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A large cell-adhesive scatter factor secreted by human gastric carcinoma cells

KAORU MIYAZAKI*, YAMATO KIKKAWA, AKIHIRO NAKAMURA, HIDETARO YASUMITSU, AND MAKOTO UMEDA

Division of Cell Biology, Kihara Institute for Biological Research, Yokohama City University, 2-120-3 Nakamura-cho, Minami-ku, Yokohama 232, Japan

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ABSTRACT **Human gastric carcinoma cell line STKM-1 secretes a large protein that induces scattering of a rat liver epithelial cell line (BRL) into disconnected individual cells in monolayer culture. This cell-scattering factor was purified from serum-free conditioned medium of STKM-1 cells and found to be composed of three disulfide-linked subunits of 140, 150, and 160 kDa. The 140-kDa peptide contains an amino acid sequence homologous to that of the laminin B2t chain. The native protein has an apparent molecular mass of >1000 kDa and a pI of 5.0. In addition to the cell-scattering activity, the purified protein stimulates attachment of BRL cells to substrate and their migration. Similar effects have been observed toward various cell lines, including nontumorigenic epithelial, endothelial, and fibroblastic cell lines and human cancer cell lines. Similar cell-scattering activity was secreted by human squamous carcinoma and gastric carcinoma cell lines and nontumorigenic epithelial and endothelial cell lines. These results indicate that the protein, named "ladsin," is probably an extracellular matrix protein that regulates cell–cell and cell–substrate interactions and cell migration.**

Cellular movement and migration play an important part in various physiological and pathological processes such as embryogenesis, tissue repair, and tumor invasion (1, 2). Several groups of proteins are known to regulate cell motility or cell migration (for review, see ref. 3). Various peptide growth factors such as epidermal growth factor, platelet-derived growth factor, fibroblast growth factors, and insulin-like growth factors stimulate not only DNA synthesis but also chemotactic migration of some specific cell types (4–6). The second group contains cell adhesion molecules such as fibronectin, laminin, and collagens that mediate cell–substrate adhesion and stimulate chemotactic migration of various kinds of cells (7). Similar extracellular matrix proteins, osteonectin (or SPARC), tenascin, and thrombospondin also stimulate cell motility and modulate cell–substrate interaction of many cell types (8–11).

In addition to the above groups of proteins, recent studies have revealed a unique group of cell motility factors. Scatter factor is a fibroblast-derived protein that stimulates cell motility and separates contiguous sheets of some kinds of epithelial cells into disconnected individual cells (i.e., scattering) (12). This factor, a disulfide-linked heterodimer of 57- and 30-kDa peptides (13, 14), was recently shown to be identical to hepatocyte growth factor (HGF) (15), the potent mitogen for hepatocytes (16). HGF/scatter factor is supposed to function as a paracrine effector of epithelial–mesenchymal interaction. Migration-stimulating factor (70 kDa) is secreted by human fetal fibroblasts and cancer patient fibroblasts and stimulates the migration of normal adult fibroblasts (17). Autocrine motility factor, a protein of ≈55 kDa purified from the conditioned medium (CM) of metastatic human melanoma cell line A2058, stimulates both random and chemotactic motilities of the producer cells in an autocrine manner (18).

Metastatic dissemination involves active locomotion of tumor cells in many invasive processes, such as liberation from a primary site, intravasation, and extravasation. It is reasonable to hypothesize that the tumor-derived motility factors are responsible for the highly motile behavior of invasive tumor cells. In the present study, we found that a metastatic gastric carcinoma cell line, STKM-1, secreted into culture medium a protein capable of dispersing nontumorigenic rat liver epithelial cells in monolayer culture. This protein, tentatively named ladsin, was purified to homogeneity from the CM of STKM-1 cells. The physicochemical and biological properties of ladsin are described in this report.

MATERIALS AND METHODS

Cells and Culture Conditions. The nontumorigenic epithelial cell line BRL has been established from the liver of a normal Buffalo rat by Coon (19). This cell line is highly sensitive to transforming growth factor β and some other growth-inhibitory factors (20, 21). The gastric adenocarcinoma cell line STKM-1, which secreted a high cell-scattering activity into culture medium, was a kind gift from S. Yanoma (Kanagawa Cancer Center, Japan).

Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2/95% air. Unless otherwise noted, Dulbecco's modified Eagle's medium (DMEM)/F-12 (GIBCO) supplemented with 15 mM Hepes, $NaHCO_3$ (1.2 mg/ml), penicillin G (100 units/ml), and streptomycin sulfate (0.1 mg/ml) was used as the basal medium. Cultures were grown in the basal medium supplemented with 10% (vol/vol) fetal calf serum (HyClone). Plastic culture dishes were gifts from Sumibe Medical (Tokyo).

Purification of Cell-Scattering Factor (CSF). The serum-free CM of STKM-1 cells was prepared in roller bottles containing RPMI 1640 medium, concentrated ≈180-fold by ammonium sulfate precipitation, and subjected to molecular-sieve chromatography on a Cellulofine GCL 2000-m column (2.6 × 98 cm; Chisso, Tokyo) preequilibrated with 20 mM Tris·HCl (pH 7.5) containing 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and 500 mM NaCl, as reported (23). Active fractions from the molecular-sieve chromatography were pooled, dialyzed against 20 mM Tris·HCl (pH 7.5) containing 1% CHAPS (Tris·HCl/CHAPS), and then applied to a heparin-Sepharose CL-6B column (1.6 × 9 cm; Pharmacia LKB) preequilibrated with the same buffer, at a flow rate of 15 ml/h. Proteins bound to the column were eluted with the Tris·HCl/CHAPS supplemented (*i*) with 250 mM NaCl and (*ii*) with 500 mM NaCl. The cell-scattering activity was eluted with the buffer containing

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Abbreviations: CSF, cell-scattering factor; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CM, conditioned medium; HGF, hepatocyte growth factor.

*To whom reprint requests should be addressed.

500 mM NaCl. The active fractions were combined, dialyzed against the Tris·HCl/CHAPS, and then applied to a Shodex QA-824 anion-exchange column (8 × 75 mm; Showa Denko, Tokyo), preequilibrated with the same buffer, at a flow rate of 0.5 ml/min. Proteins bound to the column were eluted with a linear gradient of 0–0.5 M NaCl in 25 ml of the Tris·HCl/CHAPS.

Assay of Cell-Scattering Activity. The rat liver cell line BRL was used as the indicator cell for the assay. The cells were plated into each well of 24-well plates containing 0.5 ml of DMEM/F-12 plus 10% fetal calf serum at 7000 cells per well. Serially diluted test samples (5 μl) were added to each well and incubated in a CO_2 incubator for 2 days. After the incubation, cell-scattering was judged by microscopic observation. One unit of cell-scattering activity was defined as the minimal amount of the factor that caused scattering of BRL cells in limiting dilution. In some cases, cell-scattering activity was assayed in serum-free DMEM/F-12 supplemented with insulin (10 μg/ml), transferrin (10 μg/ml), and fibronectin (5 μg/ml) (DMEM/F-12+3F) at 20,000 cells per well.

SDS/PAGE. SDS/PAGE was carried out on 7.5% slab gels (90-mm long, 90-mm wide, 0.75-mm thick) under reducing conditions (20). Proteins were stained with Coomassie brilliant blue R-250 or with a Wako silver staining kit (Wako Biochemicals, Osaka). The molecular mass markers used are nonreduced mouse laminin (870 kDa), mouse laminin A chain (440 kDa), rabbit skeletal muscle myosin (200 kDa), *Escherichia coli* galactosidase (116.25 kDa), rabbit muscle phosphorylase *b* (97.1 kDa), bovine serum albumin (66.3 kDa), hen egg albumin (42.7 kDa), bovine carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20.1 kDa), and hen egg lysozyme (14.3 kDa).

Determination of Protein Concentration. Protein concentration was determined by the dye method with a Bio-Rad protein assay kit, using bovine IgG as the standard.

Reagents. Bovine plasma fibronectin, mouse laminin, and bovine lens type IV collagen were purchased from Nitta Gelatin (Tokyo); bovine skin type I collagen and bovine placenta types III and V collagens were from Koken (Tokyo). Rabbit antiserum against bovine type I collagen was purchased from LSL (Tokyo); rabbit antiserum against human laminin was from Chemicon; rabbit antiserum against mouse laminin was from GIBCO; rabbit antiserum against human tenascin was from Telios Pharmaceuticals (San Diego); mouse monoclonal antibody against human thrombospondin was from Oncogene Science.

RESULTS

CSF Secreted by STKM-1 Cells. Malignant human gastric carcinoma cell line STKM-1 displayed extremely irregular morphology with prominent projections or pseudopodia in culture. They showed little intercellular contact. In contrast, the normal rat liver-derived epithelial cell line BRL showed regular epithelial morphology, forming contiguous cell sheets in both serum-free and -containing media. When the serum-free CM of STKM-1 was added to the BRL cell culture, cells were markedly scattered and could not form contiguous cell sheets.

To characterize the cell-scattering activity secreted by the cancer cells, we purified the active substance from the CM of STKM-1 cells. When the serum-free CM of STKM-1 cells was subjected to molecular-sieve chromatography, the activity was eluted as a single peak corresponding to an apparent molecular mass of >1000 kDa (Fig. 1*A*). Next, the cell-scattering activity was bound to a heparin-Sepharose column and eluted with 0.5 M NaCl (Fig. 1*B*). When the resultant active fractions were applied to anion-exchange HPLC, the CSF was effectively separated from contaminating proteins (Fig. 1*C*). By repeating this step, a single protein

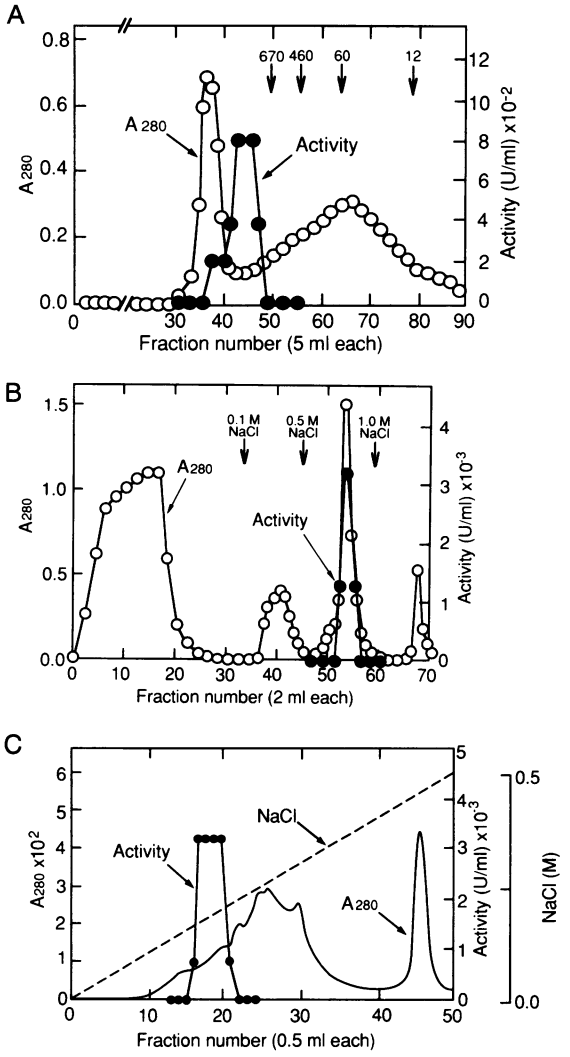

FIG. 1. Purification of CSF from CM of STKM-1 cells. (*A*) Molecular-sieve chromatography on Cellulofine GCL 2000-m column. The serum-free CM (16 liters) of STKM-1 cells was concentrated to 90 ml and divided into six equal portions. Each portion, which contained ≈150 mg of protein, was applied to the column. (*B*) Affinity chromatography on heparin-Sepharose CL-6B column. (*C*) Anion-exchange HPLC on QA 824 column. ○, A_{280}; ●, cell-scattering activity. U, unit(s).

peak of cell-scattering activity was obtained. About 0.2 mg of the CSF was purified with an activity yield of ≈4% from 16 liters of the CM.

The purified factor showed three bands of 140, 150, and 160 kDa on SDS/PAGE under reducing conditions (Fig. 2). On nonreducing SDS/PAGE, it showed a single band at ≈460 kDa (data not shown). These results suggested that the CSF was composed of three subunit peptides linked with interchain disulfide bonds. In some preparations, additional bands of 110 and 100 kDa were copurified with the three bands above. A mouse monoclonal antibody raised against the CSF cross-reacted with the 140-kDa and 100-kDa peptides, indicating that the 100-kDa peptide was a proteolytic product of the 140-kDa one. Analysis by isoelectric focusing in a sucrose density gradient column showed that the purified factor was an acidic protein with pI 5.0.

The N-terminal amino acid sequences of the CSF peptides were analyzed with an automated protein sequencer. The most probable N-terminal sequence of the 100-kDa peptide was D(28)-E(33)-N(28)-P(22)-D(26)-I(20)-E(33)-(X)-A(23)-D(29)-(X)-P(17)-I(15)-G(20)-F(15)-, where numbers in paren-

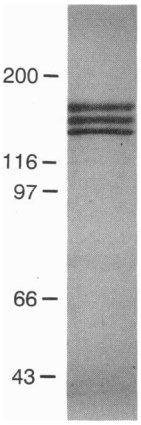

FIG. 2. SDS/PAGE of purified CSF on 7.5% gel under reducing conditions. Proteins were stained with silver. Molecular masses in kDa are shown to the left.

theses are the yield of amino acid in pmol. This sequence is consistent with an internal sequence of the laminin B2t chain from residue 435 to residue 449. The cDNA for the laminin B2t chain has recently been cloned by Kallunki *et al.* (22), but proteins containing the laminin chain have not been identified yet. The other CSF peptides gave different but less reliable sequences.

Biological Activities of Purified Factor. When the purified CSF was added into the serum-free culture of BRL cells shortly after cell plating, it caused marked scattering of the indicator cells as shown in Fig. 3. In the presence of the factor, cells did not make intercellular connections until the culture became confluent. The concentration for minimum cell scattering was ≈20 ng/ml. Cell-scattering activity of the purified factor was also observed in serum-containing culture, but to a lesser extent than in serum-free culture.

To test whether the purified CSF disrupts intercellular connection, it was added to culture medium after BRL cells had formed small contiguous cell sheets in serum-free medium. In this case, each cell gradually migrated from the cell colonies, resulting in almost complete cell scattering within 20 h (Fig. 4). This indicated that the purified protein stimulated both cell migration and the disruption of intercellular connection.

In serum-free culture, the purified protein appeared to promote cell attachment to the plastic substrate. To test this activity, plastic culture dishes were coated with the purified protein and the dishes were washed with phosphate-buffered saline (PBS). When BRL cells were plated on the treated dishes containing the protein-free basal medium, they promptly attached and spread on the dishes (Fig. 5 *Right*). In

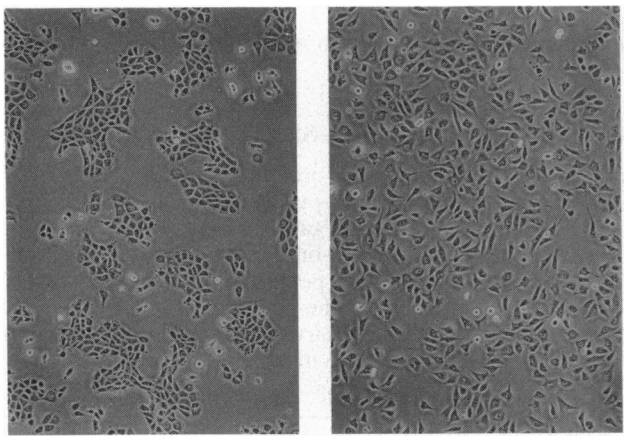

FIG. 3. Effect of CSF on morphology of BRL cells in serum-free medium. BRL cells were cultured for 2 days in a serum-free medium (DMEM/F-12+3F) without (*Left*) or with (*Right*) purified protein (60 ng/ml) and examined by phase-contrast microscopy. (×50.)

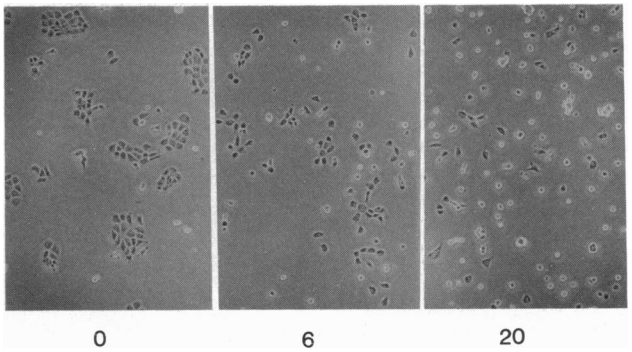

FIG. 4. Time course of scattering of BRL cells after addition of CSF in serum-free culture. BRL cells were incubated in serum-free medium for 2 days to form small contiguous cell sheets (0 h) (*Left*), and then the purified CSF (60 ng/ml) was added to the culture medium, followed by further incubation for 6 h (*Center*) and 20 h (*Right*). The photographs (×45) show the typical morphology of BRL cells. Note that the contiguous cell sheets of the indicator cells have completely been disrupted into individual cells within 20 h.

contrast, BRL cells poorly attached and spread on the nontreated control dishes (Fig. 5 *Left*). This indicated that the purified protein functioned as a cell-substrate adhesion molecule rather than a soluble cytokine. Therefore, we also tested the cell-scattering activity of fibronectin, laminin, vitronectin, and types I, II, III, IV, and V collagens toward BRL cells. These cell-substrate adhesion molecules, except for laminin, showed no cell-scattering activity (data not shown). Laminin showed a weak cell-scattering activity at >10 times the concentration of the purified CSF in serum-free medium, but not at all in serum-containing medium.

The structural relationship between purified CSF and the known cell adhesion molecules was also tested by immunoblot analysis and enzyme-linked immunoassay on microplates (data not shown). Two kinds of polyclonal antibodies against human and mouse laminin immunostained the B chains of the authentic human laminin but did not react at all with the purified factor on an immunoblot. A mouse antiserum raised against the purified factor immunostained its 140-kDa subunit but not any subunit of human laminin. These results indicated that the CSF was immunologically different from laminin. Antibodies against type I collagen, tenascin, and thrombospondin also did not cross-react with

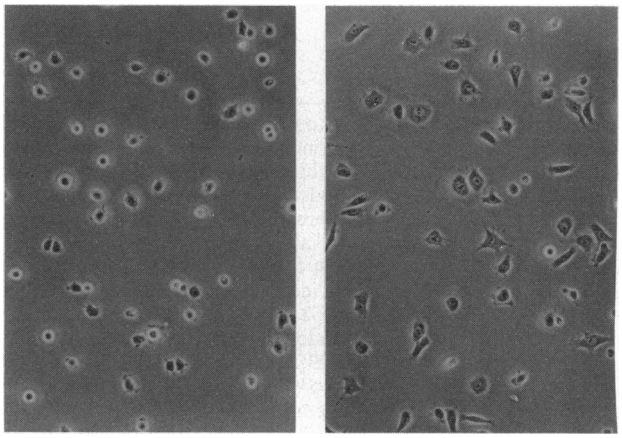

FIG. 5. Cell attachment activity of purified CSF. Plastic culture dishes (35 mm) were incubated with 1 ml of PBS alone (*Left*) or with PBS supplemented with purified CSF (0.1 μg/ml) (*Right*), incubated in a CO_2 incubator for 60 min, and then washed three times with PBS. BRL cells were plated on the pretreated dishes containing the serum-free basal medium, incubated for 45 min, and subjected to phase-contrast microscopy. (×100.)

Table 1. Sensitivities of various cell lines to purified CSF and their production of BRL-scattering activity

Cell line	Type	Sensitivity	Secretion
	Human cancer-derived cell lines		
STKM-1	Stomach ad	−	+++
MKN1	Stomach ad	+++	++
MKN28	Stomach ad	NT	−
MKN45	Stomach ad	−	++
MKN74	Stomach ad	+	++
NUGC-2	Stomach ad	+	+
KATO III	Stomach sig ca	NT	−
SCH	Stomach chorionic ca	−	−
AZ-521	Stomach ca	−	−
HLE	Hepatoma	++	−
HLF	Hepatoma	+++	−
EJ-1	Bladder ca	+++	−
T24	Bladder ca	NT	−
MIA-PaCa-2	Pancreas ca	+++	−
A549	Lung ad	++	−
CaR-1	Rectum ad	NT	−
HeLa S3	Cervic epit ca	−	++
A431	Vulva epid ca	−	++
HSC-2	Tongue sq ca	+	+
HSC-3	Tongue sq ca	−	++
HSC-4	Tongue sq ca	−	+++
C-41	Cervix epid ca	−	+++
CaSki	Cervix epid ca	−	+
HT-1080	Fibrosarcoma	+	−
YP-MeL	Melanoma	−	−
YST-3	Schwannoma	+	−
	Normal tissue-derived cell lines		
BRL	Rat liver epi	++++	−
IAR-20	Rat liver epi	+++	NT
RLC-10.P3	Rat liver epi	+++	NT
C127	Mouse mam epi	+++	++
MDCK	Canine kidney epi	++	++
BS-C-1	Monkey kidney epi	+	++
CHO-K1	CH ovary epi	+	NT
LLC-RK1	Rabbit kidney epi	+++	NT
ECV304	Human umb end	+++	−
CPA	Bovine pul end	+	++
WI-38	Human embryo fib	−	−
YH-1	Human embryo fib	−	−
NIH/3T3	Mouse embryo fib	−	−
Swiss/3T3	Mouse embryo fib	+	NT
NRK-49F	Rat kidney fib	++	NT
BHK-21	SH kidney fib	+++	NT
V79-6TG	CH lung fib	+++	NT

To test the sensitivity of cells to purified CSF, each cell line was plated at 2×10^4 cells per well in 0.5 ml of the serum-containing medium and incubated with CSF at 1, 3, 10, or 30 units per well for 2 days. The cell lines that exhibited cell scattering in response to CSF at 1, 3, 10, and 30 units/ml are expressed as ++++, +++, ++, and +, respectively. − denotes the absence of cell scattering at 30 units/ml. NT, not tested. To test the production of cell-scattering activity, confluent cultures of the indicated cell lines were incubated in serum-free DMEM/F-12 for 2 days. The resultant CM was collected, clarified by centrifugation, and concentrated 30-fold by ammonium sulfate precipitation (23). The concentrated samples were dialyzed against PBS, and 5 μl of each sample was added to the culture of BRL cells in the serum-containing medium (0.5 ml per well) at three dilutions (1:1, 1:3, and 1:9). After a 2-day incubation, the scattering of BRL cells was examined microscopically. The cell-scattering activity at 1:9, 1:3, and 1:1 dilutions is expressed as +++, ++, and +, respectively. − denotes the absence of cell scattering at a 1:1 dilution. Abbreviations: ad, adenocarcinoma; ca, carcinoma; sq ca, squamous cell carcinoma; epi, epithelium; epit, epithelioid; epid, epidermoid; umb end, umbilical vein endothelium; pul end, pulmonary artery endothelium; fib, fibroblast; CH, Chinese hamster; mam, mammary; sig, signet ring cell; SH, Syrian hamster. The sources of STKM-1 (23), C-41 (20), CaSki (20), and BRL (20) have been reported. YP-MeL and YST-3 were generous gifts from T. Funabiki and Y. Nagashima (School of Medicine, Yokohama City University), and ECV304 was from K. Takahashi (National Defense Medical College, Saitama, Japan). The other cell lines were from the Japanese Cancer Research Resource Bank.

the purified CSF, though they reacted with the respective authentic human proteins.

CSF showed little effect on the growth of BRL cells in either serum-containing or serum-free culture medium (data not shown).

Stability. Stability of the CSF was tested under various conditions. The cell-scattering activity was completely lost by heating at 100°C for 3 min, treatment with 1 M acetic acid, or reduction with 20 mM dithiothreitol, whereas 6 M urea had no effect on the activity. These results indicated that a special conformation is essential for the cell-scattering activity.

Activity of the purified factor was completely lost by incubation with trypsin but was resistant to bacterial collagenase. Analysis by SDS/PAGE showed that trypsin, but not collagenase, digested CSF into small fragments (data not shown).

We have reported that STKM-1 cells secrete at least four serine proteinases—trypsin, plasmin, tissue kallikrein, and plasminogen activator (23). However, CSF was completely separated from these proteinases during the purification procedure and did not show any proteolytic activity (data not shown).

Sensitivity of Various Cell Lines to CSF. Sensitivity of various cell lines to the purified CSF was examined in serum-containing culture (Table 1). Among normal tissue-derived cell lines tested, all 8 epithelial cell lines and 2 endothelial cell lines (CPA and ECV-304) responded to the factor to different degrees. It was difficult to judge the response of fibroblastic cells and cancer cells to the factor, because many of them did not form contiguous cell sheets. Nevertheless, 4 out of 7 fibroblastic cell lines and 11 out of 22 human cancer cell lines showed significant cell scattering when the factor was added.

The apparent lack of sensitivity to the factor in some tumor cell lines, such as the producer cell line STKM-1, MKN45, SCH, and YP-Mel, might be due to their poor intercellular contact. However, five of the six squamous cell and epidermoid carcinoma lines (A-431, HSC-3, HSC-4, CaSki, and C4I) were insensitive to the factor, despite forming contiguous epithelial sheets.

Production of CSF by Various Cell Lines. Serum-free CM of various cell lines was assayed for cell-scattering activity toward BRL cells (Table 1). Twelve of 26 human cancer cell lines (46%), including 5 of 9 gastric carcinoma cell lines, released cell-scattering activity. It is noteworthy that all 6 squamous cell and epidermoid carcinoma lines, which were mostly insensitive to purified CSF, released cell-scattering activity. Cell-scattering activity was also detected in the CM of 3 epithelial cell lines (C127, MDCK, and BSC-1) and 1 endothelial cell line (CPA) but not in the CM of the rat liver epithelial cell line BRL.

DISCUSSION

In the present study, a large protein with cell-adhesion and cell-scattering activities was purified to homogeneity from the CM of a human metastatic gastric carcinoma cell line. The purified factor was a heparin-binding acidic protein consisting of three disulfide-linked peptides of 140, 150, and 160 kDa. The molecular mass of the native protein was estimated to be >1000 kDa by molecular-sieve chromatography, suggesting that it might exist as a dimer or oligomer of the three

peptide set in CM or that, in the chromatography, the 460-kDa CSF was eluted much faster than the elution volume expected from its real molecular size due to a possibly fibrous structure.

The purified protein disrupts intercellular connection and inhibits cell–cell binding, resulting in marked dispersion or scattering of sensitive cells into disconnected individual cells. In addition, it stimulates attachment and migration of the indicator cells on plastic dishes with serum-free medium. These biological activities are observed even on plastic dishes pretreated with the purified protein. These properties suggest that the CSF is a kind of extracellular matrix protein that regulates cell–substrate and cell–cell interactions and cell migration. The cell-scattering activity may be attributed to its capacity to stimulate cell adhesion and cell migration. We propose the name ladsin for the *l*arge *ad*hesive *s*catter factor.

Cell-scattering activity has been observed in not only HGF/scatter factor but also some cytokines such as tumor necrosis factor α and acidic fibroblast growth factor and cell-adhesive proteins such as laminin and collagens for certain cell types. However, these cytokines and cell-adhesive proteins did not show cell-scattering activity on BRL cells, except for laminin, which had a weak cell-scattering activity only under serum-free conditions. Although ladsin contains an amino acid sequence homologous to the laminin B2t chain reported by Kallunki *et al.* (22), human laminin and ladsin showed different immunoreactivities to two anti-laminin and one anti-ladsin antibodies used. These results suggest that ladsin may be related to laminin, but there is a significant difference between their structures. In addition, the marked difference in their cell-scattering activities suggests that ladsin may have a different function.

Recently, two extracellular matrix molecules having a subunit structure similar to ladsin, named epiligrin and kalinin, have been identified in the culture of human keratinocytes by two groups. Epiligrin has been shown to be a ligand for cell adhesion via integrin $\alpha_3\beta_1$, which localizes in the epithelial basement membrane *in vivo* and in focal adhesions of cultured keratinocytes (24). Similarly, kalinin has been considered to be a cell adhesion component of anchoring filaments in the keratinocyte culture (25, 26). However, their primary structures and other biological activities such as cell-scattering and cell-migration-stimulating activities have not been reported. Further structural studies are required to ascertain whether the keratinocyte-derived matrix proteins and ladsin are related to each other.

Ladsin exerts cell-scattering activity toward a wide variety of cells including epithelial, endothelial, and fibroblastic cells, indicating that it may be a general cell motility factor. This is in contrast to HGF/scatter factor, which is specific to epithelial and endothelial cells. Among many human cancer cell lines, squamous cell carcinomas and gastric carcinomas secreted ladsin at high rates, though most of the squamous cell carcinomas tested were not responsive to exogenous ladsin. In tumor metastasis, the tumor cells must penetrate intercellular spaces and connective tissues. Ladsin secreted by tumor cells may contribute to their invasion to surrounding normal tissues by both autocrine and paracrine mechanisms. In the former mechanism, ladsin may increase the motility of the producer cells, stimulating their dissemination, whereas in the latter mechanism, it may cleave or loosen the intercellular junctions of surrounding cells. The latter mechanism seems especially important in the steps of intravasation and extravasation, where tumor cells must invade the barrier of vascular endothelial cells. In addition, the fact that epithelial cells and endothelial cells derived from normal tissues secrete ladsin-like activity suggests that it generally functions in cell migration and cell–cell interaction under normal physiological conditions.

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