Purification and Characterization of Human Lung Fibroblast Motility-stimulating Factor for Human Soft Tissue Sarcoma Cells: Identification as an NH2-terminal Fragment of Human Fibronectin

Mei Hu, Raphael E. Pollock, and Garth L. Nicolson

ABSTRACT

Paracrine motogenic factors, including motility cytokines and extracellular matrix molecules secreted by normal cells, can stimulate metastatic cell invasion. Both intact extracellular matrix molecules and their degradative products may exhibit these activities. We have found that human lung fibroblasts produce paracrine motility-stimulating factors for recently established human sarcoma cell strains. We purified the major fibroblast motility-stimulating factor (FMSF) from human lung fibroblast-conditioned medium by sequential heparin affinity chromatography and DEAE anion exchange chromatography. Lysylendopeptidase C digestion of FMSF and sequencing of peptides purified by reverse-phase high-pressure liquid chromatography identified FMSF as an NH2-terminal fragment of human fibronectin. Using SYN-1 sarcoma cells, FMSF predominantly stimulated chemotaxis and some chemokinesis, and it was chemotactic for a variety of human sarcoma cells, including fibrosarcoma, leiomyosarcoma, liposarcoma, synovial sarcoma, and neurofibrosarcoma cells. The FMSF activity present in human lung fibroblast-conditioned medium was completely eliminated by either neutralization or immunodepletion with a rabbit anti-human-fibronectin antibody, thus further confirming that the NH2-terminal fibronectin fragment was the FMSF responsible for the motility stimulation of human soft tissue sarcoma cells. Because human soft tissue sarcoma cells have a distinctive hematogenous metastatic pattern (predominantly lung), and lung-derived fibroblasts secrete large amounts of FMSF, FMSF and fibronectin may play a role in stimulating sarcoma invasion into lung tissue.

INTRODUCTION

Tumor cell motility is a principal requirement for malignant cells that undergo invasion during metastasis formation (1). Metastatic cell motility occurs during invasion of tumor cells across basement membranes, intravasation of the blood vasculature or lymphatics, and extravasation from the vasculature into parenchymal tissues at secondary sites (2). A variety of molecules have been found to influence tumor cell motility, including components of the extracellular matrix, such as laminin (3), FN (4), collagen (5), and elastin (6).

In addition, motogenic cytokines (7), such as platelet-derived growth factor (7), insulin-like growth factor 1 (8), HGF/SF (7), and epiregulin (9), are also important in stimulating tumor cell motility. Tumor cells can also produce AMFs, such as AMF/neureoleukin (10–12), that can stimulate both motility and growth; migration-stimulating factor (13); and autotaxin (14). These motility factors can play important roles in differentially stimulating migration of metastatic cells at primary and secondary sites.

MATERIALS AND METHODS

Materials. Liquid chromatography instruments and Macro-prep DEAE support material were purchased from Bio-Rad (Hercules, CA). Heparin-Sepharose gel was from Pharmacia (Piscataway, NJ). All other chemicals were...
purchased from Sigma Chemical Co. (St. Louis, MO) or Boehringer Mannheim Biochemical (Indianapolis, IN).

Cell Strains and Cell Lines. All human soft tissue sarcoma cell strains and lung fibroblast cell strain were established in short-term cultures directly from surgical specimens obtained at the University of Texas M. D. Anderson Cancer Center as described (23). These cell strains were cultured in DMEM/F12 (Life Technologies, Inc., Grand Island, NY) with 10% fetal bovine serum (Hyclone, Logan, UT) in 5% CO₂, 95% air at 37°C and passaged by treatment with a solution containing 0.25% trypsin (Life Technologies, Inc.) and 1 mM EDTA when they reached 80% confluence. All sarcoma cell strains were stored according to protocols described above, and the lung fibroblast cell strain was stored using passage 10. The human fibrosarcoma cell line HT1080, human leiomyosarcoma cell line SK-LMS-1 and human liposarcoma cell line SW272 were obtained from the American Type Culture Collection (Rockville, MD), cultured as described above, and were used at passages 21–24 (HT1080), passages 19–22 (SK-LMS-1), or passages 12–15 (SW272 cells). The cell strains and lines were routinely checked and found to be free of mycoplasma contamination.

Preparation of CM. For preparation of CM on an analytical scale, sub-confluent cultures of HLFs were rinsed twice with serum-free DMEM/F-12 medium and cultured in 10 ml of serum-free DMEM/F-12 medium. One day later, the culture medium was removed by aspiration, and 10 ml of fresh serum-free DMEM/F-12 medium was added to each culture. HLF-CM was collected after a 72-h incubation, and 25 mM HEPES buffer (pH 7.4), 0.5 mg/ml leupeptin, 0.7 mg/ml pepstatin, 150 mg/ml phenylmethylsulfonyl fluoride, 1 mM EDTA, 0.02% NaN₃, and 0.1% BSA (Intergen, Purchase, NY) were added. For preparation of HLF-CM on a preparative scale, roller bottle cultures of HLFs were used. HLF-CM was prepared and collected in a similar way except that the 0.1% BSA was omitted. The HLF-CM was filtered through a 0.22 μm filter (Millipore, Bedford, MA), frozen, and stored at −70°C until use.

Cell Migration Assay. Cell migration assays were performed in 24-well, 6.5-mm internal diameter Transwell cluster plates (8.0-μm pore size; Costar, Cambridge, MA) as described (23). Four random fields of each Transwell filter were counted at ×200 power, and the cell numbers were calculated as total migrated cell number per filter.

SDS-PAGE Analysis of Proteins. Discontinuous SDS-PAGE minigels (8 cm x 7 cm x 0.7 mm) with a stacking gel of 4% acrylamide/piperazine diacrylamide (39:1) and a resolving gel of 12% acrylamide/piperazine diacrylamide (39:1) were prepared and electrophoresed according to the method of Laemmli (24).

Chromatography Procedures for FMSF Purification. HLF-CM (6500 ml) was directly loaded onto a heparin-Sepharose column (7 x 2.5 cm), and the column was washed with HEPES-buffered saline (137 mM NaCl, 20 mM HEPES, pH 7.40) until absorbance at 280 nm reached baseline. The column was eluted using step gradients of 0.30, 0.40, 0.60, and 2.0 mM NaCl in 20 mM HEPES buffer, pH 7.40, and 8-ml fractions were collected. The eluates were pooled in 50 ml volumes, assessed for protein concentration by the Coomassie protein assay (Pierce, Rockford, IL), concentrated using a 10,000 molecular weight cutoff microcon unit (Amicon, Beverly, MA), resolved by SDS-PAGE, and silver stained. Motility-stimulating activity was determined in the cell migration assay using diluted eluates. The most active fractions (0.4 mM NaCl eluates) were pooled and diluted twice so that NaCl concentration was 0.2 mM. Concentrated Tris-HCl buffer, pH 8.0, was added to a final concentration of 50 mM Tris-HCl, pH 8.0. The sample was loaded onto a DEAE anion exchange column (1.5 x 15 cm) and washed with 20 mM NaCl, 20 mM Tris-HCl buffer, pH 8.0 until absorbance at 280 nm reached baseline. The column was eluted using step gradients of 0.3, 0.4, 0.5, 0.70, and 1.5 mM NaCl in 20 mM Tris-HCl buffer, pH 8.0, and 6-ml fractions were collected. The fractions were pooled according to the elution pattern, analyzed by SDS-PAGE and silver staining, and protein concentration was estimated by densitometry of silver stained protein bands using comparison with known quantities of protein standards. Motility-stimulating activity of the eluted fractions was determined using the cell migration assay. The most active fractions were pooled and dialyzed with 4 liters of 25 mM HEPES buffer, pH 7.4, and stored at −70°C. Alternatively, the samples were stored for no longer than 2 weeks at 4°C for protein sequencing.

Preparation of SDS-PAGE Purified Protein for Sequencing. Purified FMSF was concentrated with 10,000 molecular weight cutoff microcon units; mixed with reducing SDS-PAGE sample buffer so that the final concentration of buffer was 7% SDS, 10% glycerol, 5% 2-mercaptoethanol, 62.5 mM Tris-HCl, pH 6.8; and heated at 95°C for 20 min. A discontinuous SDS-PAGE gel containing a 4% stacking gel and a 12% resolving gel (24) was used. To scavenge free radicals remaining in the gel and to minimize the possibility of modification of reactive amino acid residues of the protein during electrophoresis, 0.002% thiglycolic acid was added to the electrophoresis running buffer (25, 26). To achieve a more thorough polymerization and to decrease free radicals in the gel, the resolving gels were cast 1 day in advance, and the gel solutions were thoroughly degassed prior to casting (25). The electrophoresis was performed at 200 V constant voltage for 45 min; the gel was briefly stained in a 0.25% Coomassie Blue R 250, 40% methanol, and 10% acetic acid solution for 3–5 min; and the gel was destained in a 40% methanol and 10% acetic acid solution for 15–30 min.

Microsequencing and Sequence Analysis. Protein bands were carefully cut out with a clean scalpel, washed by being vortexed briefly in 100 μl of 50% acetonitrile solution twice, and shipped moist in a clean O-ring-sealed vial (Sarstedt, Neuron, NC) with dry ice to the Harvard University Microchemistry Facility. The samples were subject to lysylendopeptidase C digestion (26), narrow-bore C18 reverse-phase high-performance liquid chromatography separation of peptides (27), and matrix-assisted laser desorption time-of-flight mass spectrometry to assess Mr, and heterogeneity of separated peptides (28). NH₂-terminal sequencing of the separated peptides was performed, and the partial amino-acid sequences were analyzed using SwissProt protein data base and the GCG program (Genetics Computer Group, Madison, WI).

Checkerboard Analysis. Three different concentrations of purified FMSF (based on its dose-response parameters) were chosen, and nine different combinations of the three concentrations were used in the upper and lower chambers of Transwells to determine motility-stimulating activities using SYN-1 cells. The result was plotted as a checkerboard analysis (29).

Analysis of Proteolytic FN Fragments and Effect of Anti-human-FN Ab. Proteolytic fragments of human plasma FN and rabbit anti-human-FN Ab were obtained from Sigma. The Ab was generated in rabbits using purified human plasma FN as immunogen and purified by FN immunofluorescent chromatography. Proteolytic fragments were diluted with DMEM/F12 medium and used in cell migration assays. To deplete the FN fragments in HLF-CM, 10 mg of affinity-purified rabbit anti-human-FN Ab per ml of CM or 10 mg purified normal rabbit Ab (control; Sigma) were used. Briefly, 1 ml of HLF-CM containing 10 mg Ab and 0.5 mg/ml leupeptin, 0.7 mg/ml pepstatin, 150 mg/ml phenylmethylsulfonyl fluoride, 1 mM EDTA, 0.02% NaN₃, 25 mM HEPES buffer (pH 7.4), and 0.1% BSA was incubated with gentle rocking at 4°C for 4 h. Protein A/G agarose beads (40 ml; Pierce) were added, and the solution was gently rocked for 2 h at 4°C. The supernatant was collected after centrifugation at 1000 x g for 5 min, dialyzed against 4 liters of 25 mM HEPES buffer overnight at 4°C, reconstituted into test medium using 5X DMEM/F-12 medium, and assayed for motility-stimulating activity using SYN-1 sarcoma cells. Alternatively, the FMSF activity was neutralized with affinity-purified rabbit anti-human-FN Ab, and the procedure was the same, except that the treatment of protein A/G agarose beads was omitted.

RESULTS

Purification of FMSF from HLF-CM. We previously found that HLF-CM contained FMSF for a variety of human soft tissue sarcoma cells, and FMSF was not related to HGF/SF (23). To study the role of human lung FMSF in invasion and lung metastasis of human soft tissue sarcomas, we purified FMSF. Because many extracellular regulatory molecules, including matogenic cytokines and extracellular matrix components, have the ability to bind heparin, heparin affinity chromatography was chosen as the first purification step. Nearly all of the motility-stimulating activity in HLF-CM was retained by heparin affinity resin and was eluted by NaCl. Most of the motility-stimulating activity was recovered in the 0.4 mM NaCl eluates (Fig. 1), and some motility-stimulating activity was found in the trailing peaks of 0.3 and 0.6 mM NaCl eluates (Fig. 1). The wide peak pattern of elution may be due to both low pressure and extensive step gradient elution. The 0.4 mM NaCl eluate pool was subject to further purification because it contained most of the motility-stimulating activity. Therefore, the 0.4
Loading sample

Migrated cell number
(Cells/µg; )

Fig. 1. Heparin affinity chromatography of FMSF. HLF-CM (6500 ml) was directly loaded onto a heparin affinity chromatography column and washed thoroughly with HEPES-buffered saline. The material was eluted with NaCl in HEPES buffer by step gradients as indicated, and eluates from the same step were pooled for analysis. Most of the motility-stimulating activity was recovered in the 0.4 M NaCl eluates.

NaCl concentration (M)

0 0.005 0.01 0.015 0.02 0.025 0.03 0.035 0.04 0.045 0.05 0.055 0.06 0.065 0.07 0.075 0.08 0.085 0.09 0.095 0.1 0.105 0.11 0.115 0.12 0.125 0.13 0.135 0.14 0.145 0.15 0.155 0.16 0.165 0.17 0.175 0.18 0.185 0.19 0.195 0.2

Migrated cell number
(Cells/µg; )

Fig. 2. DEAE anion exchange chromatography of FMSF. The 0.4 M NaCl eluates from heparin affinity chromatography were pooled, reconstituted, and loaded onto a DEAE anion exchange chromatography column. The column was eluted with NaCl step gradients, and the motility-stimulating activity was found to be eluted in the 0.4 M NaCl fraction.

NaCl concentration (M)

0 0.005 0.01 0.015 0.02 0.025 0.03 0.035 0.04 0.045 0.05 0.055 0.06 0.065 0.07 0.075 0.08 0.085 0.09 0.095 0.1 0.105 0.11 0.115 0.12 0.125 0.13 0.135 0.14 0.145 0.15 0.155 0.16 0.165 0.17 0.175 0.18 0.185 0.19 0.195 0.2

Under reducing conditions, FMSF migrated as a band with an apparent molecular weight of 34,000 and under nonreducing conditions as two bands with apparent molecular weights of 31,000 and 62,000, suggesting that FMSF had intramolecular disulfide bonds and that it formed homodimers under nonreducing conditions, possibly due to intermolecular disulfide bonds (Fig. 3). As summarized in Table 1, 6500 ml of HLF-CM containing about 120 mg of crude protein...
Chemokinesis is believed to be important in initiation and maintenance of cell migration process, and chemotaxis is thought to be important in the direction of cell migration. Thus a checkerboard analysis was performed to assess chemokinetic versus chemotactic cell migration or both. As shown in Fig. 5, FMSF predominantly stimulated chemotaxis but also stimulated some chemokinesis, suggesting that FMSF may play roles both in the initiation and maintenance and in determining the direction of sarcoma cell migration.

**FMSF Is a Chemoattractant for a Variety of Human Sarcoma Cells.** Using eight human sarcoma cell strains or cell lines representing six different tumor histopathological types, the target cell specificity of human soft tissue sarcoma cells for FMSF was studied. HT1080 fibrosarcoma, SK-LMS-1 leiomyosarcoma, and SW-872 liposarcoma cell lines were supplemented with SYN-1, SYNb-1, and SYNb-2 synovial sarcoma, NFS-2 neurofibrosarcoma, and EES-1 Ewing’s sarcoma cell strains. As shown in Fig. 6, the motility of all eight human sarcoma cells was stimulated by FMSF, although different sarcoma cells responded to different levels of FMSF. Pronounced motility responses were observed for HT1080, SK-LMS-1, and SYN-1, whereas SW-872, SYNb-1, SYNb-2, and NFS-2 cells demonstrated modest motility responses, and EES-1 cells showed poor response to FMSF, suggesting that FMSF may not be the primary lung-derived motility-stimulating factor for EES-1 sarcoma cells.

**Microsequencing of Internal Peptides of FMSF.** Because most secreted proteins of mammalian cells have blocked NH\textsubscript{2}-termini, purified FMSF was digested in gel with lysylendopeptidase C to generate internal peptides (26). The peptides were separated with narrow-bore C18 reverse-phase high-pressure liquid chromatography (27) and analyzed with matrix-assisted laser desorption time-of-flight mass spectrometry (28). Appropriate peptides were subject to NH\textsubscript{2}-terminal sequencing, and peptide sequences were analyzed. As shown in Fig. 7, two peptide sequences with molecular weights of 2113 and 1921.7, respectively, completely matched a portion of the NH\textsubscript{2}-terminal sequence of the human FN molecule. Interestingly, one peptide sequence extended beyond the NH\textsubscript{2}-terminal protein sequence of human plasma FN (32) and matched the deduced amino acid sequence from human FN cDNA (33). Because the deduced FN amino acid adjacent to the sequenced peptide on the NH\textsubscript{2}-terminal side was a lysyl residue and therefore the cleavage site for lysylendopeptidase C, the NH\textsubscript{2}-terminal sequence of FMSF may extend beyond this peptide sequence.

**Analysis of Motility-stimulating Activity of HLF-CM with Affinity-purified Anti-human-FN Ab.** Microsequencing of internal peptides of purified FMSF indicated that an NH\textsubscript{2}-terminal fragment of human cellular FN was the motility-stimulating factor for SYN-1 sarcoma cells. To confirm that the motility-stimulating activity of HLF-CM was due primarily to FMSF, affinity-purified rabbit anti-human-FN Ab was used to remove or block FMSF in HLF-CM. Using immunodepletion and neutralization with affinity-purified anti-human-FN Ab, the motility-stimulating activity in HLF-CM for SYN-1 sarcoma cells was nearly completely eliminated, confirming that an NH\textsubscript{2}-terminal FN fragment was the principal FMSF activity in HLF-CM (Fig. 8, A and B).

On the basis of the apparent molecular weight of FMSF detected in SDS-PAGE and the microsequence information, FMSF was found to be equivalent to the NH\textsubscript{2}-terminal heparin/fibrin-binding fragment of human FN. Therefore, a Mr 30,000 NH\textsubscript{2}-terminal heparin/fibrin-binding FN fragment, a Mr 45,000 gelatin-binding fragment, and a 70,000 heparin/gelatin-binding FN fragment were generated by sequential limited cathepsin D and trypsin digestion of human plasma FN, purified by affinity, and analyzed for their motility-stimulating activity using SYN-1 sarcoma cells. None of these FN fragments showed significant motility-stimulating activities (data not shown).

**Additional Characterization of FMSF.** Human FN and its different proteolytic fragments have been shown to have adhesive activity for murine melanoma cells (19) and fibroblasts (34, 35). To test the cell adhesive activity of FMSF, a substrate-coated microwell cell adhesion assay was used, and adherent cells were quantitated with crystal violet staining. FMSF (100 ng) had significant cell adhesive activity.
assayed for the motility stimulation of human sarcoma cells, and the sarcoma motility potency for SYN-1 sarcoma cells in comparison with other tumor cell motility-stimulating factors.

Microsequencing of internal peptides of purified FMSF and analysis with affinity-purified antihuman-FN Ab resulted in the finding that FMSF was an NH2-terminal fragment of human cellular FN. Interestingly, plasma FN has been demonstrated to stimulate the motility of fibroblasts (17), neural crest cells (18), and murine B16 melanoma cells (4). The FN domains responsible for motility stimulation have been mapped to the central cell-binding and the COOH-terminal heparin-binding domains (17–19). Recently, a gelatin-binding fragment of FN was shown to stimulate migration of adult fibroblasts into collagen gels (36). Here, we found that the NH2-terminal fragment of human cellular FN was the FMSF responsible for the motility stimulation of human sarcoma cells, and the sarcoma motility-stimulating activity of FN may be released after its proteolytic generation either extracellularly or on the cell surface (20, 37).

activity for SYN-1 sarcoma cells compared to the BSA control but was less adhesive than 5 mg of FN (Sigma), consistent with previous findings on the NH2-terminal FN fragment (34, 35). In addition, FMSF did not bind to gelatin agarose resin (Pharmacia; data not shown).

DISCUSSION

In the present study, we purified a FMSF from HLF-CM using sequential heparin affinity chromatography and DEAE anion exchange chromatography. FMSF appeared to form homodimers under nonreducing conditions, and characterization of FMSF-mediated tumor cell motility indicated that FMSF predominantly stimulated chemotaxis in addition to some chemokinesis and that FMSF had medium motility potency for SYN-1 sarcoma cells in comparison with other tumor cell motility-stimulating factors.

### Table 1 Summary of FMSF purification

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein yield (%)</th>
<th>Activity yield (%)</th>
<th>Specific activity (units/µg)</th>
<th>Purification factor (-fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6500 ml HLF-CM</td>
<td>100</td>
<td>100</td>
<td>0.447</td>
<td>1</td>
</tr>
<tr>
<td>Heparin chromatography</td>
<td>0.083</td>
<td>10.0</td>
<td>12.0</td>
<td>120.6</td>
</tr>
<tr>
<td>DEAE chromatography</td>
<td>99.59a</td>
<td>0.75</td>
<td>10.0</td>
<td>120.6</td>
</tr>
</tbody>
</table>

* One unit of motility-stimulating activity was defined as the stimulation of net migration per 4 h of 100 cells to the lower chamber of a Transwell apparatus when 2 x 10^5 cells were added to the upper chamber, and net migration was defined as actual number of migrated cells subtracted by background migration (negative control).

* Protein was quantitated by Coomassie protein assay (Pierce) using BSA as standard.

* Protein was quantitated by densitometry of silver stained SDS-PAGE using Novex Mark 12 protein standards as standard.

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Fig. 4. Dose response of FMSF. Using serial dilution of purified FMSF in the cell migration assay, a dose-response curve was generated. FMSF was active at as low as 25 ng/ml (0.75 mM) and reached full activity at about 300 ng/ml (9.0 mM). Assays were performed in duplicate and repeated; data points, mean; bars, SD.

Fig. 5. Checkerboard analysis of FMSF. Nine different combinations of the three concentrations of FMSF were used in the upper and lower chambers of Transwells to determine motility-stimulating activities using SYN-1 cells. The result was plotted as a checkerboard analysis and demonstrated that FMSF predominantly stimulated chemotaxis but also stimulated some chemokinesis. Assays were performed in duplicate and repeated. Values are means, with SD in parentheses.

Fig. 6. Motility-stimulating activity of FMSF for different types of human soft tissue sarcoma cells. Motility of all eight human sarcoma cells was stimulated by FMSF, although different sarcoma cells responded to different degrees to FMSF. Assays were performed in duplicate and repeated; columns, mean; bars, SD.

Fig. 7. Comparison of internal peptide sequences of FMSF with deduced amino acid sequence of human FN. Two peptide sequences completely matched a portion of the NH2-terminal sequence of the human FN molecule. Underlined peptide sequence, propeptide sequence of human FN.
It is interesting to note that intact FN can be eluted by 0.5 M NaCl in 10 mM Tris-HCl (pH 7.4) buffer from a heparin-Sepharose affinity column (38) and by 0.19 M NaCl in 10 mM Tris-HCl (pH 8.1) buffer from a DEAE anion-exchange column (39). Therefore, proteolytic processing of FN may alter the binding properties of FN fragments to these matrices. Ab analysis using both immunodepletion and neutralization assays with a rabbit antihuman-FN Ab indicated that the Ab completely eliminated the motility-stimulating activity present in HLF-CM for sarcoma cells, further confirming that FMSF was the motility-stimulating factor present in HLF-CM for SYN-1 sarcoma cells. Characterization of the rabbit antihuman-FN Ab by immunoblotting indicated that the Ab recognized human FN and its different proteolytic fragments, including FMSF and the M, 30,000 NH2-terminal heparin/fibrin-binding fragment. Although FMSF may be the major motility-stimulating factor present in HLF-CM for SYN-1 sarcoma cells, intact FN also stimulated the motility of SYN-1 sarcoma cells (data not shown). Thus, the complete elimination of motility-stimulating activity by both immunodepletion and neutralization assays suggests that in addition to FMSF, FN may account for the remaining motility-stimulating activity in HLF-CM and that the motility-stimulating activity present in HLF-CM for SYN-1 sarcoma cells may be due exclusively to FMSF and its precursor FN.

Microsequencing of FMSF peptides suggested that the NH2-terminal sequence of FMSF extended beyond the NH2-terminal protein sequence of human plasma FN. This was a demonstration that the deduced propeptide sequence of the human FN molecule may be present at the protein level and may be retained in a form of FN, possibly due to differential posttranslational proteolytic processing (40). Previous protein sequencing of FN was performed mainly using the plasma form of human FN, and multiple differences were known to exist between the plasma and cellular forms of human FN, including differences in posttranslational glycosylation (41), acylation (42), solubility (43), and domain structures (43, 44). The extended NH2-terminal sequence of FN may be another difference between these two forms. Because FMSF has an apparent molecular weight of 34,000 under reducing conditions, FMSF represents an NH2-terminal fragment of human cellular FN, most likely due to posttranslational proteolytic processing or differential RNA splicing. Studies on FN gene structure, however, indicate that differential splicing of human FN RNA does not occur in the NH2-terminal sequence (45).

Interestingly, the enzymatically produced NH2-terminal fragments of human plasma FN did not show significant motility-stimulating activities with SYN-1 sarcoma cells. Because there are primary sequence differences between the purified cellular FN and the plasma form of the NH2-terminal FN fragments, and multiple biochemical differences between the cellular and plasma forms of FN occur as well, the lack of motility-stimulating activity of the human plasma FN fragments may be an important difference between two FN forms. In addition, plasma FN is produced mainly in the liver (40), whereas human sarcomas have a distinct hematogenous metastatic pattern, predominantly to the lung (22). Therefore, the lack of motility-stimulating activity of human plasma FN fragments with SYN-1 sarcoma cells may be important with respect to the clinical pattern of metastasis formation of human sarcomas. Alternatively, the biological properties of human plasma FN may be altered during its exposure to urea, a protein-denaturing agent that was used to elute FN from gelatin affinity chromatography during its purification (40). As a result, the renatured plasma FN may have lost the motility-stimulating activity with SYN-1 sarcoma cells.

The NH2-terminal heparin/fibrin-binding fragment of the FN molecule has been well characterized for its essential role in FN matrix assembly. Although the cell-binding domain and the NH2-terminal matrix assembly domain of FN participate in FN matrix assembly (46), recombinant FN molecules containing only the intact NH2-terminal and COOH-terminal regions without the RGDS cell-binding domain can form a significant fibrillar matrix in vitro (47), and the M, 29,000 NH2-terminal domain containing the five type I FN repeats is able to inhibit FN matrix assembly (48). Interestingly, the NH2-terminal domain of FN interacts with the matrix assembly receptor of fibroblasts with a KD of 25 nM (49, 50), and the matrix assembly receptor has been purified as a M, 66,000–67,000 protein from both human U937 histiocytic lymphoma cells (51) and chick myoblasts (52). Because the matrix assembly receptor interacts with the NH2-terminal domain of FN and its KD value is similar to the active concentration range of FMSF for SYN-1 sarcoma cells, the malignant counterparts of mesenchymal cells that are normally involved in extracellular matrix assembly and express the matrix assembly receptor, this receptor may be the receptor for FMSF on sarcoma cells.

In addition to its role in FN matrix assembly, the NH2-terminal fragment of FN is also required for cytoskeletal reorganization, formation of actin stress fibers, and spreading of fibroblasts on FN matrix (34, 35), and it also stimulates adipose differentiation of ST-13...
preadipocytes (53). The matrix assembly receptor appears to participate in chick myoblast differentiation (54).

It is interesting that several extracellular matrix molecules, including FN, laminin, and elastin, possess some similarities in their receptors and associated biological functions. For example, FN, laminin, and elastin all have two types of cellular receptors. The first type of receptor is the low-affinity integrin family receptor, such as $\alpha_5\beta_1$ for FN or $\alpha_6\beta_1$ for laminin (55, 56). The binding affinity of integrins can be increased, however, by inside-out cell signaling (57). The second type of receptor is of high-affinity type and is represented by nonintegrin receptors, such as the $M_f$ 66,000–67,000 matrix assembly receptor for FN or the $M_f$ 67,000–69,000 laminin/elastin receptor for laminin and elastin (55, 58). The matrix assembly receptor of FN is mediated by the heparin-binding FN fragments and functions in fibroblast FN matrix assembly (59) and is required for fibroblasts to form actin stress fibers and fully spread on FN matrix (34, 35). The laminin/elastin receptor functions in elastin matrix assembly in aortic smooth muscle cells (60) and is required for endothelial cells to fully spread on laminin matrix (61). In pathological states, such as tumor invasion and metastasis, the laminin/elastin receptor has been found to mediate human melanoma cell motility stimulated by laminin (3) and Lewis lung carcinoma cell motility stimulated by elastin fragments (6). The laminin/elastin receptor has been proposed to be important in lung metastasis of melanoma cells in vivo (62) and is a tumor progression marker in human colon cancer (63). In the present study, an $NH_2$-terminal fragment of FN was found to mediate sarcoma cell motility, possibly by interaction with the FN matrix assembly receptor. On the basis of the striking similarity between the receptors and functions of these extracellular matrix molecules, it is possible that the $NH_2$-terminal FN fragment/FMSF may function in sarcoma cell motility through the FN matrix assembly receptor in a manner analogous to the laminin/elastin receptor, and the FN matrix assembly receptor may be involved in metastasis formation and progression of human soft tissue sarcomas in vivo similar to the laminin/elastin receptor on melanoma and colon cancer cells.

The aberrant interactions between malignant tumor cells and their extracellular matrix is one of the fundamental changes occurring during malignant transformation, tumor progression, and metastasis formation (64). Benign tumor cells are normally restrained by their surrounding extracellular matrix in the form of tumor capsules, and FN has been shown to be a principal component of the extracellular matrix surrounding tumor cells (65). Metastatic tumor cells are able to produce extracellular degradative enzymes that dissolve their surrounding extracellular matrix barrier containing FN to generate passageways for invasion (2, 64). Metastatic cells are also modulated by the degradative products of extracellular matrix (66), and the degradative products of FN may possess novel biological activities that stimulate tumor cell growth (20) and motility, thereby affecting malignant cell properties. Human soft tissue sarcomas have a distinct hematogenous metastasis pattern, predominantly to the lung (22). Aberrant interactions between metastatic sarcoma cells and the extracellular matrix of lung may also occur during invasion and metastasis of human soft tissue sarcomas to the lung, and the $NH_2$-terminal FN fragment/FMSF and FN may play a role in this process.

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