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Evidence for the identity of human scatter factor and human hepatocyte growth factor

(motility factor/tumor cell invasion/chromosomal localization/c-met tyrosine kinase receptor/lung fibroblast-derived mitogen)

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ABSTRACT **Scatter factor (SF), a secretory protein of fibroblasts, dissociates and increases the motility of epithelial cells and may be involved in cell migration processes during embryogenesis and tumor progression. Hepatocyte growth factor (HGF), a protein isolated from serum of patients with liver failure, is a potent mitogen for hepatocytes and is thought to play a role in liver regeneration. Here we present structural and functional evidence that human SF and human HGF (and also the human lung fibroblast-derived mitogen) are identical proteins encoded by a single gene, since (*i*) no major difference could be found by protein sequencing, by cDNA analysis, and by immunological comparison and (*ii*) SF in fact acts as a hepatocyte growth factor—i.e., stimulates DNA synthesis of primary hepatocytes—whereas HGF exhibits scatter factor activity—i.e., dissociates and induces invasiveness of various epithelial cells. The human SF/HGF gene was localized to chromosome bands 7q11.2–21. These results have important consequences for further studies on the involvement of SF/HGF as a modulator of cellular growth and motility in embryonal, malignant, and regenerative processes.**

Cell motility factors have been described as a group of cytokines that selectively stimulate cell migration with little or no effect on cell proliferation. This group of proteins includes scatter factor (SF), which dissociates epithelial cells and acts in a paracrine fashion (1–3), autocrine motility factor, which is derived from melanoma and breast carcinoma cells (4), and migration-stimulating factor, which specifically affects fibroblasts (5). It is not known whether these factors exhibit structural similarities or whether they represent a collection of diverse proteins with related biological activities. Growth factors, on the other hand, often influence both proliferation and cell motility. For example, platelet-derived growth factor or transforming growth factor β promotes or inhibits cell migration, respectively (6, 7).

We have recently reported that SF purified from conditioned medium of human MRC5 fibroblasts is a 92-kDa glycoprotein, which can be proteolytically cleaved into disulfide-linked 62- and 34/32-kDa subunits (ref. 3; compare also with refs. 8 and 9). Sequence analysis of tryptic peptides from both subunits of SF revealed a strong similarity to sequences of human hepatocyte growth factor (HGF; ref. 3).** Initially, HGF was discovered in the serum of patients with fulminant hepatic failure and was purified from plasma of those patients (11–13). Recently, the cDNA sequence of human HGF has been determined by independent laboratories (14, 15). It encodes a protein of 728 amino acid residues including a putative signal peptide at the N terminus. Heavy and light chains are then produced from the common translation product by proteolytic processing. The mature heavy chain consists of an N-terminal region followed by four kringle modules; the light chain shows homologies to the serine protease domain of plasminogen. However, 2 amino acid residues essential for the catalytic properties of serine proteases, histidine and serine, are replaced by glutamine and tyrosine, respectively, in HGF (14, 15). Recent observations indicate that the sequence of a human lung fibroblast-derived mitogen with a broad cell-type specificity is also identical to HGF (16). Furthermore, HGF binds to and activates the c-*met* protooncogene product, a transmembranous tyrosine kinase receptor (refs. 17 and 18; L. Naldini, K.M.W., W.B., G. Michalopoulos, and P. M. Comoglio, unpublished data).

In the present communication we present evidence that human SF and human HGF are structurally and functionally identical proteins encoded by a single gene located on chromosome bands 7q11.2–21.††

MATERIALS AND METHODS

Origin and Assays of SF and HGF. SF was purified from conditioned medium of human MRC5 fibroblasts (3); human HGF was a recombinant protein produced in Chinese hamster ovary (CHO) cells (14). The cell dissociation and cell invasion assays were performed as described (3, 19, 20). [^{3}H]Thymidine incorporation into rat primary hepatocytes was measured according to ref. 13. Western blot analysis of SF was performed with a monoclonal antibody against human HGF. This antibody is species specific and does not cross-react with related proteins such as human plasminogen. Recombinant HGF and purified SF were subjected to SDS gel electrophoresis (4–20% gradients) under nonreducing conditions and were electroblotted onto nitrocellulose membranes. The membranes were incubated with anti-HGF monoclonal antibody and specifically stained by using a Vectastain ABC kit (Vector Laboratories).

Cloning of SF cDNA from Human Fibroblasts and Chromosomal Localization of the Human SF Gene. A PCR product was generated from human MRC5 fibroblast cDNA by using degenerated oligonucleotide primers designed from SF peptides b and d (Fig. 1)—i.e., GCTGGGAT/CTCTCAA/GTAT/CCCICA (I, inosine) (the first T is position 985; cf. refs. 14 and 15) and GCTCIGGT/CTCCCAA/GAAG/

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Abbreviations: SF, scatter factor; HGF, hepatocyte growth factor; EGF, epidermal growth factor.

**Sequence similarity between the N-terminal amino acids of the light chains of mouse SF and rat HGF (21 of 24 residues) was also observed by Gherardi and Stoker (10).

††The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M73239 and M73240).

TATG/ATG (the first T is position 1292). PCR was run for 30 cycles (denaturation, 2 min at 94°C; anealing, 3 min at 55°C; amplification with *Taq* DNA polymerase, 5 min at 72°C). Various SF cDNA clones were isolated from a human MRC5 fibroblast cDNA library constructed in λgt10 according to ref. 21. SF cDNA inserts were cloned into the Bluescript SK$^+$ plasmid (Stratagene) and sequenced by using T7 and T3 oligonucleotide primers and T7 DNA polymerase (Pharmacia). For Northern blot analysis, poly(A)$^+$ RNA (2 μg) blotted onto Hybond-N membranes (Amersham) was hybridized with the PCR product and the various cloned cDNA probes as described (22). For Southern blot analysis, human placental DNA was digested with *Pst* I or *Sac* I, and 5 μg per lane was analyzed on 0.6% agarose gels, followed by blotting onto nitrocellulose filters and hybridization with random-primed cDNA probes as described (22). The last two washing steps were with 0.5× standard saline citrate/0.1% SDS at 64°C for 15 min each.

Chromosomal *in situ* hybridization was performed as described (23). A human SF cDNA probe covering the entire coding region was radiolabeled by nick-translation with two tritiated nucleotides ([^{3}H]dCTP and [^{3}H]dTTP) to a specific activity of 1.2×10^7 dpm per μg of DNA.

RESULTS

Identity of Amino Acid Sequences of Human SF and Human HGF. The predicted domain structure of mature HGF (14, 15) is shown in Fig. 1*B*; the heavy (H) chain is composed of a short N-terminal region followed by four kringle modules, and the light (L) chain is produced by proteolytic cleavage of the precursor peptide at the indicated Arg-Val but remains covalently linked to the heavy chain via a disulfide bridge. The seven tryptic peptides we have sequenced from human SF (Fig. 1*A*; see also ref. 3) correspond to the N-terminal region of human HGF (peptide a), to kringles 3 and 4 of the heavy chain (peptides b–e), and to sequences of the light chain (peptides f and g). Of the 72 amino acids that could be identified unequivocally (representing 10% of the full-length protein), no differences to HGF were detected (for details, see also legend of Fig. 1). Furthermore, a monoclonal antibody raised against and specific for human HGF recognizes human SF on Western blots (Fig. 2).

SF and HGF Exhibit Identical Biological Activities. To further evaluate the degree of relationship between the two proteins, we tested HGF in the motility assays and SF in the hepatocyte proliferation assay. We used SF purified from conditioned medium of human MRC5 fibroblasts (3); human HGF was a recombinant protein produced in CHO cells (14).

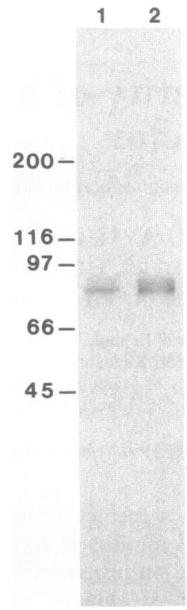

FIG. 2. Western blot analysis of human SF with a monoclonal antibody raised against human HGF. This antibody is species specific and does not crossreact with related proteins such as human plasminogen; 6.3 ng each of recombinant HGF (lane 1) and purified SF (lane 2) was analyzed with the anti-HGF monoclonal antibody. Molecular mass markers are in kDa.

In fact, HGF dissociates SF-sensitive Madin–Darby canine kidney (MDCK) epithelial cells, Hs 766T human pancreas carcinoma cells, and A 549 human lung carcinoma cells in tissue culture (Fig. 3). HGF also induces the invasion of MDCK epithelial cells into collagen matrices in the same dose-dependent manner as SF (Fig. 4) and promotes the invasion of various human carcinoma cell lines (data not shown). Conversely, SF promotes the growth of hepatocytes in a fashion similar to that of HGF (Fig. 5). In combination with epidermal growth factor (EGF), SF and HGF exhibit a strong synergistic effect on hepatocyte proliferation.

The biological assays thus clearly demonstrate that SF is a hepatocyte growth factor and, vice versa, that HGF is a scatter factor. Furthermore, since the two proteins also have the same molecular mass, the same subunit structure (3, 13), and the same amino acid sequence within the 10% analyzed, and since they are immunologically related, we presume that SF and HGF are identical proteins.

Isolation and Characterization of the SF cDNA from Human Fibroblasts. Further evidence for the identity of the two proteins was obtained by characterization of the SF cDNA and by Northern and Southern blot analyses. Initially, we amplified a part of the SF cDNA by PCR with two oligonucleotide pools of limited degeneracy designed according to sequences of SF peptides b and d (Fig. 1). A 0.31-kilobase

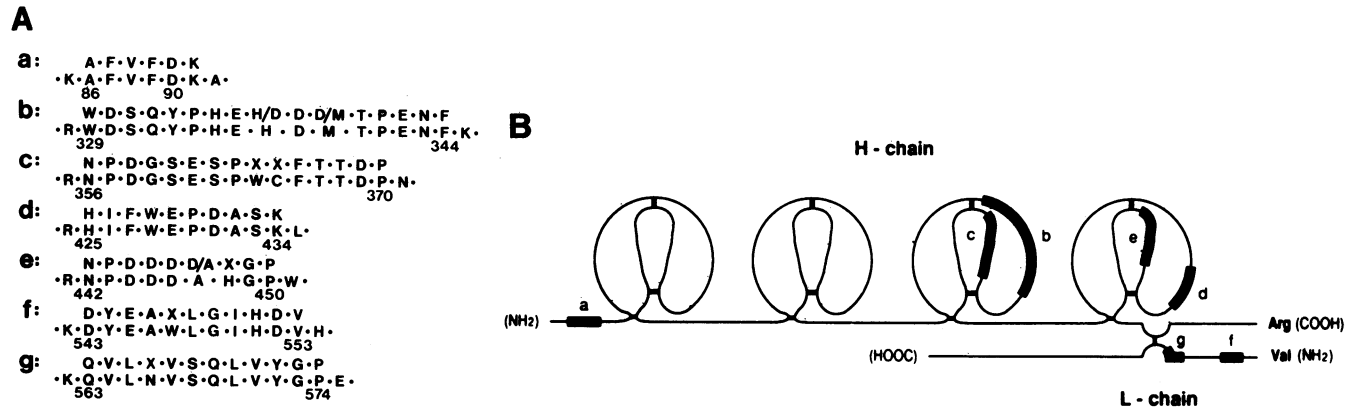

FIG. 1. Amino acid sequences of tryptic peptides of human SF and their localization within the sequence of human HGF. (*A*) Peptides a–g of SF (upper lines) were sequenced as described (3) and are compared with the corresponding regions of HGF (lower lines, see also ref. 14). X indicates the absence of any identifiable residue. In three cases (i.e., in peptides b and e), two amino acids were found equally in the particular sequencing cycle. Note that all clearly identifiable residues of SF are identical to the corresponding sequences of HGF. (*B*) Location of the peptides within HGF (the scheme is generated from ref. 14).

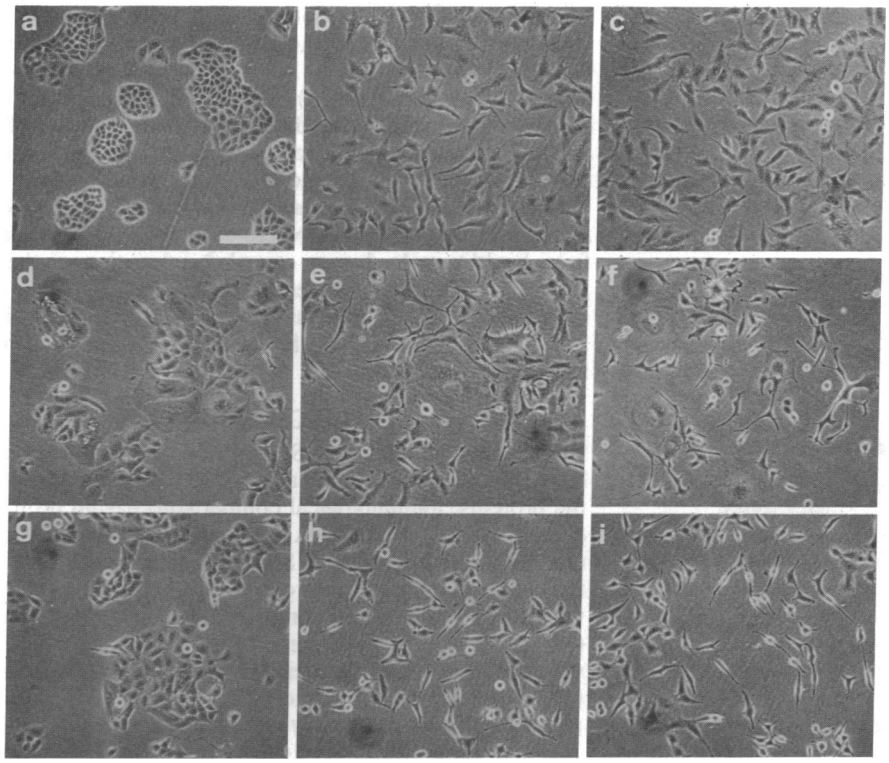

FIG. 3. SF (*b*, *e*, and *h*) and HGF (*c*, *f*, and *i*) dissociate colonies of various epithelial cell lines: MDCK (*a–c*), Hs 766T (*d–f*), and A 549 (*g–i*). Factors (10 ng/ml) were added and left overnight under the conditions described in ref. 3. (*a*, *d*, and *g*) Respective controls without factor. For the origin of the cell lines see ref. 3. (Bar = 100 μm.)

(kb) DNA fragment was obtained when cDNA from human fibroblasts was amplified. When this PCR fragment (probe I; Fig. 6*A*) was used as a probe for Northern blot analysis, RNA transcripts 6 and 3 kb long, as well as a minor transcript 4.5 kb long, were detected in RNA from human fibroblasts (Fig. 6*B*, lane a). No hybridization signal was obtained when RNA from human epithelial cells was analyzed (lanes b and c). A cDNA library from human fibroblast RNA was then synthesized, and various SF cDNA clones were isolated with oligonucleotides and the PCR product as probes and were characterized by sequence analysis. One of the isolated cDNA clones contained an insert 2.7 kb long encoding the entire SF protein. Two fragments of this cDNA clone were isolated and used as probes for Northern blot analysis (fragments II and III indicated in Fig. 6*A*). Again, RNA transcripts 3 and 6 kb long were observed (Fig. 6*B*, lanes d and e). However, when a different cDNA clone corresponding to sequences of the 3′ untranslated region was used as a probe (probe IV), the 3-kb transcript was not detected. This indicates that the two SF mRNA species presumably are created by differential poly(A) site usage (cf. ref. 25). In accordance with this, one of our cDNA isolates contains only a short 3′ untranslated sequence followed by a stretch of poly(A) (data not shown).

Southern analysis with two SF cDNA fragments as probes (III and V indicated in Fig. 6*A*) revealed single hybridizing bands in total human DNA (Fig. 6*C*, lanes a–d). A third cDNA fragment (probe II) hybridized to multiple fragments in genomic human DNA (Fig. 6*C*, lanes e and f); this fragment corresponds to sequences encoding three kringle domains and might therefore be encoded by several exons. Together,

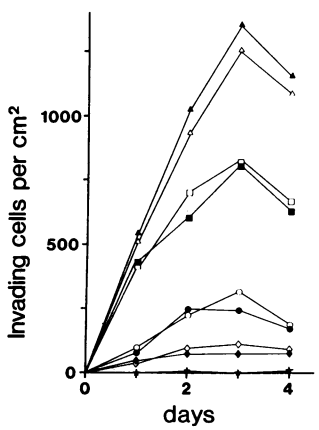

FIG. 4. SF and HGF induce the invasion of MDCK epithelial cells into collagen matrices. Cells were plated on gels of collagen type I and invading cells were counted under light microscopy (3, 24). Open symbols, SF; solid symbols, HGF; ★, without factor; ◇ and ◆, 1 ng/ml; ○ and ●, 3 ng/ml; □ and ■, 10 ng/ml; △ and ▲, 20 ng/ml.

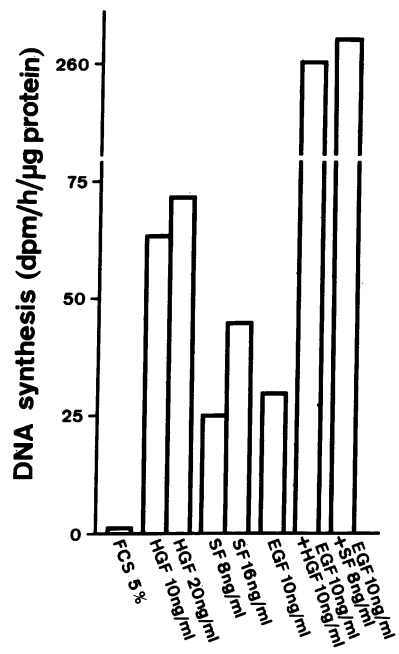

FIG. 5. HGF and SF stimulate DNA synthesis of cultured primary rat hepatocytes as determined by [^{3}H]thymidine incorporation (cf. ref. 11). Under the conditions shown, HGF and SF promoted DNA synthesis 30- to 50-fold; stimulation in combination with EGF was 200-fold. FCS, fetal calf serum.

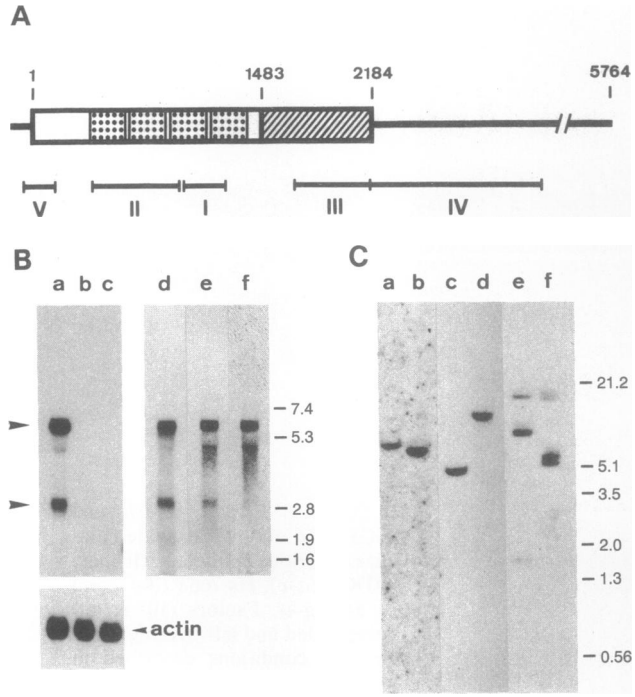

FIG. 6. Northern and Southern blotting of SF. (*A*) Localization of the probes used within the SF/HGF cDNA (nucleotides are numbered according to ref. 14, the coding region is boxed, kringles are dotted, and the protease domain is hatched). Probe I is a PCR product generated from human MRC5 fibroblast cDNA using degenerated oligonucleotide primers designed from SF peptides b and d. Probes II, III, and V are derived from a SF cDNA clone covering the protein coding region. Probe IV was isolated from a clone extending into the 3′ noncoding region. All clones were isolated from a human MRC5 fibroblast cDNA library (positions: II, 400–968; III, 1747–2176; IV, 2177–3352; V, −65–121; cf. refs. 14 and 15). (*B*) Northern blot analysis. Poly(A)$^+$ RNA from MRC5 human fibroblasts (lane a), RT4 human bladder carcinoma cells (lane b), and LX-1 human lung carcinoma cells (lane c) was probed with the PCR product (probe I). Poly(A)$^+$ RNA from MRC5 human fibroblasts was probed with probe II (lane d), probe III (lane e), and probe IV (lane f). Equal loading in lanes a–c was checked by actin staining. (*C*) Southern blot analysis. Human placental DNA digested with *Pst* I (lanes a, c, and e) and *Sac* I (lanes b, d, and f) was analyzed with probe V (lanes a and b), with probe III (lanes c and d), and with probe II (lanes e and f). RNA and DNA size markers are in kb.

these data indicate that a single gene for SF exists in the human genome and that the different transcripts in MRC5 fibroblasts are most likely the product of a single transcription unit. The cDNA and putative amino acid sequence of SF from human MRC5 fibroblasts (Fig. 7) was found to be identical to human HGF (14, 26) and to human lung fibroblast-derived mitogen (16). All seven tryptic peptides of SF (Fig. 1, underlined in Fig. 7) are found in this sequence. One of three cDNA clones encoded a variant SF with an in-frame deletion of five amino acid residues in the first kringle module (Fig. 7; see also refs. 16 and 26).

Chromosomal Localization of the Human SF/HGF Gene. *In situ* hybridization of SF cDNA to 66 lymphocyte metaphases from healthy male donors revealed a location of the SF/HGF/lung fibroblast-derived mitogen gene on human chromosome 7q11.2–21. Of 188 silver grains, 13.8% were found to be specifically located in this region. χ^2 values are highly significant ($P < 0.001$).

DISCUSSION

In this study, we show that SF and HGF are indistinguishable by the following criteria: analysis of the biological activity of the proteins, immunological comparison, partial protein sequence analysis, and cDNA sequence comparison. A molecular characterization of the SF gene supports this further. In the human genome, a single copy of the SF/HGF gene exists, which is located on chromosome 7. Two major mRNA transcripts of this gene were found in human fibroblasts, which are presumably generated by differential poly(A) site usage. Determination of the SF cDNA sequence and comparison with the previously characterized human HGF (14, 26) and lung fibroblast-derived mitogen (16) sequence showed complete agreement. The meaning of the variations in the HGF sequence described in ref. 15 is unclear at this point. Together, these data indicate that SF, HGF, and lung fibroblast-derived mitogen are structurally and functionally identical. The physiological role of possible structural microheterogeneities (e.g., the five-amino acid deletion in kringle 1) remains to be analyzed further.

It is well known that several growth factors, besides promoting cell proliferation, can also influence differentiation and cell motility—e.g., EGF, platelet-derived growth factor, transforming growth factor β, fibroblast growth factors, nerve growth factor (refs. 6 and 7; see also refs. 27 and 28 for reviews). Apparently, SF/HGF/lung fibroblast-derived mitogen represents another cytokine that can exert such bimodal activity. It is remarkable, however, that SF/HGF seems to mediate different effects in different biological systems: SF is not mitogenic for some epithelial cell lines tested (2, 19), while HGF is a potent mitogen for primary hepatocytes, and lung fibroblast-derived mitogen stimulates proliferation of various cell types. On the other hand, SF clearly modulates the motility of various epithelial and endothelial cell lines (1–3, 29). It is possible that the different

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1                                31
MWVTKLLPALLLQHVLLHLLLLPIAIPYAEG
32                                                                  127
QRKRRNTIHEFKKSAKTTLIKIDPALKIKTKKVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYENKDYIRN

128                                                            210
CIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGKDLQENYCRNPRGEEGGPWCFTSNPEVRYEVCDIPQCSEVE
211                                                                 304
CMTCNGESYRGLMDHTESGKICQRWDHQTPHRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPLETTE
305                                                            390
CIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGSESPWCFTTDPNIRVGYCSQIPNCDMSHGQD
391                                                                                             494
CYRGNGKNYMGNLSQTRSGLTCSMWDKNMEDLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNLDHPVISCAKTKQLR

495                                                                                             594
VVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRDLKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFV
595                                                                                             694
STIDLPNYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAGAEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPG
695                              728
RGCAIPNRPGIFVRVAYYAKWIHKIILTYKVPQS*
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FIG. 7. Predicted amino acid sequence of human SF (signal peptide, positions 1–31; N-terminal region, positions 32–127; four kringle domains and connecting spacers, positions 128–494; light chain exhibiting similarity to serine proteases, positions 495–728). The seven sequenced tryptic peptides are underlined. The five-amino acid deletion in the first kringle domain found in a variant cDNA clone is boxed.

biological effects of SF/HGF are mediated by either different cell-surface receptors or different intracellular signal cascades, or that identical signal cascades activate different target genes in the various cell types. However, a common receptor for SF/HGF/lung fibroblast-derived mitogen has recently been discovered—i.e., the c-*met* protooncogene product (refs. 17 and 18; L. Naldini, K.M.W., W.B., G. Michalopoulos, and P. M. Comoglio, unpublished data). Binding of the factors to this surface receptor results in the activation of the cytoplasmically located tyrosine kinase.

The different sources of production of SF/HGF might also suggest multiple functions of the factor. SF was identified in human mesenchymal cells of tissue culture—i.e., in fibroblasts and smooth muscle cells (1–3, 16). SF was also detected in the second trimester human placenta and the corresponding amniotic fluid and, furthermore, primary fibroblasts isolated from human placenta were found to be a source (29). HGF activity was detected in serum and plasma of patients with various liver diseases (14). Furthermore, HGF is present in human placenta (14), human liver (15), and human leukocytes (26). In experimental animals, HGF was detected in additional cells and tissues. For instance, rat HGF was purified from platelets (24) and was also measured by Northern analysis in lung, kidney, thymus, and brain (30). Hepatopoietin A, which is the rabbit homologue of human HGF, was immunohistochemically localized to acinar cells of the pancreas, to C cells of the thyroid, to ductal cells of the salivary glands, to Brunner's glands of the duodenum, and to some large neurons of the brain (31). In these latter tissues—e.g., glandular epithelia and neurons—SF/HGF might thus even exert additional and as yet unknown functions.

We have discovered another activity of SF/HGF—i.e., the induction of invasiveness of epithelial (carcinoma) cells into extracellular matrices (ref. 3; this work). We have also shown that the invasion capacity of the carcinoma cells correlates with the degree of dedifferentiation (20, 22). We could therefore hypothesize that SF/HGF, which changes epithelial cells toward a more fibroblastoid phenotype, might also be involved in the progression of carcinomas toward a more malignant stage *in vivo*. Liver regeneration also requires extensive dedifferentiation of tissue, and HGF has been suggested to be involved in this process (11, 12). The common denominator might thus be the involvement of SF/HGF in both types of dedifferentiation events, one being a highly uncontrolled process leading to increased metastatic potential of epithelial cells, and the other being a tightly controlled process, responsible for ordered regeneration of liver tissue. A contribution of SF/HGF to other dedifferentiation/differentiation processes—for example, during epithelial–mesenchymal transitions in development (cf. ref. 32)—would be possible.

The human gene for SF/HGF/lung fibroblast-derived mitogen has here been localized to the chromosome segment 7q11.2–21. A number of genes involved in cell growth and differentiation as well as in tumor invasion and metastasis have been mapped to chromosome 7 (33). Numerical and structural aberrations concerning chromosome 7 occur nonrandomly in different hematological and solid tumors (34): The chromosome bands 7q11.2–21 are found to be affected by deletions, inversions, and translocations in acute nonlymphocytic leukemia, in chronic myeloid leukemia and other chronic myeloproliferative disorders, in myelodysplastic syndromes, and in acute lymphocytic leukemia. In malignant lymphomas and in several solid tumors, 7q11–21 aberrations were also observed but with lower frequencies than in hematopoietic malignancies. Whether the gene for SF/HGF is affected in these malignancies remains to be shown.

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