Hepatocyte Growth Factor/Scatter Factor Induces a Variety of Tissue-Specific Morphogenic Programs in Epithelial Cells

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Abstract. Hepatocyte growth factor/scatter factor (HGF/SF) is the mesenchymal ligand of the epithelial tyrosine kinase receptor c-Met. In vitro, HGF/SF has morphogenic properties, e.g., induces kidney epithelial cells to form branching ducts in collagen gels. Mutation of the HGF/SF gene in mice results in embryonic lethality due to severe liver and placenta defects. Here, we have evaluated the morphogenic activity of HGF/SF with a large variety of epithelial cells grown in three-dimensional collagen matrices. We found that HGF/SF induces SW 1222 colon carcinoma cells to form crypt-like structures. In these organoids, cells exhibit apical/basolateral polarity and build a well-developed brush border towards the lumen. Capan 2 pancreas carcinoma cells, upon addition of HGF/SF, develop large hollow spheroids lined with a tight layer of polarized cells. Collagen inside the cysts is digested and the cells show features of pancreatic ducts. HGF/SF induces EpH4 mammary epithelial cells to form long branches with end-buds that resemble developing mammary ducts. pRNS-1-1 prostate epithelial cells in the presence of HGF/SF develop long ducts with distal branching as found in the prostate. Finally, HGF/SF simulates alveolar differentiation in LX-1 lung carcinoma cells. Expression of transfected HGF/SF cDNA in LX-1 lung carcinoma and EpH4 mammary epithelial cells induce morphogenesis in an autocrine manner. In the cell lines tested, HGF/SF activated the Met receptor by phosphorylation of tyrosine residues. These data show that HGF/SF induces intrinsic, tissue-specific morphogenic activities in a wide variety of epithelial cells. Apparently, HGF/SF triggers respective endogenous programs and is thus an inductive, not an instructive, mesenchymal effector for epithelial morphogenesis.

DURING embryonal development, histogenesis of epithelial organs is dependent on diffusible and membrane-bound mesenchymal factors as well as on signals originating from the surrounding extracellular matrix. This was shown for developing organs such as kidney, lung, mammary gland, prostate, digestive system, and salivary gland (Grobstein, 1953; Saxén and Sariola, 1987; Kratochwil, 1983; Cunha, 1986; Sakakura, 1991). The molecular nature of some of these factors was identified: for instance, the extracellular matrix component laminin was shown to be essential for the differentiation of mammary gland and kidney epithelia (Ekblom, 1992; Streuli et al., 1995). Epimorphin was suggested to be a mesenchymal factor for branching morphogenesis of embryonic skin and lung epithelia (Hirai et al., 1992; but see also Pelham 1993; Spring et al., 1993). Furthermore, a series of epithelial tyrosine kinase receptors and their mesenchymal ligands are involved in mesenchymal-epithelial interactions during development (Stoker, 1987; Press et al., 1990; Miki et al., 1991; Montesano et al., 1991*b*; Peles et al., 1992; Sonnenberg et al., 1991, 1993; Weidner et al., 1993; Alarid et al., 1994; Schuchardt et al., 1994; Peters et al., 1994; Schmidt et al., 1995; Uehara et al., 1995; for a review see Birchmeier and Birchmeier, 1993). Since these tyrosine kinase receptors were first discovered due to their transforming potential, they can mediate mitogenic signals. However, recently it became evident that tyrosine kinases also give signals that direct differentiation, cell movement, and morphogenesis, i.e., these receptors and their ligands are able to regulate decisive events in epithelial development.

An importance of tyrosine kinase receptors in mesenchymal-epithelial interactions during kidney and lung development was demonstrated also by genetic experiments: a targeted mutation of the c-Ret gene, which is expressed during embryogenesis at the tips of the branching ureter buds, results in severe hypoplasia or aplasia of the kidneys (Schuchardt et al., 1994). The ligand for c-Ret is yet unknown but was proposed to be of mesenchymal origin. Furthermore, a dominant-negative receptor for keratinocyte growth factor (KGF) was expressed specifically in embryonic lungs in transgenic mice. Mice that carry the transgene show long epithelial tubes that extend from the bifurcation of the trachea to the diaphragm, but form no alveoli (Peters et al., 1994).

To study molecular mechanisms responsible for differ-

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entiation of various epithelial cells into highly organized multicellular units, three-dimensional matrices reconstituted from extracellular matrix components like matrigel or collagen have widely been used (Yang et al., 1979; Bennet, 1980; Chambard et al., 1981; Hall et al., 1982; Montesano et al., 1983; Hadley et al., 1985; Barcellos-Hoff et al., 1989). However, the epithelial structures formed in these gels often differ considerably from the composition in the original tissues, e.g., MDCK kidney cells grown in collagen form hollow cysts, a structure not encountered in the developing kidney. Apparently, this in vitro system lacks additional stimuli for the induction of proper histogenesis. A more complex morphogenic program of MDCK cells could be induced by the mesenchymal hepatocyte growth factor/scatter factor (HGF/SF)[1]: addition of HGF/SF to MDCK cells in a collagen matrix leads to outgrowth of branching tubuli from the cysts, a course of events similar to the elongation and branching of the epithelial ureteric buds during kidney organogenesis (Montesano et al., 1991*a*,*b*).

HGF/SF is a paracrine factor produced by mesenchymal cells that induces, in addition to morphogenesis, various biological responses in target cells such as proliferation, motility, and angiogenesis (Nakamura et al., 1987; Stoker et al., 1987; Nakamura et al., 1989; Zarnegar and Michalopoulos, 1989; Miyazawa et al., 1989; Noji et al., 1990; Weidner et al., 1990, 1991; Bussolino et al., 1992; Rosen et al., 1993; Sonnenberg et al., 1993). The diverse biological effects of HGF/SF are transmitted by one high-affinity receptor, the receptor tyrosine kinase c-Met, which is expressed preferentially on epithelial and endothelial cells (Bottaro et al., 1991; Naldini et al., 1991; Weidner et al, 1993). In the developing embryo, c-Met is present in the *anlagen* of various epithelial organs, while HGF/SF is produced in the mesenchymal stroma directly adjacent (Sonnenberg et al., 1993). Transgenic mice deficient in the HGF/SF gene were found to die around day 15 of embryogenesis and display defects in development of the liver and placenta (Schmidt et al., 1995; Uehara et al., 1995). In addition, HGF/SF and c-Met null mutant mice lack limb muscles; apparently, HGF/SF also controls migration of myogenic precursor cells from the somites into the developing extremities (Christ et al., 1977; Bladt et al., 1995). In vivo, HGF/SF thus affects proliferation, migration, and morphogenesis of cells and ensures a controlled development.

To address the question of the morphogenic potential of HGF/SF, we set out to examine a series of epithelial cell lines in an in vitro organogenesis system. Seventy-three epithelial cell lines from colon, pancreas, mammary gland, prostate, lung, and other organs were tested for their ability to form, in response to HGF/SF, organoid structures in collagen gels. We found that cell lines from virtually each of the epithelial tissues respond to HGF/SF by morphogenesis. In most cases, structures that resemble the epithelial organization of the organ of origin were found. The results indicate that HGF/SF can induce morphogenesis in diverse epithelial cells, but that intrinsic programs of the epithelia determine exact morphogenic events.

1. *Abbreviation used in this paper*: HGF/SF, hepatocyte growth factor/scatter factor.

Materials and Methods

Recombinant HGF/SF

Recombinant HGF/SF was produced by inserting the HGF/SF cDNA (Weidner et al., 1991) into the pBlueBac vector followed by expression in Sf9 insect cells using the Maxbac baculovirus system (Invitrogen, San Diego, CA). After transfection of Sf9 cells with the HGF/SF vector and wild-type baculovirus DNA by calcium phosphate coprecipitation, the culture medium was assayed for scattering activity in the MDCK cell assay (Weidner et al., 1990). Recombinant baculoviruses were selected by plaque screening and positive plaques were propagated. Sf9 cells were infected with recombinant baculovirus and cultured in serum-free Excell 401 medium (Seralab, Crowley Down, England) supplemented with 0.1% Pluronic F-68 (Life Technologies, Gaithersburg, MD). 3 d after infection, conditioned medium was clarified by centrifugation, loaded onto a Heparin Sepharose column (Pharmacia, Uppsala, Sweden) and eluted by a linear NaCl gradient (0.6–1.8 M). This one step purification resulted in pure HGF/SF with a specific activity of 5 scattering units/ng protein. Recombinant HGF/SF was also produced by transient expression in Neuro 2A cells (Hartmann et al., 1992).

Autocrine Stimulation of c-Met

Epithelial cell lines EpH4 and LX-1 were stably transfected with the HGF/SF cDNA (pBAT-SFtag together with the pSV-2 neomycin resistance plasmid; Hartmann et al., 1994) in order to examine autocrine stimulation of the Met receptor. Resistant clones were selected in G 418 at a concentration of 0.8 mg/ml medium and analyzed for HGF/SF expression. To inhibit autocrine stimulation, a sheep antibody against HGF/SF was used (kindly provided by Dr. Ermanno Gherardi, Cambridge), which blocked HGF/SF activity in the organogenesis assay at a concentration of 20 μg/ml.

Cell Lines

MKN 45 and Okajima cells were from Dr. G. F. Vande Woude (Frederick, MD); CSG 120/7 cells were from Dr. L. M. Franks (London, UK); EpH4 cells were from Dr. E. Reichmann, (Lausanne); NMuMG cells were from Drs. M. Mareel, Gent, and R. Derynck (San Francisco, CA); pRNS-1-1 and 267B1SV40 cells were from Dr. J. S. Rhim (Frederick, MD); SW 1222 and SW 1116 cells were from Dr. T. Braulke, Göttingen; CAL 51 and CT 7/3 cells were from Dr. J. Kopp (Berlin). The other cell lines were purchased from Amer. Type Culture Collection (Rockville, MD) or are described in Frixen et al. (1991). Epithelial cells were cultivated in DMEM

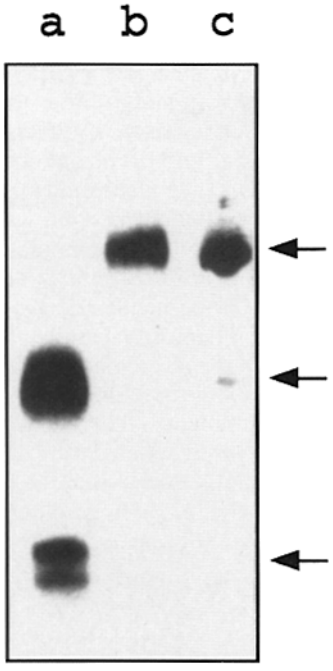


Figure 1. Recombinant HGF/SF purified from murine Neuro 2A cells (lanes *a* and *b*) and from baculovirus-infected Sf 9 insect cells (*c*) by Heparin Sepharose chromatography. In Neuro 2A cells (*a*), wild-type HGF/SF is proteolyzed into a 60-kD heavy chain and a 30-kD light chain doublet. In *b*, HGF/SF with a cleavage site mutation was expressed in order to indicate the non-cleaved form (Hartmann et al., 1992). The baculovirus-produced factor (*c*) is a 90-kD form which is easily cleaved in the assay by serum components (Naldini et al., 1992). *a* and *b* shows auto radiograms of [^{35}S]methionine-labeled factor, (*c*) is a silver-stained gel.

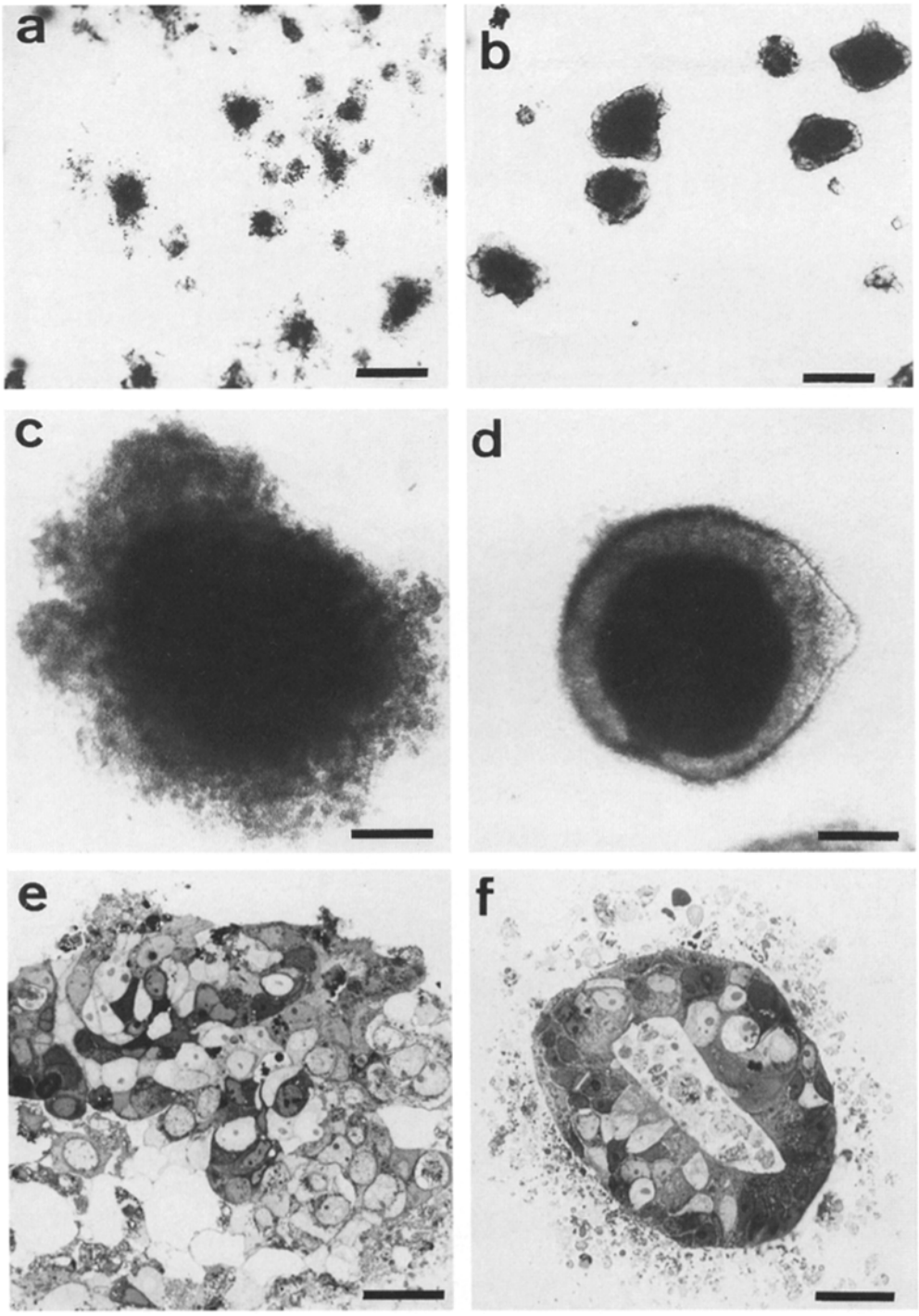


Figure 2. SW 1222 colon carcinoma cells grown in collagen gels form loose aggregates (*a*, *c*, and *e*). In the presence of HGF/SF, the cells rearrange to form crypt-like structures (*b*, *d*, and *f*). (*a–d*) Micrographs of living cells using Nomarski optics, (*e* and *f*) Toluidine blue–stained semithin sections analyzed by light microscopy, Bars: (*a* and *b*) 250 μm; (*c* and *d*) 100 μm; (*e* and *f*) 50 μm.

supplemented by 10% FCS; pRNS-1-1 and 267B1 cells (Lee et al., 1994; Kaighn et al., 1989) were cultivated in keratinocyte growth medium 041–17005 M (GIBCO BRL) and 3101/4131 (Clonetics, San Diego, CA), respectively.

Organogenesis Assay

To test for morphogenic potential, the epithelial cells were cultivated in a three-dimensional lattice of collagen. The gelling mixture was prepared using 7 parts of collagen stock solution (90% type I/10% type III, Biochrom), and 1 part each of 10 × concentrated DMEM, 10% FCS, and $NaHCO_3$ (22 mg/ml). After neutralization with NaOH, 300 μl/well of cold mixture was plated onto 24-well plates (Nunc, Roskilde, Denmark) and allowed to gel at 37°C for 20–30 min. Trypsinized epithelial cells were suspended in another 300 μl aliquot of collagen gelling mixture and seeded on top of the first layer. After gelling, 1 ml/well culture medium was added and changed twice weekly. After the cells had adapted to the growth in collagen (usually after 3–7 d), HGF/SF at a concentration of 20 ng/ml (100 U/ml) was added, and the cells were cultured further for 10–20 d.

Light and Electron Microscopy

Morphological characteristics of the cell lines were examined using an inverted light microscope (Zeiss Axiovert) with Nomarski interference optics. To further analyze the structure of organoids, cells were fixed with 2.5% glutaraldehyde (Sigma), and small blocks cut out of the gel. After postfixation with OsO_4, the blocks were contrasted with tannic acid and uranyl acetate. The specimens were dehydrated in a graded ethanol series and embedded in Epon 812. Ultrathin sections (50–70 nm) were contrasted with lead citrate and analyzed in a Zeiss EM 10 electron microscope.

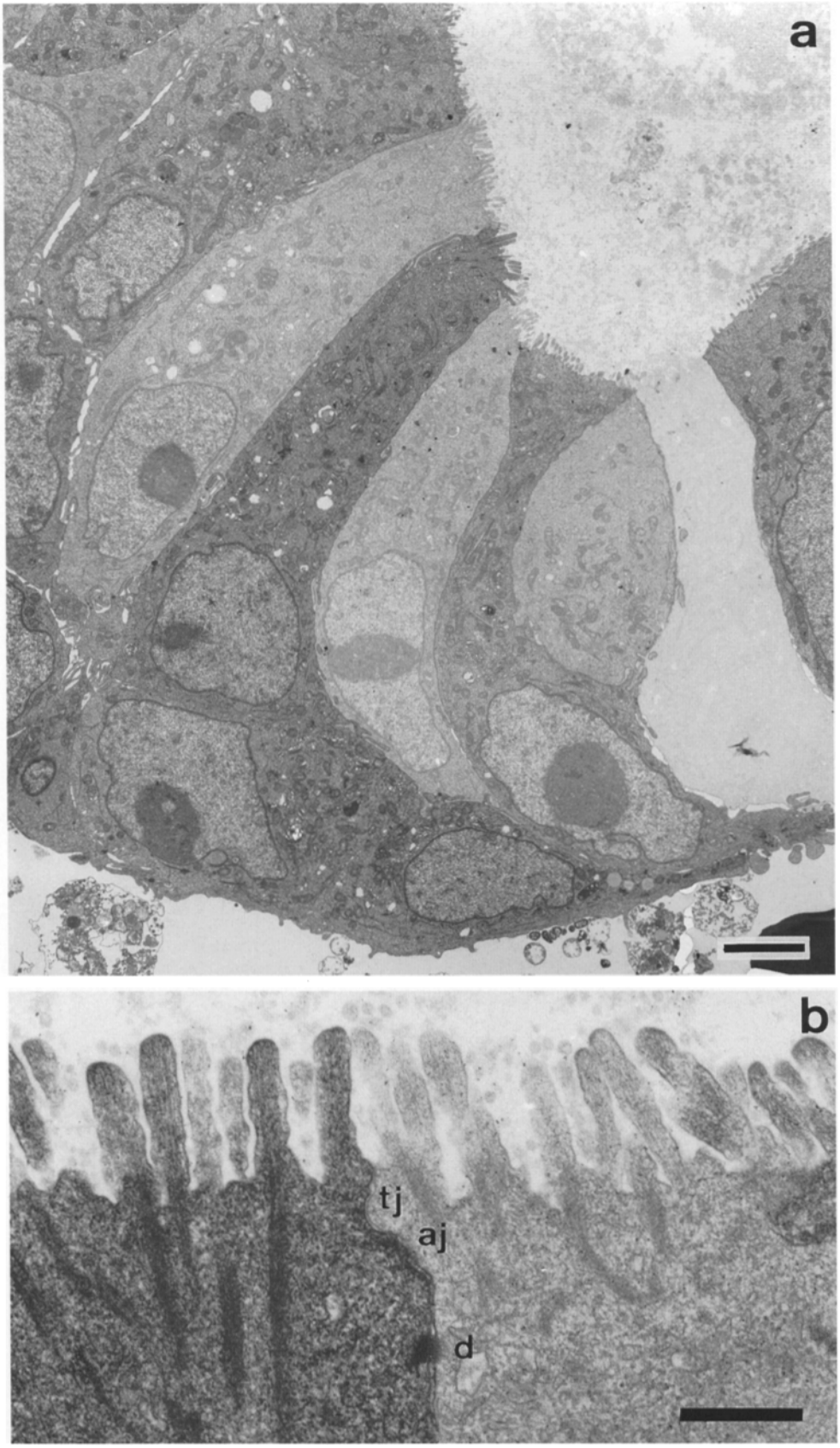
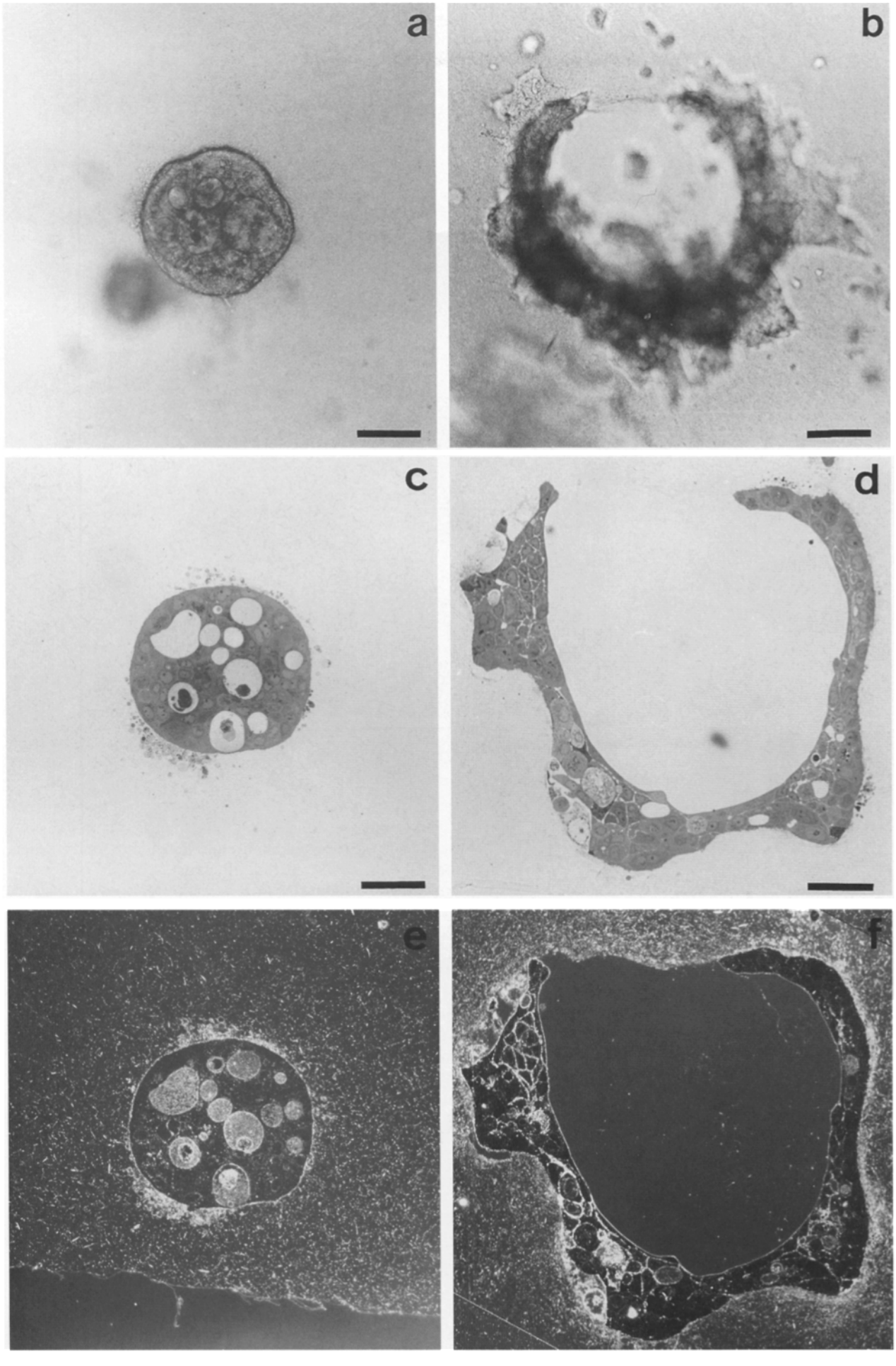


Figure 3. Electron micrograph of a HGF/SF-induced crypt-like organoid of SW 1222 cells. The cells are polarized with basal nuclei and an apical brush border lining the central lumen (*a*). The induced cells bear features of absorptive enterocytes, i.e., they form a brush border towards the lumen (*b*) and develop junctional complexes composed of tight (*tj*), and adherens junctions (*aj*) and of desmosomes (*d*). Bar, 5 μm in *a*; 1 μm in *b*.

Figure 4. Capan 2 pancreas carcinoma cells cultured in collagen gels form aggregates with intercellular lumina (*a*, *c*, and *e*). In the presence of HGF/SF, the cells loosen their contacts and begin to form cup-like structures (*b*, *d*, and *f*). The collagen inside the cup becomes degraded as revealed by dark-field microscopy (*f*). (*a* and *b*) Light microscopy of living cells, (*d–f*) semithin sections as analyzed by bright- (*c* and *d*) and dark-field microscopy (*e* and *f*). Bar, 100 μm in *a* and *b*; 50 μm in *c–f*.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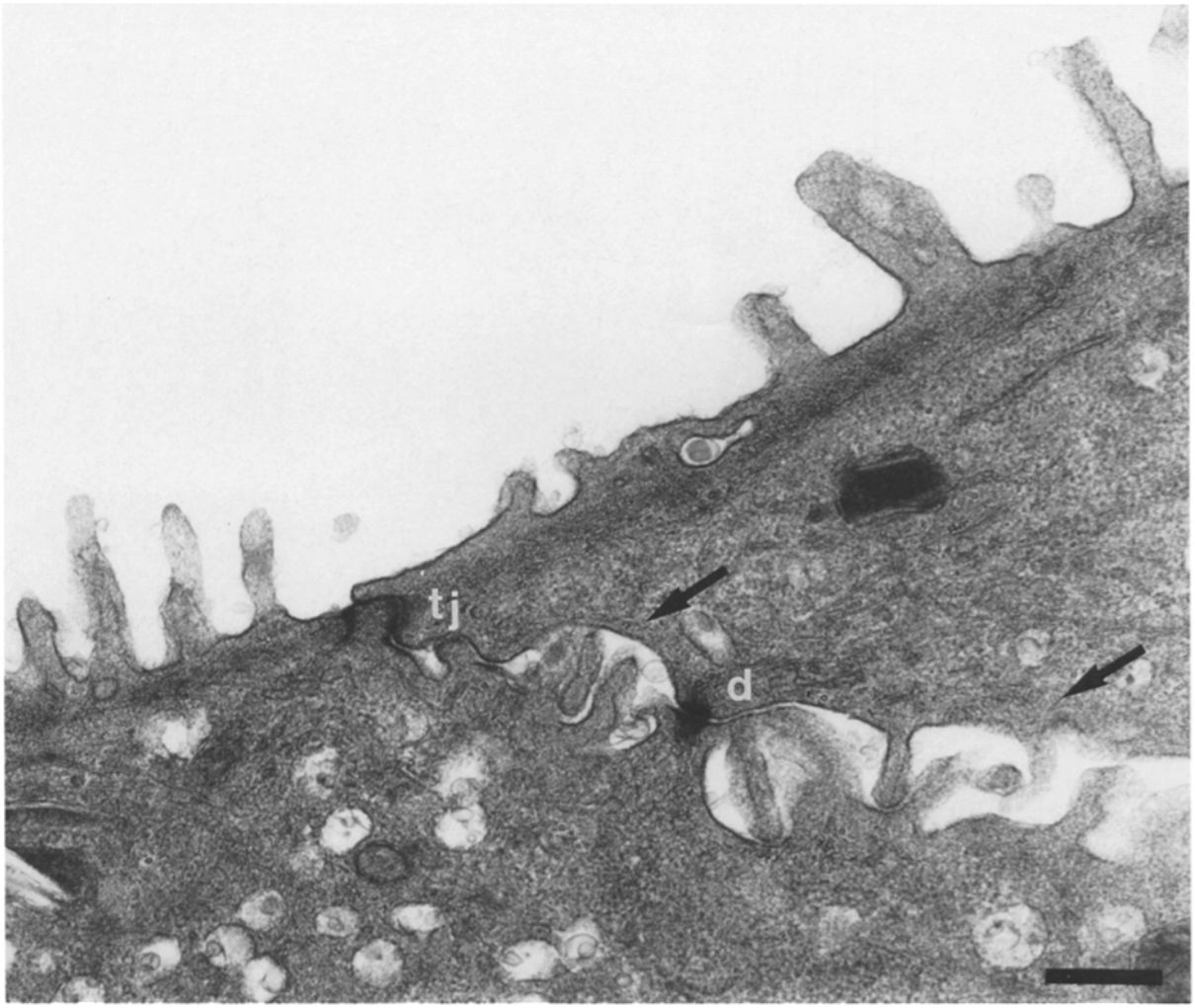

Figure 5. Detail of hollow spheroids of Capan 2 pancreas carcinoma cells. The cell layer is tight towards the lumen, and the cells are interconnected by desmosomes. The lateral membranes are highly interdigitated (*arrows*). On the apical surface, small microvilli are sparsely distributed. *tj*, tight junctions; *d*, desmosome. Bar, 0.5 μm.

Semithin sections (0.5 μm) were stained with Toluidine blue and analyzed with a Zeiss Axiophot light microscope.

Ligand-induced Tyrosine Phosphorylation of the Met Receptor

Cells in monolayer cultures were grown to semiconfluency and stimulated by adding 20 ng/ml of recombinant HGF/SF to the culture medium. After 6 or 10 min, 5×10^6 cells were lysed in RIPA-kinase lysis buffer (50 mM Hepes, pH 7.2, 10 mM EDTA, 0.1% SDS, 1% NP-40, 0.5% deoxycholate, 50 mM Na-pyrophosphate, 100 mM Na-fluoride, 2 mM Na-orthovanadate, 30 mM phenyl phosphate, 1 mM Zn-chloride, 50 μM ammonium molybdate, 35 μM phenyl arsine oxide, 1.25 mM PMSF, 10 μg/ml aprotinin). Debris was removed by centrifugation, and the Met tyrosine kinase was immunoprecipitated using polyclonal rabbit antisera and protein G coupled to Sepharose beads (Weidner et al., 1993). The precipitated proteins were separated on 7% SDS-PAGE using the Laemmli system (Laemmli, 1970) and were blotted onto nitrocellulose. The sheets were blocked in 3% skimmed milk in PBS and probed with an anti-phosphotyrosine monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Bound antibody was detected using the ECL system (Amersham).

Results

To evaluate the role of HGF/SF in morphogenesis of epithelial organs, we have examined epithelial cell lines from various tissues in an in vitro organogenesis assay. The cells were allowed to grow in three-dimensional collagen gels that provide an extracellular matrix largely devoid of factors which stimulate growth and differentiation (Yang et al., 1979; Bennet, 1980; Hall et al., 1982; Daniel et al., 1984; Del-Buono et al., 1991; Montesano et al., 1991*b*; Santos and Nigam, 1993). We found that in the presence of HGF/SF, a variety of cell lines developed complex organoid structures which are analyzed in detail below. The recombinant HGF/SF used was purified from two expression systems: Mammalian and insect cells (Fig. 1). The factor obtained from murine Neuro 2 A cells consisted of the proteolyzed αβ heterodimer with molecular weights of 60 and 30 kD (Hartmann et al., 1992). The baculovirus-produced HGF/SF is a 90-kD single chain component; it has previously been shown that this single chain factor is proteolyzed in medium by serum components (Naldini et al., 1992).

HGF/SF Induces Tissue-Specific Organoids from SW 1222 Colon Cells

SW 1222 human colonic carcinoma cells exhibit an epithe-

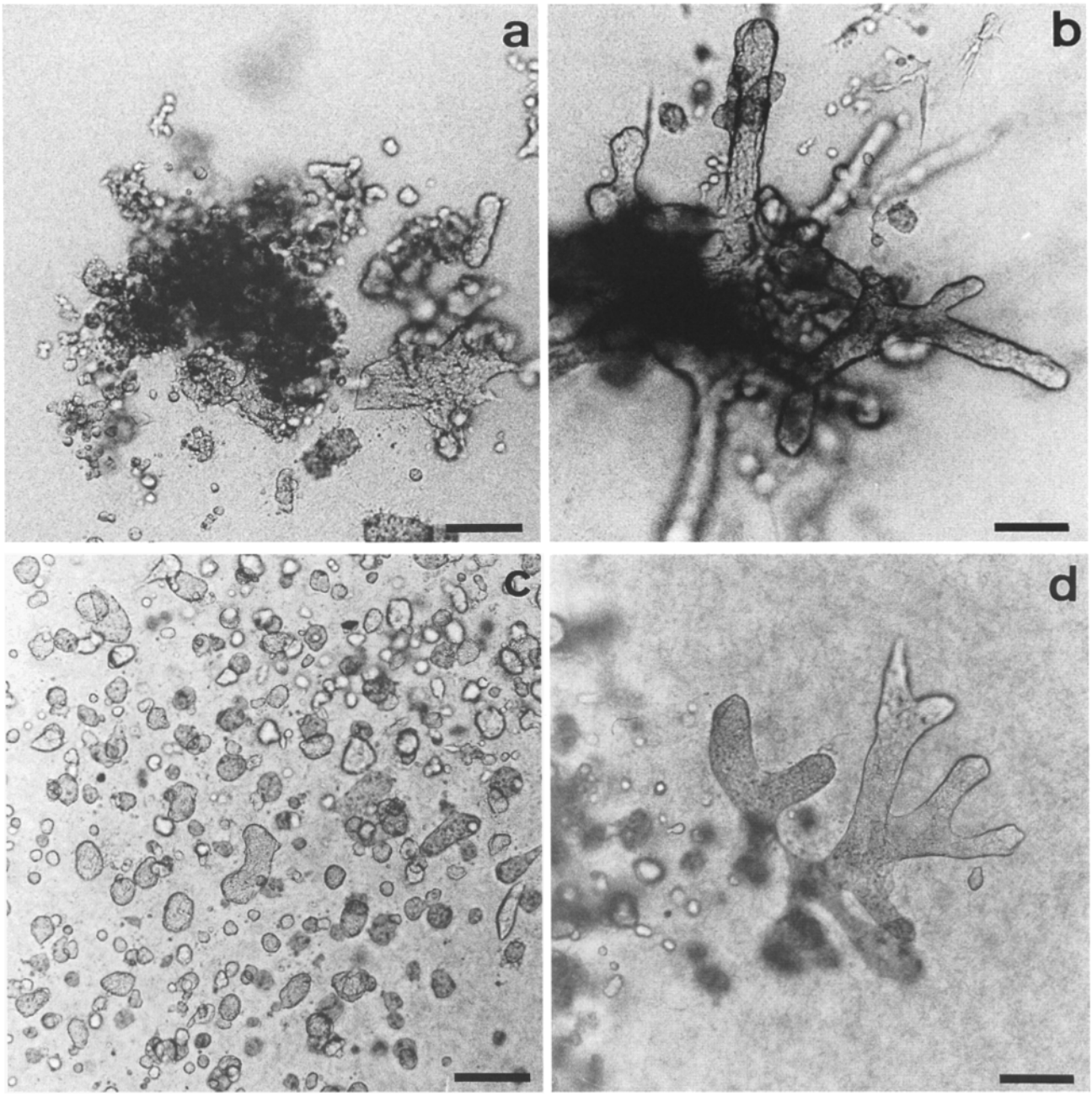

Figure 6. EpH4 mammary epithelial cells grow as small aggregates when cultured in collagen gels (*a*). HGF/SF induces branching morphogenesis leading to long extensions (*b*). Spontaneous outgrowth of extensions occurs after stable transfection of EpH4 cells with HGF/SF cDNA (*d*), which can be inhibited by anti-HGF/SF antibody (*c*). Bar, 100 μm.

lial phenotype, express E-cadherin, and readily form dome-like structures in monolayer culture (Pignatelli and Bodmer, 1989). When SW 1222 cells were plated in collagen gels for two weeks, they formed loose aggregates with no multicellular organization of higher order (cf. Fig. 2 *a* for an overview and Fig. 2, *c* and *e* for the morphology of single structures). The borders of the aggregates were uneven and showed signs of cellular disorganization (*c*). In contrast, HGF/SF at a concentration of 20 ng/ml (100 U/ml in the MDCK scattering assay) induced rearrangement of the majority of the aggregates into hollow spheroids with smooth surfaces (cf. Fig. 2 *b* for an overview and Fig. 2 *d*, for the morphology of single structures). Quantitative analysis revealed that 79% of the aggregates were rounded and had a smooth surface; 60% of these had a clear lumen; remaining aggregates were small and irregular. Reorganization into hollow spheroids in the presence of HGF/SF took place within 1–2 wk. Fine analysis showed that these structures contain polarized cells and that the central lumen is lined by a continuous and well-developed brush border (Fig. 3 , *a* and *b*). The cytoskeleton of the microvilli extends into the apical cytoplasm, i.e., forms a terminal web. Apical and basolateral surfaces are separated by junctional complexes with well visible tight junctions, adherens junctions, and desmosomes (Fig. 3 *b*). The polarized cells are highly elongated and the nuclei located at the

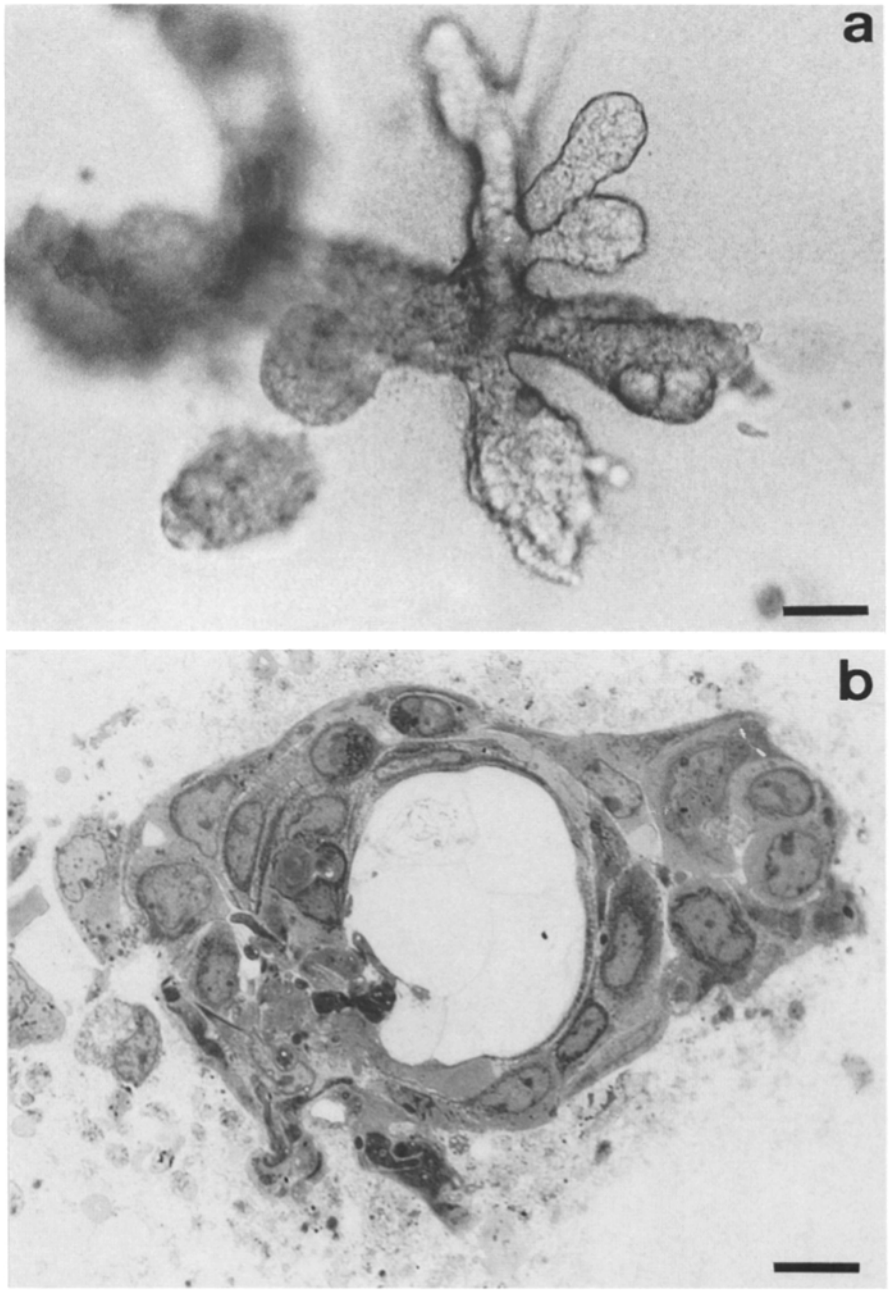

Figure 7. EpH4 cells cultured in the presence of HGF/SF frequently form end bud-like structures (*a*) which contain a lumen (*b*). Bars: 50 μm in *a*; 10 μm in *b*.

basal side and thus resemble absorptive enterocytes (Fig. 3 *a*). The cells in the spheroids exhibit distinct staining intensities (cf. Fig. 3 *a*), the reason for this remarkable difference is not known to us. Such hollow aggregates were never observed in the absence of HGF/SF. The architecture of these organoids in transverse section is thus similar to that of colonic crypts; the crypts represent the major organization unit of colon epithelia (Roth and Gordon, 1990).

HGF/SF Induces Cysts from Capan 2 Human Pancreas Adenocarcinoma Cells

Capan 2 cells express E-cadherin and form continuous monolayers in tissue culture (Frixen et al., 1991). In the absence of HGF/SF, the cells form aggregates with several lumens and smooth surfaces (Fig. 4, *a*, *c*, and *e*). In the presence of HGF/SF, the aggregates generally flatten and in average 60% extend to form cup-like structures within 1–2 wk (Fig. 4, *b*, *d*, and *f*). In the course of this process, the extensions which initially contain several layers of cells, thin out and finally form a single cell layer which is tight towards the lumen; in the intermediary stage, cells facing the outside of the structure are more loosely associated (Fig. 4 *d* and Fig. 5). As revealed by dark field microscopy (Fig. 4 *f*) and by the absence of fibers in ultrathin sections (Fig. 5), collagen inside the cups seems to be modified, i.e., degraded. Finally, the extensions meet and the gap is closed to form a hollow spheroid lined by a single layer of epithelial cells. Fine analysis of this cell layer revealed short microvilli facing the lumen (Fig. 5). The lateral membranes have tight junctions, are highly interdigitated and connected by desmosomes at particular contact points. Taken together, HGF/SF induces cysts from pancreatic

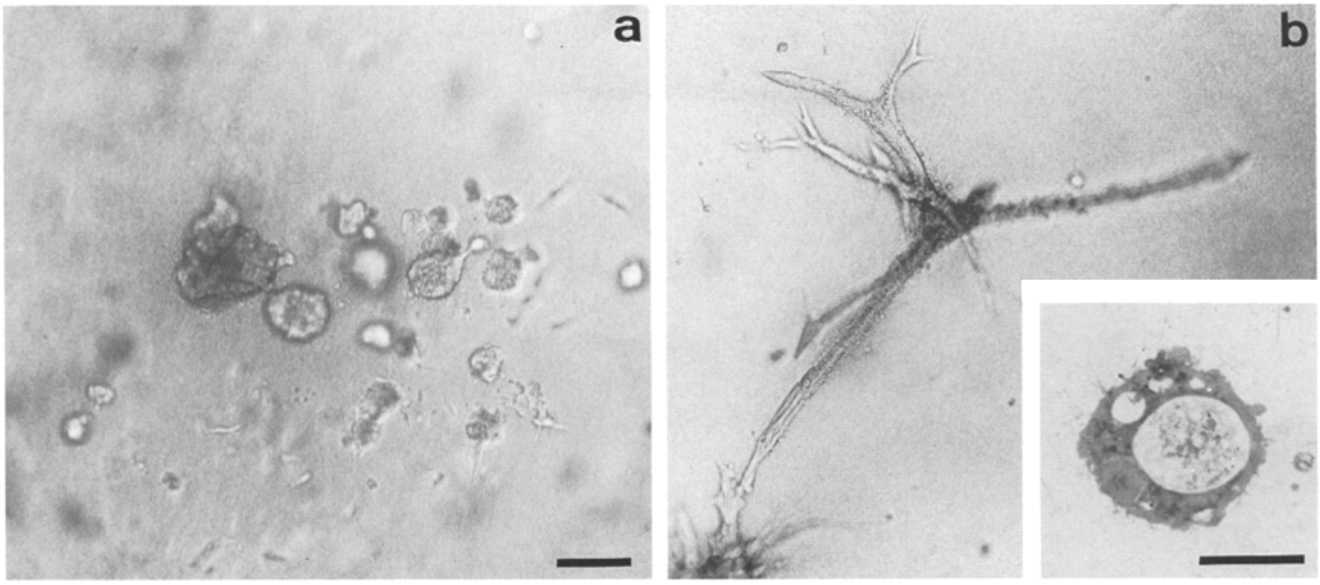

Figure 8. pRNS-1-1 control cultures form solid spheres in collagen (*a*), whereas in the presence of HGF/SF, they develop long ducts with distal branching (*b*). The insert in *b* demonstrates lumen formation. Bars: (*a* and *b*) 100 μm; (*insert*) 10 μm.

carcinoma cells that bear characteristic cellular features of pancreatic ducts.

HGF/SF Induces Ductular Structures from EpH4 Murine Mammary Epithelial Cells

EpH4 cells, a derivative of IM-2 cells, were originally isolated from mammary tissue of a mid-pregnant mouse and form continuous epithelial monolayers (Reichmann et al., 1989; Beug, H., personal communication). Control cultures of EpH4 cells in collagen gels form small compact aggregates (Fig. 6 *a*). In the presence of HGF/SF, branching morphogenesis of the aggregates occurs which leads to outgrowth of long extensions within 1–2 wk (Fig. 6 *b*). EpH4 cells were also transfected with a HGF/SF expression plasmid and clones that produce HGF/SF (5–8 U/10^6 cells in 24 h) were selected. When these clones are employed in the organogenesis assay, branching morphogenesis occurs spontaneously, indicating the formation of an autocrine loop (Fig. 6 *d*). Branching morphogenesis is inhibited by the addition of 20 μg/ml of a polyclonal anti-HGF/SF antibody (Fig. 6 *c*). Frequently, the extensions are terminated by end bud-like structures (Fig. 7 *a*). As found in cross sections, these end buds can form lumina (Fig. 7 *b*). Interestingly, HGF/SF induces an amazing network of intermediate filaments in the protrusions which might serve as mechanic support (data not shown, cf. also Reichmann et al., 1989). HGF/SF does not induce the production of milk proteins in EpH4 cells (cf. also Yang et al., 1995).

HGF/SF Induces Branching Morphogenesis from pRNS-1-1 Human Prostate Epithelial Cells

The pRNS-1-1 cell line is derived from normal adult prostate epithelium and was immortalized by transfection with a plasmid containing an origin-defective SV40 genome (Lee et al., 1994). The cells express E-cadherin and grow as continuous epithelial monolayers when cultured in keratinocyte growth medium (cf. Materials and Methods). In collagen matrix, pRNS-1-1 cells form round aggregates (Fig. 8 *a*), which frequently disintegrate after one week of culture. In the presence of HGF/SF, long ducts develop from the spheres that sparsely arborize at the distal ends (Fig. 8 *b*). These branched organoids did not show any signs of degeneration during the culture period of 20 d. The branching pattern is thus similar to the one seen in the developing prostate (Timms et al., 1994).

HGF/SF Induces LX-1 Lung Carcinoma Cells to Form Alveolar-like Structures

LX-1 lung carcinoma cells were transfected with HGF/SF cDNA and stable clones secreting HGF/SF (10–12 U/10^6 cells in 24 h) were analyzed in the organogenesis assay. After 8–10 d, numerous prominent intracellular lumens developed in 75% of the cell aggregates (Fig. 9, *b* and *d*). Several lumens cluster into separate units; most of the cytoplasm and the nuclei were dislodged to the unit surface. The lumens were lined by extremely thin layers of cytoplasm, thus resembling developing alveoli (terminal sacs, cf. Chen and Little, 1987). When anti-HGF/SF antibody (at 20 μg/ml) was added to the cultures, less than 7% of the clusters formed lumens, which were much smaller (Fig. 9, *a* and *c*). This indicates that lumen formation in HGF/SF-transfected LX-1 cells is due to autocrine stimulation.

Evaluation of the Morphogenesis Potential of HGF/SF in a Variety of Carcinoma Cell Lines of Different Tissue Origin

We show here that particular cell lines from virtually all analyzed organs are responsive towards HGF/SF (Table I). The most pronounced examples for organogenesis were described above, i.e., SW1222, Capan 2, EpH4, pRNS-1-1, and LX-1 cells; tubulogenesis of MDCK (kidney) and 1-7HB2 or NMuMG mammary epithelial cells has been

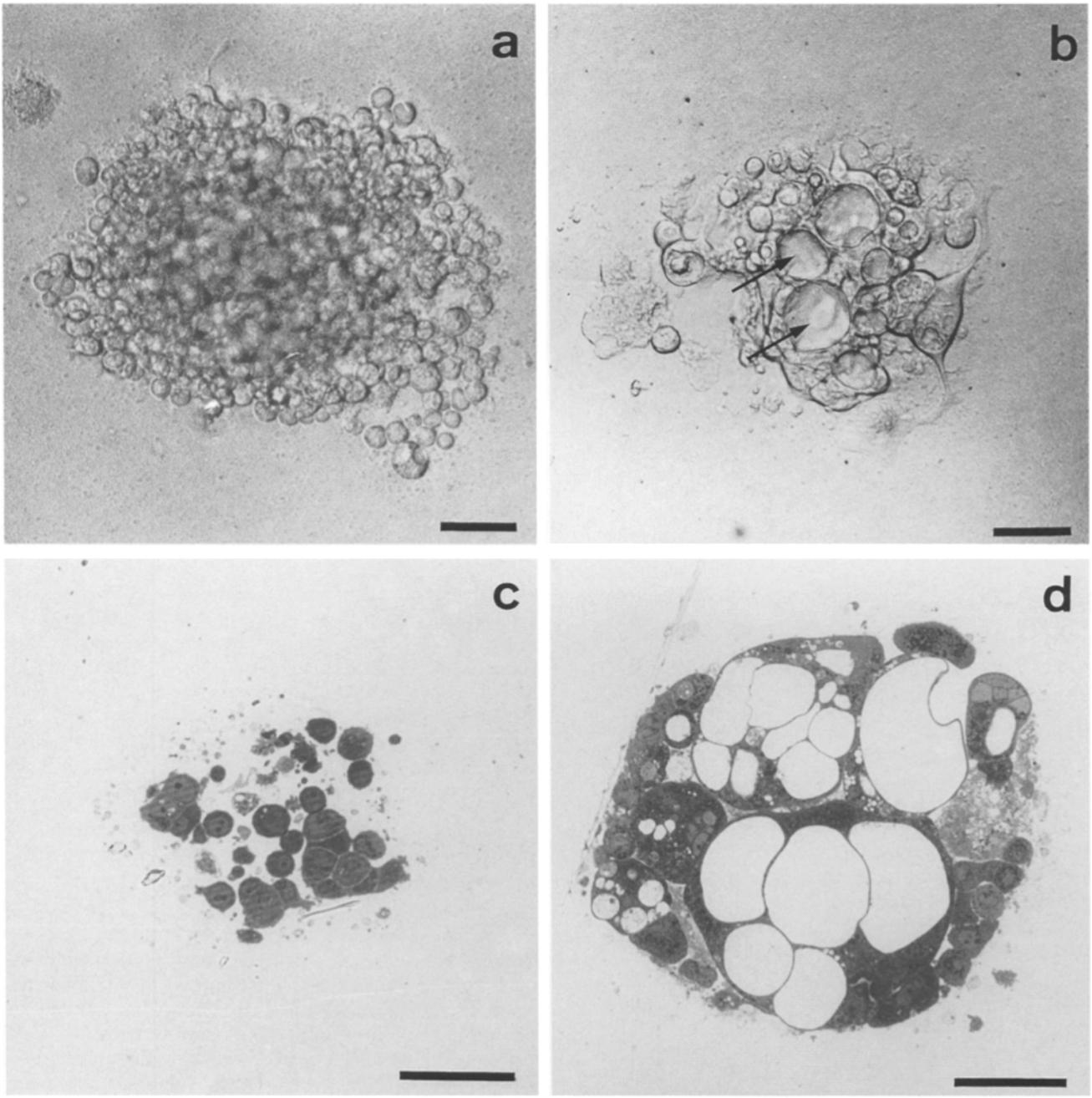

Figure 9. LX-1 lung carcinoma cells develop large alveolar-like structures (*arrows* in *b*) when transfected with HGF/SF cDNA. This morphogenic effect can be largely inhibited by anti-HGF/SF antibody (*a* and *c*). Bar, 50 μm.

reported before (Montesano et al., 1991 *a,b*; Berdichevsky et al., 1994; Soriano et al., 1995). Our extensive analysis allows the following conclusions to be drawn: (*1*) Only differentiated cell lines which formed well developed epithelial monolayers and expressed the cell–cell adhesion molecule E-cadherin in tissue culture (Frixen et al., 1991) can assemble into complex organoid structures. The degree of Met expression also varied between the different cell lines, but no correlation to the responsiveness in the organogenesis assay was apparent. (*2*) The response towards HGF/SF resembles the origin of the cell line (cf. above). Exceptions are CX-1 colon carcinoma cells that respond to HGF/SF with branching morphogenesis or the bladder carcinoma cell line RT 4 that form sheets, which in the presence of HGF/SF, disassemble and degenerate. Certain poorly differentiated cell lines, e.g., MDA-MB 435 S (breast) or ME-180 (cervix) carcinoma cells, form small aggregates with some protrusions. (*3*) We have tested various carcinoma-derived and noncarcinoma-derived cell lines in the organogenesis assay: Out of 64 carcinoma-derived cells (cf. Table I), only 3 responded with HGF/SF-induced morphogenesis, whereas out of 9 noncarcinoma-derived cells, 4 showed a response.

The Cell Lines Responsive in the Organogenesis Assay Express c-Met That Shows Ligand-dependent Phosphorylation

It has been shown previously that the morphogenesis of

Table I. HGF/SF Induced Morphogenesis: Survey of the Various Epithelial Cell Lines Tested

Cell line	Tissue of origin	Response to HGF/SF
SW 1222	colon	++
SW 1116	colon	+
SW 948	colon	+
CX-1	colon	+*
HT-29	colon	+
WiDr	colon	−
Caco-2	colon	−
Colo 201	colon	−
Colo 205	colon	−
HCT 116	colon	−
SW 48	colon	−
SW 620	colon	−
SW 1088	colon	−
CX-2	colon	−
FHs74Int	small intestine	−
Capan-2	pancreas	++
Hs 766T	pancreas	+
Capan-1	pancreas	−
DANG	pancreas	−
MIA PaCa-2	pancreas	−
MTB 13	pancreas	−
EpH 4	breast	++
MDA-MB-435S	breast	+‡
CT 7/3	breast	+
NMuMG	breast	+
BT 549	breast	−
HS 578T	breast	−
T-47D	breast	−
MCF7	breast	−
MDA-MB 134-VI	breast	−
MDA-MB-231	breast	−
MDA-MB 361	breast	−
MDA-MB-436	breast	−
MDA-MB 453	breast	−
MDA-MB 468	breast	−
HBL-100	breast	−
CAL 51	breast	−
MKN 45	stomach	+‡
Okajima	stomach	−
Hep-G2	liver	+
Hep 2B	liver	−
pRNS-1-1	prostate	++
269B1	prostate	+
LNCaP	prostate	+
PC-3	prostate	+
Du 145	prostate	+
SIRC	cornea	+‖
MRI-H 196	cervix	+
ME-180	cervix	+‡
C-33 A	cervix	−
MS 751	cervix	−
Ca Ski	cervix	−
MRI-H 186	cervix	−
HeLa	cervix	−
A 431	vulva	−
HEp-2	larynx	−
BA	larynx	−
WE	larynx	−
FaDu	hypo pharynx	−
CSG 120/7	salivary gland	−
A 431	vulva	−
FRTL 5	thyroid	−
MDCK	kidney	++
MRI-H 121	kidney	+
LX-1	lung	++
A 549	lung	+
L2	lung	−
A-427	lung	−
LXF 289	lung	−
SK-MES-1	lung	−
RT 4	bladder	+§
RT 112	bladder	−
EJ 28	bladder	−

Table I. (continued)

Cell lines are derived from human carcinomas (Frixen et al., 1991) except for EpH4 and NMuMG (from murine mammary epithelium), pRNS-1-1 and 267B1 (from human prostate epithelium, transfected with SV 40), SIRC (from rabbit cornea epithelium), CSG 120/7 (from murine salivary gland epithelium), and MDCK (from canine kidney epithelium). Rating in the morphogenesis assay: ++, cells develop organoid structures; +, cells show minor morphologic changes after the addition of HGF/SF; −, cells not responsive to HGF/SF.

*Branching morphogenesis was seen.

‡Cells form small aggregates with protrusions.

§Cells formed sheets which disassembled in the presence of HGF/SF.

‖Formed sheets of high prismatic cells in the presence of HGF/SF.

MDCK epithelial cells requires activation of the Met receptor: tubulogenesis in collagen gels could be induced by NGF in cells transfected with a trk-Met hybrid receptor (Weidner et al., 1993). SW 1222 colon and Capan 2 pancreas carcinoma cells as well as EpH4 and MDCK epithelial cells were treated here with HGF/SF, and the Met receptor was immunoprecipitated and analyzed for tyrosine phosphorylation (Fig. 10). In all cell lines tested, HGF/SF induced rapid autophosphorylation of the 140-kD β-chain of the Met receptor.

Discussion

HGF/SF can influence morphogenesis of various epithelial cells in reconstituted extracellular matrices. It was previously shown that kidney cells form branching tubules in the presence of HGF/SF (Montesano et al., 1991 *a,b*). The systematic study presented here demonstrates further that colon cells rearrange to organoids with features of colonic crypts, pancreas cells develop hollow cysts, mammary gland cells build ducts with end buds and lung cells form alveolar-like structures. The organoids induced by HGF/SF resemble thus organization of epithelial cell units in the respective organ of origin. Apparently, HGF/SF triggers epithelial cells to accomplish a morphogenic program; however, the exact shape is determined by the tissue of origin and depends on other, so far unknown factors. The data thus suggest that HGF/SF is an inductive and less an instructive mesenchymal factor for epithelial morphogenesis.

A striking example for the morphogenic potential of HGF/SF is the induction of intestinal cells to build crypt-like organoids with well developed brush borders. This complex assembly is produced by rearrangement of previously unstructured cell aggregates (compare Fig. 2 *a*, *c*, *e* and *b*, *d*, *f*). Richman and Bodmer (1988) have previously studied the morphogenic potential of a variety of colon carcinoma cells in the presence of fibroblast-conditioned medium. Some of the cell lines were promoted to develop

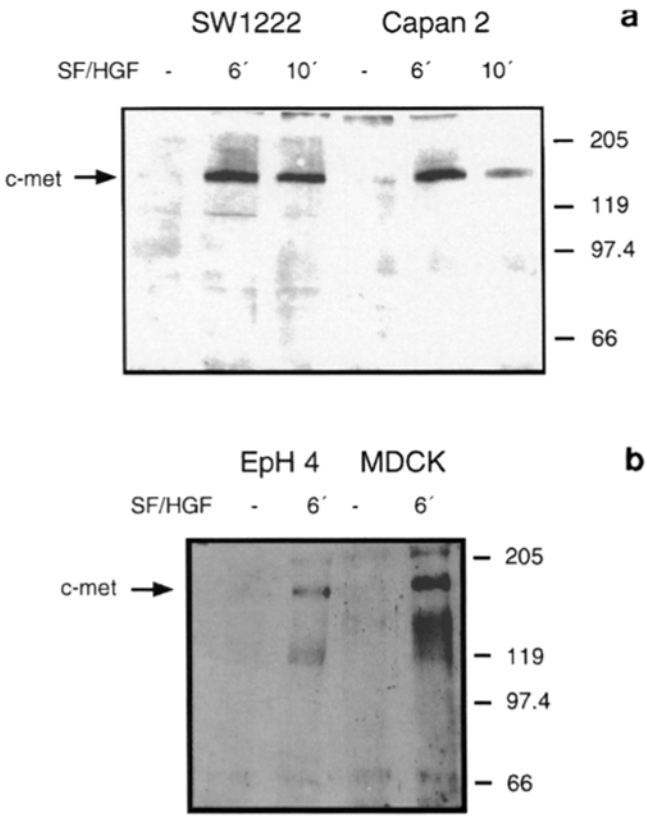

Figure 10. Autophosphorylation of the Met receptor in SW 1222 and Capan 2 carcinoma cell lines (*a*) as well as in EpH4 and MDCK cells (*b*) is induced by HGF/SF. Cells were stimulated with HGF/SF for 6 min or 10 min.

glandular structures, but the nature of the inductive factors had not been further characterized. During development, the *anlage* of the gastrointestinal tract consists first of a poorly differentiated, stratified epithelium that is surrounded by mesenchymal cells. In the perinatal phase, the multilayered epithelium is converted into the single-layered villous epithelium and simultaneously, terminal differentiation takes place (Mathan et al., 1976). The first sign of these morphogenic processes is the dissociation of cell–cell contacts and the formation of secondary lumens in the multilayered epithelium. This rearrangement is governed by mesenchymal-epithelial interactions, and correspondingly, mesenchymal cell clusters can be found in the vicinity which secrete abundant amounts of HGF/SF (Sonnenberg et al., 1993). Our data show that it is possible to simulate the rearrangement of intestinal cell clusters and induce cellular differentiation in vitro, and that HGF/SF is critical for this process. Apparently, SW 1222 cells bear an intrinsic potential to fulfill both aspects of colon development: they are able to rearrange in three dimensions and to differentiate into absorptive enterocytes.

We show here that Capan 2 pancreas cells in collagen form hollow cysts after addition of HGF/SF. Cells facing the lumens of the cysts develop short, sparsely distributed microvilli and the lateral cell surfaces are highly interdigitated. These features are characteristic for pancreatic duct cells (Ide et al., 1993; Githens et al., 1994). However, the overall architecture of the organoids is different from the in vivo situation, i.e., no tubular structures are formed, indicating that only part of the intrinsic morphogenic program of pancreatic duct cells is induced. Acinar structures were not induced by HGF/SF. Accordingly, the vast majority of pancreas carcinomas derive from ducts of the exocrine pancreas (Lack, 1989). Capan 2 cells also seem to originate from ducts; they express CD44 (V. Brinkmann, data not shown), in contrast to pancreatic acini that lack CD44 expression (Heider et al., 1993). During embryogenesis, the pancreas develops as an extension of the foregut. Elongation and branching of epithelia leads to tubular structures followed by formation of acini (Hisaoka et al., 1993). Epithelial-mesenchymal interactions have been shown to be important for proper morphogenesis of the pancreas; interestingly, the mesenchymal contribution is not organ specific (Fell and Grobstein, 1968). Met transcripts are produced in the epithelia of the developing pancreas, whereas HGF/SF is expressed in the adjacent mesenchyme (Sonnenberg et al., 1993). In our in vitro morphogenesis system, we can thus simulate a first phase of pancreatic development by the action of HGF/SF; the factors for later stages, such as the formation of acini, are presently unknown.

When mammary epithelial cells were employed in the organogenesis assay, we were able to induce even further steps of development: in the presence of HGF/SF, EpH4 mammary cells developed branched ducts with end buds which frequently carry a lumen. Other investigators also demonstrated branching morphogenesis of mammary gland epithelial cell lines upon addition of HGF/SF (Berdichevsky et al., 1994; Soriano et al., 1995); in the latter case, lumen formation was enhanced by the addition of hydrocortisone. We found that in EpH4 cells, lumen formation was not promoted by hydrocortisone (data not shown). Mammary gland development is under control of mesenchymal-epithelial interactions (DeOme et al., 1958; Sakakura et al., 1991). In embryogenesis, mammary buds develop from the ventral epidermis, grow into the underlying fat pad, and begin to branch. During adolescence, elongation and branching is strongly intensified leading to the fully developed glandular tree. During pregnancy, lobulo-alveolar morphogenesis takes place, i.e., the functional units of milk production are formed. We could recently show that HGF/SF promotes branching morphogenesis of mammary gland organ cultures, and that HGF/SF transcription is upregulated during puberty in the mammary gland mesenchyme. Interestingly, lobulo-alveolar morphogenesis and the production of milk proteins was inhibited by HGF/SF, but stimulated by neuregulin, another mesenchymal ligand of epithelial tyrosine kinase receptors (Yang et al., 1995). We conclude that the first phase of mammary gland development, branching morphogenesis, can be simulated in three-dimensional collagen gels; it needs to be shown whether in this assay, lobulo-alveolar differentiation can be induced by neuregulin.

As in the mammary gland, mesenchymal-epithelial interaction in the prostate begins during fetal development and continues into adulthood. During invasion into the surrounding mesenchyme, the primary ducts elongate and branch peripherally, thus forming an extensive glandular network. Androgen induces the production of keratinocyte growth factor in the mesenchyme which can elicit a paracrine signal (Cunha et al., 1994; Cunha, 1994; for a re-

view). Here we show that HGF/SF induces branching morphogenesis of the human epithelial cell line pRNS-1-1 which resembles prostatic branching patterns, i.e., long extensions with distal arborizations are formed (Cunha et al., 1986; Timms et al., 1994). Thus, our data suggest a potential role of HGF/SF in mesenchymal-epithelial signaling of the prostate gland, particularly since the branching is characteristically different to that of kidney and breast epithelial cells.

During alveolar development of the lung, drastic changes in epithelial cell shape occur from columnar to flattened cellular arrangement. This results in an extreme enlargement of the surface area, which is a prerequisite for the respiratory function of lung cells. Again, these processes are regulated by mesenchymal-epithelial interactions (Wessels, 1977; Bernfield et al., 1984; Chen and Little, 1987). When we cultured LX-1 lung carcinoma cells producing HGF/SF in an autocrine fashion, we observed a similar change to flattened cells lining large cavities. It appears that a part of lung development can thus be simulated in the organogenesis assay by HGF/SF. It was previously shown that during development, HGF/SF is expressed in the lung mesenchyme in close vicinity to bronchiolar epithelium (Sonnenberg et al., 1993).

There are two types of mesenchymal-epithelial interactions known: in the first, different mesenchymes induce one particular program such as in the exocrine pancreas (Fell and Grobstein, 1968). In a second, the different mesenchymes can be instructive, e.g., mammary gland epithelia develop features of salivary glands in the presence of salivary gland mesenchyme (Kratochvil 1983). Our data suggest that HGF/SF is not an instructive factor. HGF/SF induces epithelial cells from various organs to develop characteristic tissue-specific programs and is thus a nonrestrictive inducer, i.e., activates the respective program but does not dictate it. Apparently, the induced morphogenic programs are endogenous to the various epithelial cells that seem to have a "memory" which form is appropriate; the exact shape is dictated by the tissue of origin. This conclusion is in line with the finding that HGF/SF is expressed in a wide variety of mesenchymes during embryogenesis (Sonnenberg et al., 1993). We might thus speculate that the general function of HGF/SF is to allow epithelial cells to rearrange. A prerequisite for such rearrangements might be the ability of HGF/SF to promote scattering and invasiveness of various epithelial cells. It is also known that HGF/SF induces the production of matrix-degrading proteases (Pepper et al., 1992), which would allow such rearrangement. In conclusion, our data suggest HGF/SF to be a general epithelial morphogen with a comprehensive function. The induced programs, however, depend on the origin of the epithelial cells.

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