulation of DNA Synthesis and Cell Proliferation of Human Mammary Myoepithelial-like Cells by Hepatocyte Growth Factor/Scatter Factor Depends on Heparan Sulfate Proteoglycans and Sustained Phosphorylation of Mitogen-activated Protein Kinases p42/44*

Nicolas Sergeant‡, Malcolm Lyon§, Philip S. Rudland‡, David G. Fernig‡, and Maryse Delehedde‡¶

From the ‡School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB and the §Cancer Research Campaign Department of Medical Oncology, University of Manchester, Christie Hospital NHS Trust, Wilmslow Road, Manchester M20 4BX, United Kingdom

Hepatocyte growth factor/scatter factor (HGF/SF) is a heparan/dermatan sulfate-binding growth factor produced by stromal cells that acts as a paracrine effector on neighboring epithelia. HGF/SF stimulated DNA synthesis in human mammary (Huma) 109 myoepithelial-like cells grown on collagen I and fibronectin substrata but not when grown on plastic. Dual phosphorylation of mitogen-activated protein kinases (p42/44MAPK) was required for this stimulation of DNA synthesis. In Huma 109 cells cultured on plastic, HGF/SF stimulated a transient phosphorylation of p42/44MAPK, which reached a maximum at 10 min after addition of the growth factor and returned to near basal levels after 20 min. In contrast, the phosphorylation of p42/44MAPK stimulated by HGF/SF in cells cultured on collagen I or fibronectin was sustained over 45 min. In Huma 109 cells deficient in sulfated glycosaminoglycans, HGF/SF failed to stimulate p42/44MAPK phosphorylation or DNA synthesis on any substratum, even when soluble heparan sulfate proteoglycans purified from the cells or from the culture medium were added. However, HGF/SF stimulated DNA synthesis and a sustained phosphorylation of p42/44MAPK in sulfated glycosaminoglycan-deficient Huma 109 cells plated on a substratum of medium HS proteoglycans but not cell HS proteoglycans. The HGF/SF-induced proliferation is thus highly dependent on heparan sulfate proteoglycans in myoepithelial-like cells.

Hepatocyte growth factor/scatter factor (HGF/SF) is synthesized by cells in the stroma and stimulates the growth, motility, and morphogenesis of epithelial cells. It is thus an important mediator of stromal-epithelial communication (1, 2). In the case of the mammary gland, HGF/SF is produced by stromal fibroblasts, and it has been shown to have a potent morphogenic activity on cultured mammary epithelial cells and epithelial rudiments (3, 4). HGF/SF has two distinct types of receptors, a transmembrane tyrosine kinase, which corresponds to the product of the c-met protooncogene and proteoglycans bearing the glycosaminoglycans heparan sulfate (HS) or dermatan sulfate (5–7). The binding site for HGF/SF in HS is distinct from those of other HS-binding growth factors such as fibroblast growth factor-2 (FGF-2) (7–10). After HGF/SF binding, which is thought to induce c-met dimerization, the c-met receptor undergoes autophosphorylation on a number of tyrosine residues. The latter provide docking sites for proteins with phosphotyrosine recognition domains, which recruit and activate a number of signaling pathways (11–14). One such pathway involves the mitogen-activated protein kinases (MAPKs) (15, 16). The MAPK family includes extracellular signal-regulated kinases 1 and 2, also called p44 and p42, respectively. Phosphorylation of p42/44MAPK on both threonine and tyrosine in the TEY motif by a cytoplasmic dual specificity MAPK kinase, MEK-1, activates these kinases (16–18). The activated p42/44MAPK then phosphorylate a number of cellular substrates, which include Ser/Thr effector kinases. If it is to stimulate cell division or cell migration, HGF/SF must interact with a dual receptor system consisting of the c-met receptor and the HS (or dermatan sulfate) proteoglycan receptor (5, 10, 19, 20). However, the mechanism by which the HS receptors or the more recently described dermatan sulfate receptors contribute to the delivery of growth-stimulatory signals by HGF/SF is unclear. The resting mammary ductal epithelium is composed of two main cell types: epithelial cells, which line the lumen of the ducts, and myoepithelial cells, which lie between the epithelial cells and the basement membrane (21). Because HGF/SF is a product of the mammary stroma that acts on the epithelium (10, 22, 23), it will first come into contact with the myoepithelial cells. We have therefore examined how HGF/SF regulates in vitro the proliferation of human mammary myoepithelial-like cells (Huma 109). When cells were cultured on plastic, HGF/SF did not stimulate DNA synthesis and only elicited a transient phosphorylation of p42/44MAPK. DNA synthesis was stimulated by HGF/SF when Huma 109 cells were cultured on collagen I or on fibronectin, and it was always associated with a sustained phosphorylation of p42/44MAPK. We have also demonstrated that the HS receptor is required for HGF/SF-induced proliferation. Indeed HGF/SF only stimulated DNA synthesis...
and sustained phosphorylation of p42/44MAPK in chlorate-treated cells, when they were cultured on a substratum of medium-derived HS proteoglycans.

**EXPERIMENTAL PROCEDURES**

**Materials**—FGF-2 and HGF/SF were obtained from R & D Systems (Abingdon, UK) and mouse epidermal growth factor (EGF) was obtained from Pepayn (Liverpool, UK). Reagents for electrophoresis were purchased from Bio-Rad, and other reagents were purchased from BDH (Poole, UK). Cell culture reagents, including sulfate-free Dulbecco’s modified Eagle’s medium, were obtained from Life Technologies, Inc. PD98059 was from Calbiochem (Nottingham, UK). Antibodies against the ubiquitin protein were purchased from Cell Signaling (Buckingham, UK), whereas antibodies against the MAPKs were purchased fromp (Burlington, MA). Secondary peroxidase-labeled antibodies to IgG were from Amersham Pharmacia Biotech. Chrondroitinase ABC (EC 4.2.2.4), fibronectin, DNase I, and bovine pancreatic RNase were from Sigma. Sialidase enzyme collection (EC 3.2.1.18) and α-fucosidase (EC 3.2.1.51) were from Oxford GlycoScience (Oxford, UK). Endo-β-galactosidase (EC 3.2.1.103) and heparinase III were obtained from Roche Molecular Biochemicals and from Grampian Enzymes (Orkney, UK), respectively. Collagen I from rat tail was from Beckton Dickinson (London, UK).

**Cell Culture and DNA Synthesis Assays—**Huma 109 myoepithelial-like cells were cultured as described previously (24). For DNA synthesis assays, cells were seeded into 24-well plates and were allowed to attach for 24 h. They were then washed twice in PBS and grown in serum-free medium containing 0.2% (w/v) bovine serum albumin for 24 h before adding growth factors. [methyl-3H]Thymidine (40 μCi/ml at 0.8 μCi/ml) (ICN, Basingstoke, UK) was added directly to the culture medium 18 h later. At 18 h, whole cell monolayers were all in S phase and then were capable of incorporation of [3H]thymidine. DNA was precipitated with 5% (w/v) trichloroacetic acid, and the radioactivity incorporated into DNA was determined by liquid scintillation counting. In parallel experiments, cells plated at equal density into 24-well plates were harvested after 48 h in presence of growth factors by trypsinization and subjected to a hemocytometer. Statistics were performed using Student’s t test. In some experiments, wells were first coated at room temperature with collagen I (50 μg/ml), fibronectin (10 μg/ml), or HSPGs (3 μg/ml). After 4 h, the wells were rinsed twice with PBS before seeding the cells. Sulfated glycosaminoglycan-deficient Huma 109 cells were prepared as described previously (5, 9, 25). Cells were incubated for 4 h in sulfate-free Dulbecco’s modified Eagle’s medium supplemented with 10% dialyzed fetal calf serum (v/v) and 15 mM NaClO3. Following trypsinization, the cells were seeded in 24-well plates or 10-cm dishes as described for DNA synthesis or Western blot analysis, respectively, except that sulfate-free Dulbecco’s modified Eagle’s medium supplemented with 15 mM NaClO3 was used throughout. As others have described, cells can be kept viable in sulfate-free medium containing chlorate for up to 1 month (25, 31, 32). Checks were always made on cell death and protein synthesis. DNA synthesis and cell proliferation could be induced normally in such chlorate-treated cells when they are treated with non-heparin-binding growth factors such as EGF and more commonly by adding fetal calf serum to the cells. In some experiments, cells were seeded onto culture substrata coated with HSPGs purified from the Huma 109 cells (3 μg/ml) or from the Huma 109 cell culture medium (3 μg/ml).

**Western Blotting—**Cells were seeded at equal densities in 10-cm diameter cultured dishes and treated identically as for the DNA synthesis assays up to the addition of 30 ng/ml HGF/SF in fresh serum-free medium. In parallel experiments, PD98059, an inhibitor of MEK1 (10 mM stock solution in Me2SO) was added directly to the medium at a final concentration of 50 μM and 15 min before the addition of HGF/SF. Cells were washed twice with ice-cold PBS and lysed in 300 μl of lysis buffer (50 mM Tris-HCl, pH 6.8, 2% (v/v) SDS, 0.2% (v/v) β-mercaptoethanol, protease inhibitor mixture (Roche Molecular Biochemicals), and bromphenol blue). Equal amounts of protein in the cell lysates were subjected to electrophoresis in 15% (v/v) polyacrylamide gels in the presence of SDS. Cell lysates were subjected to Western blotting as described previously (26). Finally, immunoreactive proteins were revealed with the SuperSignal chemiluminescent detection system (Pierce and Warriner, Chester, UK) on Hyperfilm (Amersham Pharmacia Biotech).

**Purification of Heparan Sulfate Proteoglycans—**HS proteoglycans were purified as described (9) with minor modifications. The medium from 90% confluent cultures of Huma 109 myoepithelial-like cells grown in 15-cm diameter dishes was collected, and the cell monolayers were rinsed twice with 10 ml of PBS, pH 6.8. The medium was clarified by centrifugation at 2000 rpm for 15 min. Cells were lysed in 10 ml of lysis buffer (PBS, pH 6.8, containing 6% urea and 0.5% (v/v) Triton X-100). The lysate was centrifuged at 15,000 rpm for 30 min. The pellet was resuspended in lysis buffer and centrifuged at 15,000 rpm for 30 min, and the two supernatants were combined. Before applying the samples onto a 20 ml DEAE Sepharose Fast Flow column (Amersham Pharmacia Biotech) equilibrated with PBS, pH 6.8 (medium samples), or lysis buffer (cell lysates), the medium was diluted with one volume of PBS, pH 6.8, and the cell lysate was diluted with 5 volumes of lysis buffer. The column was then extensively washed with 300 mM NaCl in the same buffer. Strongly anionic molecules, including proteoglycans, were eluted with 2 mM NaCl in the same buffer. A second DEAE Fast Flow column (2 ml) was used to concentrate the eluate. The 2 mM NaCl fractions were desalted on a 75-ml G-25 Sephadex (Amersham Pharmacia Biotech) column equilibrated in 100 mM NH4HCO3. After freeze-drying, the proteoglycans were treated sequentially with sialidases, chondroitinase ABC, α-fucosidase, endo-β-galactosidase, and nucleases to remove anionic contaminants (9). The proteoglycans were concentrated on a 3 ml DEAE Fast Flow column and the 2 mM NaCl fraction was desalted using a 10 ml G-25 Sephadex column, as described above. After freeze-drying, the powders were weighed, and stock solutions were made at 1 mg/ml of either medium HSPGs or cell HSPGs in PBS. Purified HSPGs were always boiled 5 min before adding to the culture medium (9).

**RESULTS**

**Effect of HGF/SF on DNA Synthesis in Huma 109 Myoepithelial-like Cells—**DNA synthesis in Huma 109 myoepithelial-like cells was stimulated by addition of fetal calf serum and EGF (Fig. 1A), as described for other myoepithelial-like cells (27). In contrast, the HS-binding growth factors FGF-2 and HGF/SF had no stimulatory effect on DNA synthesis in these cells plated on plastic (Fig. 1A). When cells were plated on fibronectin or collagen I, HGF/SF stimulated DNA synthesis by 1.5- and 3-fold, respectively (Student’s t test, p < 0.05), whereas FGF-2 remained without effect (Fig. 1B). The [3H]thymidine incorporation in untreated cells was 0.4 ± 0.1 dpm/cell (n = 3), and the effect of FGF-2 (10 ng/ml) was significant by Student’s t test (t = 5.1, p < 0.05). Results are the means ± S.E. of triplicate experiments. Ctrl, control.
midine incorporation was always higher in cells plated on substrate compared with cells plated directly on plastic (Fig. 1). In parallel experiments, changes in total cell numbers were assessed 48 h after addition of growth factors (Fig. 2A). HGF/SF induced a similar 3-fold increase in the number of cells grown on collagen I compared with cells grown on plastic (Student’s t test, p < 0.05). No change in cell morphology was observed when cells were grown on collagen I or fibronectin (data not shown). The c-met polypeptide, the receptor for HGF/SF, was clearly detected in Huma 109 cells by Western blotting using an antibody against the N terminus of the human polypeptide (Fig. 2B). As controls, the antibody also strongly detected c-met in HT29 cells, which are known to express this receptor (28), but not in T47D cells, which barely express c-met (29). The higher very faint band seen in T47D cells did not correspond to the size of c-met and hence was presumably due to cross-reacting molecules present at low levels. No change was observed in the amount of c-met polypeptide by Western blotting and in the localization of c-met by laser scanning confocal microscopy, when Huma 109 cells were grown on plastic, fibronectin, or collagen I (data not shown).

**p42/44MAPK Phosphorylation Is Activated by HGF/SF in Huma 109 Myoepithelial-like Cells—**PD98059, the well established inhibitor of MAPK kinase 1 (MEK1) (30) and also an antibody that recognized the doubly phosphorylated pTEpY motif and hence the enzymatically active forms of p42/44MAPK (17), were used to analyze the activation of the MAPK pathway in Huma 109 cells. PD98059 clearly abolished the stimulation of DNA synthesis induced by HGF/SF in cells plated on collagen I or fibronectin substrata (Fig. 3A). Thus the MAPK pathway is required for the growth-stimulatory effects of HGF/SF in myoepithelial-like cells. As shown in Fig. 3B, the addition of HGF/SF caused an increase in the phosphorylation of p42/44MAPK (detected as a doublet of 42 and 44 kDa), even though it did not stimulate DNA synthesis when cells were grown on plastic. In fact, the levels of dually phosphorylated p42/44MAPK were substantially up-regulated by HGF/SF, when cells were grown on all three substrata, but with the collagen I and fibronectin substrata giving a slightly increased response over

**FIG. 3. Effects of HGF/SF on phosphorylation of p42/44MAPK in Huma 109 cells.** A, PD98059 (50 μM), a specific inhibitor of MEK1, was added 15 min before HGF/SF. DNA synthesis was performed as described in the legend to Fig. 1. Data shown are the mean ± S.E. of triplicate experiments. B, Huma 109 cells were plated on substrata of plastic (NC), fibronectin (Fn), or collagen I (Coll I) and treated with 30 ng/ml HGF/SF for 15 min. Whole cell lysates were resolved by SDS-polyacrylamide gel electrophoresis, and doubly phosphorylated p42/44MAPK were detected by Western blotting. C, Western blot analysis of p42/44MAPK phosphorylation in Huma 109 cells grown on collagen I treated as above except that PD98059 was added, where shown, 15 min before the growth factors. Ctrl, control.

**HGF/SF Induces a Sustained Dual Phosphorylation of p42/44MAPK**

**When Huma 109 Cells Are Plated on Fibronectin and Collagen I—**We then investigated the kinetics of p42/44MAPK dual phosphorylation following the addition of HGF/SF to Huma 109 cells plated on plastic, fibronectin, or collagen I substrata (Fig. 4). When cells were grown on plastic, HGF/SF induced a rapid and transient phosphorylation of p42/44MAPK, which reached a maximum at 10 min and then decreased to near basal levels by 20 min. In contrast, the kinetics of phosphorylation of p42/44MAPK were different when the cells were grown on dishes coated with fibronectin or collagen I. When the cells were grown on fibronectin-coated dishes, HGF/SF induced a maximum phosphorylation of p42/44MAPK at 15 min, which then decreased slightly to 95% of the maximum by 30 min and then further decreased to 80% of the maximum at 45 min (Fig. 4). Moreover, this level of phosphorylation stayed at 50% of its maximum level for at least up to 2 h (data not shown). In the case of the collagen I substratum, the initial increase in phosphorylation of p42/44MAPK was faster and was detected as early as 1 min (Fig. 4). The maximum phosphorylation of p42/44MAPK was observed at 10 min and then was maintained at 90% of this...
maximal level (Fig. 4). On both collagen I and fibronectin substrata, a sustained phosphorylation of p42/44MAPK was observed after addition of HGF/SF. This result suggested that for HGF/SF to stimulate DNA synthesis in myoepithelial-like cells a sustained dual phosphorylation of p42/44MAPK was required and that this could be achieved on collagen I or fibronectin substrata but not on plastic where only a transient dual phosphorylation was elicited, which did not result in DNA synthesis.

Requirement of Immobilized HSPGs for the Stimulation of DNA Synthesis by HGF/SF in Huma 109 Cells—To determine whether proteoglycans played a role in the promotion of the growth-stimulatory activity of HGF/SF by collagen I substrata, sulfated glycosaminoglycan-deficient Huma 109 cells were generated by growing cells in medium containing sodium chlorate. Chlorate is a potent inhibitor of sulfation (31–33) widely used to address HS function (5, 9, 25, 34, 35). Following this treatment, cells were serum-starved prior to the addition of 30 ng/ml HGF/SF in the presence or in the absence of 3 μg/ml soluble medium-derived HSPGs (M-HSPG). Cells were serum-starved prior to the addition of 30 ng/ml HGF/SF in the presence or in the absence of 3 μg/ml soluble medium-derived HSPGs (M-HSPG). DNA synthesis was determined in triplicate wells as described under “Experimental Procedures.” HGF/SF induced DNA synthesis on Huma 109 cells when they were grown on 3 μg/ml medium-derived HSPGs (Student’s t test, p < 0.05). For A, as for B, except that some of the wells coated with Huma 109 medium-derived HSPGs were incubated for 2.5 h with heparinase III at 37 °C prior to seeding the cells (Core PG). DNA synthesis was determined in triplicate wells. C, chlorate-treated Huma 109 cells grown on substrata of 3 μg/ml medium-derived HSPGs (M-HSPG) or on plastic, were serum-starved prior to the addition of 30 ng/ml HGF/SF. Cells were exposed to HGF/SF for 5–30 min as indicated, and dually phosphorylated p42/44MAPK were detected by Western blotting. Ctrl, control.

Stimulation of Proliferation by HGF/SF Requires HS

HGF/SF. HSPGs purified from the Huma 109 cells (cell-derived HSPGs) or their culture medium (medium-derived HSPGs) were then added to cultures of chlorate-treated Huma 109 cells in attempt to restore the growth-stimulatory activity of HGF/SF. Addition of cell- or medium-derived HSPGs in solution (3 μg/ml) failed to restore the stimulation of DNA synthesis by HGF/SF on cells grown on collagen I (Fig. 5A). When chlorate-treated cells were plated onto wells coated with cell-derived HSPGs, no effect of addition of HGF/SF was observed on DNA synthesis. The presence of soluble medium-derived HSPGs was similarly ineffective in restoring DNA synthesis in chlorate-treated cells, when they were plated onto wells coated with cell-derived HSPGs (Fig. 5A). In marked contrast, when chlorate-treated cells were plated onto wells coated with medium-derived HSPGs, HGF/SF now stimulated DNA synthesis (Fig. 5A). A 2-fold increase in [3H]thymidine incorporation was ob-

FIG. 4. Effects of HGF/SF on phosphorylation of p42/44MAPK in Huma 109 cells on different substrata. A, cells were serum-starved for 24 h on fibronectin (Fn) or collagen I (Coll I)-coated plates. HGF/SF (30 ng/ml) was then added in serum-free medium for 1–45 min, as indicated. Cell lysates were resolved by SDS-polyacrylamide gel electrophoresis and doubly phosphorylated p42/44MAPK were detected by Western blotting. B, densitometric quantification of the level of immunoreactive dually phosphorylated p42/44MAPK as a percentage of the maximum phosphorylation observed at 10 min after the addition of HGF/SF. Ctrl, control; NC, plastic.

FIG. 5. Effect of HSPGs on the stimulation of p42/44MAPK phosphorylation and DNA synthesis by HGF/SF in chlorate-treated Huma 109 cells. A, chlorate-treated Huma 109 cells were grown on plates coated with the following: collagen I (Coll I), 3 μg/ml of purified Huma 109 cell-derived HSPGs (C-HSPG), or 3 μg/ml of Huma 109 medium-derived HSPGs (M-HSPG). Cells were serum-starved prior to the addition of 30 ng/ml HGF/SF in the presence or in the absence of 3 μg/ml soluble medium-derived HSPGs (M-HSPG). DNA synthesis was determined in triplicate wells as described under “Experimental Procedures.” HGF/SF induced DNA synthesis on Huma 109 cells when they were grown on 3 μg/ml medium-derived HSPGs (Student’s t test, p < 0.05). A, as for B, except that some of the wells coated with Huma 109 medium-derived HSPGs were incubated for 2.5 h with heparinase III at 37 °C prior to seeding the cells (Core PG). DNA synthesis was determined in triplicate wells. C, chlorate-treated Huma 109 cells grown on substrata of 3 μg/ml medium-derived HSPGs (M-HSPG) or on plastic, were serum-starved prior to the addition of 30 ng/ml HGF/SF. Cells were exposed to HGF/SF for 5–30 min as indicated, and dually phosphorylated p42/44MAPK were detected by Western blotting. Ctrl, control.
Stimulation of Proliferation by HGF/SF Requires HS

HGF/SF is a stroma-derived growth factor, which is well described to act on neighboring epithelia in the mammary gland (22, 23, 36). HSPGs that bind HGF/SF have been purified from both the cell-associated and culture medium fractions of Huma 109 myoepithelial-like cells (7). The Huma 109 myoepithelial-like cells also possess the c-met tyrosine kinase receptor for HGF/SF (Fig. 1). These results suggest that myoepithelial cells may be a target for HGF/SF, because HGF/SF is known to require a dual receptor system consisting of both its HS and c-met receptors (10). However, the mechanism of action of the HGF/SF dual receptor system and the nature of the signaling pathways activated by c-met are not well understood. Intriguingly, HGF/SF did not stimulate DNA synthesis in Huma 109 myoepithelial-like cells cultured on plastic. In contrast, when the cells were plated on collagen I or fibronectin substrata, HGF/SF was able to stimulate DNA synthesis. This result was not due to the extracellular matrix substrata altering the level of expression of c-met or its cellular location. We first showed that there were striking differences in the kinetics of the p42/44MAPK phosphorylation by HGF/SF in cells plated on plastic, collagen I, or fibronectin. In cells plated on collagen I or fibronectin substrata, HGF/SF stimulated both a sustained dual phosphorylation of p42/44MAPK and DNA synthesis. The stimulation of DNA synthesis induced by HGF/SF was clearly dependent upon the activation of the MAPK pathway because it was inhibited by PD98059 (Fig. 4) and because DNA synthesis was always associated with a sustained phosphorylation of p42/44MAPK (Figs. 3 and 5C). A number of studies have also suggested a link between sustained MAPK phosphorylation and mitogenesis with various mitogens (37–40) including HGF/SF (41, 42). We furthermore showed that sustained phosphorylation of p42/44MAPK and consequently DNA synthesis was dependent upon the anchorage of medium-derived HSPGs in chlorate-treated Huma 109 cells (Fig. 5). Chlorate was added to the culture medium of the cells as we and others have described previously (5, 9, 25, 34, 35). Chlorate has been shown to abolish sulfation on proteins and on carbohydrate residues in intact cells without inhibiting cell growth or protein synthesis (31, 32). The positive growth-stimulatory response of chlorate-treated cells with EGF or with serum (data not shown) confirms that chlorate treatment in itself is not inhibitory to DNA synthesis. It has been well described that exposure of cells to chlorate markedly reduces the binding of FGF-2 to its receptors, without affecting appreciably other cellular functions including their ability to be stimulated to synthesize DNA with non-heparin-binding growth factors such as EGF (9, 25, 34, 35, 43, 44). However, we showed that addition of the same amounts of soluble cell- or medium-derived HSPGs to the culture medium did not restore the growth-stimulatory activity of HGF/SF on chlorate-treated H109 cells. HGF/SF thus differs markedly from the FGF family of HS-binding growth factors. Moreover, not all HGF/SF-binding HS species were able to restore the growth-stimulatory activity of HGF/SF in HS-deficient Huma 109 cells. Indeed cell-derived HSPGs, which bind HGF/SF (7), were ineffective in restoring the HGF/SF effect on DNA synthesis even when they were coated on the substratum. Structural differences within the glycosaminoglycan chains may be responsible for the differences in activity of HGF/SF, and these questions will need further investigation.

Both HGF/SF and HS chains have been shown to interact with macromolecules from the extracellular matrix (6, 10, 45, 46). Binding studies in an optical biosensor have shown that collagen I and the Hep 1 domain of fibronectin do indeed bind to HS chains purified from Huma 109 cells. Furthermore, our results suggest that the growth-stimulatory activity of HGF/SF may require that the HSPG receptors be immobilized either directly or via interaction with collagen I or fibronectin at the basal membrane level and then facilitate interactions between the growth factors and their receptors. When Huma 109 myoepithelial-like cells are grown on collagen, on fibronectin, or even on medium-derived HSPGs, they had a better uptake of [3H]thymidine (Figs. 1 and 4) than when they are grown on plastic. Myoepithelial-like cells are known to produce their own growth factors (21, 27), and their retention by the substratum may result in a residual level of DNA synthesis higher than that for cells grown on plastic. The requirement for anchored HSPG is then in favor of a preferential localization of the binding sites of HGF/SF on the basement membrane to induce HGF/SF signaling. Interestingly, the stimulation of cell scattering by HGF/SF in chlorate-treated MDCK epithelial cells has recently been demonstrated to require also an immobilized HSPG receptor (5). Correct orientation of glycosaminoglycan chains seems to be crucial to cellular events such as cell proliferation or cell scattering.

DISCUSSION

HGF/SF is a stroma-derived growth factor, which is well described to act on neighboring epithelia in the mammary gland (22, 23, 36). HSPGs that bind HGF/SF have been purified from both the cell-associated and culture medium fractions of Huma 109 myoepithelial-like cells (7). The Huma 109 myoepithelial-like cells also possess the c-met tyrosine kinase receptor for HGF/SF (Fig. 1). These results suggest that myoepithelial cells may be a target for HGF/SF, because HGF/SF is known to require a dual receptor system consisting of both its HS and c-met receptors (10). However, the mechanism of action of the HGF/SF dual receptor system and the nature of the signaling pathways activated by c-met are not well understood. Intriguingly, HGF/SF did not stimulate DNA synthesis in Huma 109 myoepithelial-like cells cultured on plastic. In contrast, when the cells were plated on collagen I or fibronectin substrata, HGF/SF was able to stimulate DNA synthesis. This result was not due to the extracellular matrix substrata altering the level of expression of c-met or its cellular location. We first showed that there were striking differences in the kinetics of the p42/44MAPK phosphorylation by HGF/SF in cells plated on plastic, collagen I, or fibronectin. In cells plated on plastic, HGF/SF stimulated a transient dual phosphorylation of p42/44MAPK but failed to stimulate DNA synthe-

2 H. Rahmoune and D. G. Fernig, unpublished observations.
Taken together the results suggest that the stimulation of proliferation of Huma 109 myoepithelial-like cells by HGF/SF requires a sustained dual phosphorylation of p42/44MAPK, which in turn requires the cells to possess both c-met and an immobilized HSPG receptor for HGF/SF. Whether the HSPG receptors are located in the extracellular matrix/basement membrane, at the surface of the responsive cell, or at the surface of an adjacent cell is an intriguing question that remains to be elucidated.

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Nicolas Sergeant, Malcolm Lyon, Philip S. Rudland, David G. Fernig and Maryse Delehedde

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