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Purification of the migration stimulating factor produced by fetal and breast cancer patient fibroblasts

(development/tumor progression/cell–matrix interactions/cancer susceptibility)

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ABSTRACT **We have previously shown that (*i*) human skin fibroblasts of fetal and adult origin display distinctive migratory phenotypes, (*ii*) this difference in cell behavior results from the production of a soluble "migration stimulating factor" (MSF) by fetal cells, and (*iii*) skin fibroblasts from breast cancer patients commonly resemble fetal fibroblasts both in migratory phenotype and in production of MSF. Data are now presented indicating that MSF present in the conditioned medium of fetal and cancer patient fibroblasts is precipitated at 10% saturation ammonium sulfate and binds to heparin and cation-exchange resins. Based on this information, we have devised a scheme for the purification of MSF involving the sequential application of ammonium sulfate precipitation, heparin affinity, gel filtration, and reverse-phase chromatography. Purified MSF has an estimated molecular mass of 70 kDa; amino acid analysis reveals a relatively high level of proline (13.34 residues per 100). Our results further suggest that skin fibroblasts from breast cancer patients produce an additional factor with migration stimulating activity; this factor is precipitated at higher concentrations of ammonium sulfate and binds to anion-exchange resins. We have previously discussed the possible direct involvement of fetal-like fibroblasts in cancer pathogenesis. The availability of MSF obtained from cancer patient fibroblasts provides a potential means with which to examine the complex cellular interactions contributing to this process as well as develop a screening regime for identifying individuals at elevated risk of developing cancer.**

Fetal fibroblasts differ from their normal adult counterparts by a number of phenotypic characteristics, which are often reexpressed in transformed cells (1). Such "onco-fetal" traits include colony formation in semisolid medium (2), production of transforming growth factors (3), and synthesis of specific isoforms of matrix macromolecules (4, 5). We have previously demonstrated that fetal fibroblasts also differ from normal adult cells in terms of the migratory phenotype they display on collagen gel substrata (6). This difference is particularly apparent in cultures plated at confluent cell densities; under these conditions, fetal fibroblasts migrate into the three-dimensional gel matrix to a significantly greater extent than do normal adult cells. Subsequent studies revealed that the elevated migratory activity of the fetal fibroblasts results from the production by these cells of a soluble "migration stimulating factor" (MSF), which is not made by adult fibroblasts (7).

Ostensibly normal fibroblasts obtained from cancer patients have been reported to display various onco-fetal characteristics (8–10). Our own work in this area has demonstrated that tumor-derived fibroblasts exhibit a fetal-like mode of migratory behavior in ≈50% of patients with sporadic breast cancer (11); in addition, skin fibroblasts from these patients also exhibited a fetal-like migratory phenotype, suggesting the systemic nature of this abnormality (11). Fetal-like skin fibroblasts were subsequently demonstrated in ≈90% of patients with familial breast cancer (12, 13) and 50% of the clinically unaffected first-degree relatives of familial breast cancer patients (13). Recent work has indicated that the fetal-like fibroblasts of breast cancer patients also produce MSF (7). These observations have led us to speculate that the dysfunction in normal epithelial–mesenchymal interactions resulting from the presence of these aberrant fibroblasts in cancer patients might contribute directly to disease pathogenesis (14).

Programmed fetal-to-adult transitions occur in various biochemical and behavioral aspects of cellular phenotype during the course of normal development (15). Our results suggest that there is a developmentally regulated cessation in MSF production. An apparently similar transition occurs spontaneously in fetal fibroblasts "aged" *in vitro*; these cells abruptly begin to express an adult pattern of migratory behavior (6) and simultaneously cease producing MSF (7) after 50–55 population doublings *in vitro*. The fetal-like fibroblasts of breast cancer patients do not undergo such a spontaneous transition in migratory phenotype and continue to produce MSF for the duration of their *in vitro* life-span (16). Such observations suggest that breast cancer patient fibroblasts may express a defect in the genetic mechanism responsible for the developmental regulation of MSF production.

The principal objective of the present study has been to purify and further characterize MSF produced by both fetal and breast cancer patient skin fibroblasts. The potential clinical implications of this work are far-reaching and are discussed in terms of (*i*) screening for individuals at elevated risk of developing cancer, and (*ii*) the development of therapeutic modalities targeted specifically at the aberrant "fetal-like" fibroblast population of cancer patients.

MATERIALS AND METHODS

Cells and Culture Conditions. Fibroblast lines were established from explant cultures (6). The following cell lines were used in this study: FSF37 foreskin fibroblasts obtained from a 6-year-old male donor; FS6 fetal limb dermal fibroblasts obtained from a 12-week female fetus; BSF11 forearm dermal fibroblasts obtained from a 50-year-old female patient with familial breast cancer. The FSF37 foreskin fibroblasts display a characteristically adult pattern of migratory behavior and do not produce MSF (7); these cells are therefore referred to as adult fibroblasts in this communication. We have previously shown that the migratory phenotype expressed by a particular fibroblast line is independent of donor sex or site of tissue biopsy (6). Stock cultures were grown in 90-mm

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Abbreviation: MSF, migration stimulating factor.

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plastic tissue culture dishes in minimal essential growth medium supplemented with 15% aseptic newborn calf serum, glutamine, nonessential amino acids, sodium pyruvate, penicillin, and streptomycin (6). Cultures were incubated at 37°C in a humidified incubator gassed with 5% CO_2/95% air and passaged at a split ratio of 1:3 approximately once a week when confluence was achieved. Fibroblasts between passages 5 and 10 were used in this study.

To obtain conditioned medium (CM), confluent fibroblasts growing in 90-mm tissue culture dishes were washed five times with serum-free growth medium (SF-MEM) and incubated with 5 ml of SF-MEM for 72 hr. The resultant CM was then collected and passed through a 0.22-μm Millipore filter to remove cellular debris and was stored at −70°C until required.

Cell Migration Assay. Type I collagen was extracted from rat tail tendons in 3% acetic acid, dialyzed for 2 days against distilled water, and used to make 2-ml collagen gels in 35-mm plastic tissue culture dishes (17). In our standard migration assay, collagen gels were overlaid with 1 ml of either SF-MEM (controls), neat CM, or SF-MEM containing the various CM fractions to be tested. FSF37 fibroblasts were used as target cells in the assay since they do not produce MSF but are responsive to it. These fibroblasts were plated onto collagen gels in 1 ml of growth medium containing 20% serum at confluent cell density (2.5×10^4 cells per cm^2). Considering the 2-ml volume of the collagen gel, this procedure gives a final concentration of 5% serum in all cultures and 25% neat CM or CM fraction. Migration data are expressed as the percentage of fibroblasts found within the three-dimensional gel matrix after 4 days of incubation. These values were determined by counting the number of cells on the gel surface and within the collagen matrix in 10 randomly selected fields using a Leitz Labovert microscope (17). Greater than 1000 cells were counted in each culture and replicate cultures were used to obtain each percentage point. Only mean values are presented since the standard errors were consistently <15% of the mean.

Biochemical Procedures Used to Fractionate CM. *Ammonium sulfate precipitation.* Proteins were precipitated from fetal and breast cancer patient CM by the stepwise addition of solid ammonium sulfate. To obtain the first fraction (0–10% saturation), solid ammonium sulfate (0.056 $g \cdot ml^{-1}$) was slowly added to neat CM; this sample was then vigorously agitated on a Vortex mixer and left to form a precipitate at 4°C for a minimum of 2 hr. The sample was then centrifuged at $10,000 \times g$ for 5 min, the supernatant was aspirated off and saved for the next step, and the pellet was resuspended in 20 mM Tris·HCl (pH 7.4) containing 0.1 M NaCl (Tris buffer). The following fractions were then obtained by adding the indicated amount of solid ammonium sulfate to the supernatant and repeating the above procedure in a stepwise fashion: 10–20% saturation (0.057 $g \cdot ml^{-1}$); 20–30% saturation (0.059 $g \cdot ml^{-1}$); 30–50% saturation (0.127 $g \cdot ml^{-1}$); 50–80% saturation (0.214 $g \cdot ml^{-1}$). The final supernatant (>80% saturation) was also retained for assessment of biological activity in the migration assay. One-milliliter aliquots of the resuspended precipitates in Tris buffer were dialyzed at 4°C for 36 hr against 3 liters of Ringer's balanced salt solution (Oxoid, Basingstoke, U.K.) and then for 24 hr against SF-MEM. The dialyzed fractions were sterilized by Millipore filtration and used in the migration assay.

Heparin affinity chromatography. Samples were fractionated by heparin affinity chromatography (18), using 1-ml prepacked columns of heparin-agarose (0.4–0.5 mg of heparin per ml of gel, 4% beaded agarose; Pierce). One-milliliter aliquots of sample were loaded on columns equilibrated with Tris buffer. The columns were then washed with this buffer until no further protein was eluted, as assessed by A_{280} (≈5 ml). The bound proteins were eluted in a stepwise fashion by the sequential addition of Tris buffer containing 0.3, 0.6, 1.5, and 3.0 M NaCl; buffer containing each concentration of NaCl was added in successive 1-ml aliquots until the A_{280} returned to zero (total volume, ≈5 ml), at which time elution with the next higher concentration of NaCl commenced. The columns were washed with 20 mM Tris·HCl (pH 7.0) containing 3.0 M NaCl to remove all bound protein prior to being reused. Eluates containing the maximum amount of protein for each NaCl concentration were extensively dialyzed, as described for ammonium sulfate precipitation, and used in the migration assay.

Ion-exchange chromatography. Samples were fractionated using the following Affi-Sep prepacked columns (Anachem, Luton, U.K.): the anion-exchange resin Affi-Sep DEAE (catalogue no. SEN 004) and the cation-exchange resin Affi-Sep CM (catalogue no. SEN 003). In each case, 1 ml of sample was passed through a column that had previously been equilibrated with Tris buffer. The bound proteins were eluted in a stepwise fashion by the sequential application of Tris buffer containing 0.3, 0.6, 1.5, and 3.0 M NaCl. The collection procedure and subsequent processing for assessment of biological activity in the migration assay were as described above for heparin affinity chromatography.

Gel-filtration chromatography. Samples were fractionated as described (7) using a fast-protein liquid chromatography system (FPLC; Pharmacia) fitted with a Superose-12 gel filtration column (HR10/30). Samples were applied so as not to exceed the column capacity (5 mg of protein). Column calibration and processing of collected fractions for assessment in the migration assay were also as described (7).

Reverse-phase chromatography. Samples were fractionated using an FPLC system (Pharmacia) fitted with a ProRPC column (HR5/10). The column was equilibrated with 0.1% (vol/vol) trifluoroacetic acid in water. Samples were injected in a vol of 500 μl (containing ≈10 μg of protein per ml). The column was then washed with 4 ml of 0.1% trifluoroacetic acid in water to remove unbound material. Bound proteins were eluted with a linear gradient of 0–50% (vol/vol) acetonitrile over 20 ml at a flow rate of 0.2 $ml \cdot min^{-1}$. One-milliliter fractions were collected and then dialyzed over a 4-day period against Ringer's balanced salt solution (7 liters), followed by 1 day against SF-MEM (2 liters), using a 28-well multichamber microdialysis system (GIBCO–BRL; catalogue no. 1200MD) fitted with a dialysis membrane (catalogue no. 1202MA). These dialyzed samples were then assessed for biological activity in the migration assay.

Concentration by Amicon filtration. Where indicated in *Results*, material was concentrated by ultrafiltration using an Amicon filtration cell fitted with a YM5 25-mm Diaflo membrane (catalogue no. 13512; Amicon) operated under N_2 pressure.

PAGE. Samples were analyzed by SDS/PAGE according to the method of Laemmli (19) using 10% resolving gel and 3% stacking gel. Electrophoresis was performed under nonreducing conditions and gels were silver stained as described by Morrissey (20). The following molecular mass standards were used: carbonic anhydrase, 29 kDa; egg albumin, 45 kDa; bovine serum albumin, 66 kDa; phosphorylase B, 97.4 kDa.

Amino acid analysis. Amino acid analysis of purified MSF was kindly performed by P. J. Barker (Microchemical Facility, Agricultural and Food Research Council, Institute of Animal Physiology and Genetic Research, Cambridge Research Station) using an LKB Alpha Plus amino acid analyzer. No attempt was made to protect cysteine residues.

RESULTS

Characterization of Migration Stimulating Activity in CM Obtained from Fetal and Breast Cancer Patient Fibroblasts. Data are presented concerning the effects of standard frac-

tionation procedures on the recovery of migration stimulating activity from neat CM produced by both fetal and breast cancer patient fibroblasts. In the first instance, the two types of CM were fractionated by ammonium sulfate precipitation and the resultant samples were assessed for biological activity in the collagen gel migration assay. As shown in Table 1, both types of neat CM stimulated adult fibroblast migration ≈5-fold. All of the migration stimulating activity in fetal CM was recovered in the 0–10% saturation ammonium sulfate fraction; all other fractions were devoid of activity. In contrast, migration stimulating activity was recovered in two fractions of the breast cancer fibroblast CM, these being 0–10% saturation ammonium sulfate (as with the fetal CM) and 20–30% saturation.

The results of heparin affinity chromatography of neat CM are presented in Table 2. All of the migration stimulating activity present in both fetal and breast cancer patient fibroblast CM was adsorbed to the heparin affinity column and eluted in the 0.3 and 0.6 M NaCl fractions.

Fetal and breast cancer patient fibroblast CM was fractionated by both cation- and anion-exchange chromatography. The results presented in Table 3 indicate that migration stimulating activity in neat fetal CM (first column) only adsorbed to the cation-exchange resin and was eluted at concentrations of NaCl between 0.3 and 0.6 M. Migration stimulating activity in neat breast cancer fibroblast CM (second column) also adsorbed to the cation-exchange resin and was eluted at the same concentrations of NaCl; an additional fraction of migration stimulating activity was also present in the breast cancer patient fibroblast CM, this being bound to the anion-exchange resin and eluting at high salt concentration (3.0 M NaCl).

The distribution of migration stimulating activity in the different fractions obtained by Superose-12 gel filtration chromatography of fetal, control adult, and breast cancer patient fibroblast CM is presented in Fig. 1. A single peak of activity was found in fractions 11 and 12 of the fetal fibroblast CM (corresponding to column retention volumes of 13 and 14 ml, respectively). Migration stimulating activity was also found at this position in fractionated breast cancer patient fibroblast CM, with additional higher molecular weight peaks also observed in fractions 5 and 7 (column retention volumes, 7 and 9 ml, respectively). No migration stimulating activity was found in the fractionated CM of normal adult fibroblasts.

Table 1. Precipitation of migration stimulating activity by different concentrations of ammonium sulfate

Treatment of CM	% adult fibroblasts in gel matrix	
	Fetal CM	Patient CM
Control (no CM)	2.7	2.7
Neat CM	13.7*	14.8*
Ammonium sulfate		
0–10%	14.4*	15.0*
10–20%	2.4	3.1
20–30%	3.6	9.8*
30–50%	3.8	3.6
50–80%	3.6	2.6
>80%	1.9	1.9

CM obtained from both fetal and breast cancer patient skin fibroblasts were precipitated with increasing concentrations of ammonium sulfate (indicated as % saturation). The precipitated proteins were redissolved and assessed for their ability to stimulate the migration of a target adult cell line in our standard collagen gel migration assay. Data are expressed as the percentage of adult fibroblasts within the collagen gel after 4 days of incubation.
*Samples that gave a significant level of stimulation compared to controls.

Table 2. Binding of migration stimulating activity to heparin

Treatment of CM	% adult fibroblasts in gel matrix	
	Fetal CM	Patient CM
Control (no CM)	1.9	1.9
Neat CM	9.0*	10.5*
Heparin affinity		
Unbound CM	2.0	1.8
0.3 M NaCl	6.3*	10.1*
0.6 M NaCl	6.8*	7.1*
1.5 M NaCl	1.6	1.4
3.0 M NaCl	2.6	1.0

CM obtained from both fetal and breast cancer patient skin fibroblasts were processed by heparin affinity chromatography. The presence of migration stimulating activity was assayed in CM recovered directly after passage through the column (unbound CM), as well as in bound material eluted by the different concentrations of NaCl. Data are expressed as % adult fibroblasts within the gel matrix after 4 days of incubation.
*Samples that gave a significant stimulation compared to controls.

Purification of MSF. On the basis of the above results, we have devised the following fractionation scheme for the purification of MSF to apparent homogeneity:

(*i*) *Ammonium sulfate precipitation of neat CM.* Collect material precipitated at 0–10% saturation ammonium sulfate, resuspend in 2 ml of 20 mM Tris buffer containing 0.1 M NaCl, and use this sample for:
(*ii*) *Heparin affinity chromatography.* Collect bound material that is eluted at 0.3 M NaCl, concentrate this sample to volume of 0.5 ml by filtration in Amicon cell, and fractionate by:
(*iii*) *Gel-filtration chromatography.* On Superose-12 column (FPLC), load 0.5-ml aliquots and collect material eluting at 13-ml column retention volume; pooled samples may be concentrated to a volume of 0.5 ml by filtration in Amicon cell and fractionated by:
(*iv*) *Reverse-phase chromatography.* On ProRPC column (FPLC), MSF present in main UV peak (column retention volume, 13–15 ml).

The UV profile and corresponding biological activity of MSF obtained from fetal fibroblast CM in the final reverse-phase chromatography step of the purification protocol are indicated in Fig. 2. There is a single major UV peak (fractions 13 and 14), which corresponds to all of the migration stimulating activity.

MSF prepared by this scheme was assessed by SDS/PAGE (Fig. 3). Purified MSF gave a single band with an estimated molecular mass of 70 kDa. Preliminary amino acid sequence data indicate the presence of a single N-terminal sequence—i.e., Ala-Pro-Ile-Pro (B. Coles, CRC Protein Sequencing Facility, personal communication).

The ability of purified fetal and breast cancer MSF to bind to cation- and anion-exchange resins is presented in Table 3 (columns 3 and 4, respectively). MSF purified from both sources binds to the cation-exchange resin but not the anion-exchange resin.

The amino acid analysis of purified fetal fibroblast MSF is presented in Table 4. Particular attention is drawn to the relatively high content of proline (13.34 residues per 100) and absence of methionine.

DISCUSSION

Data presented in this communication indicate that MSF produced by both fetal and breast cancer patient fibroblasts (*i*) is precipitated by ammonium sulfate at 10% saturation, (*ii*)

Table 3. Binding of migration stimulating activity to cation- and anion-exchange resins

Treatment	% adult fibroblasts in gel matrix			
	Fetal CM	Patient CM	Fetal MSF	Patient MSF
Control (no CM)	1.4	2.8	2.3	2.5
Neat CM or MSF	10.5*	11.4*	9.9*	10.5*
Cation exchange				
Unbound	2.0	1.9	1.8	2.8
0.1 M NaCl	1.7	2.3	2.5	2.7
0.3 M NaCl	6.3*	9.0*	11.0*	13.1*
0.6 M NaCl	4.7*	8.0*	9.7*	11.4*
3.0 M NaCl	1.9	2.6	2.1	2.0
Anion exchange				
Unbound	7.5*	13.1*	11.2*	13.3*
0.1 M NaCl	2.6	2.4	2.5	2.9
0.3 M NaCl	1.8	2.7	2.5	2.3
0.6 M NaCl	1.4	4.7	2.1	2.6
3.0 M NaCl	1.4	9.4*	1.8	2.6

CM and purified MSF obtained from both fetal and breast cancer patient skin fibroblasts were processed by cation- and anion-exchange chromatography. The presence of migration stimulating activity was assayed in CM and MSF recovered directly after passage through the respective ion-exchange columns (unbound), as well as in bound material eluted by the indicated concentrations of NaCl. Data are expressed as % adult fibroblasts within the collagen gel after 4 days of incubation.

*Samples that gave a significant level of stimulation compared to controls.

binds to cation-exchange resins, (*iii*) binds to heparin with moderate affinity, (*iv*) has a molecular mass of ≈70 kDa, and (*v*) contains an unusually high content of proline and lacks methionine. Our current efforts are directed toward cloning the gene for MSF and obtaining its complete sequence. The data presented here further suggest that breast cancer patient fibroblasts produce a second factor (or factors) with migration stimulating activity, this (these) being anionic and precipitated by ammonium sulfate at 20–30% saturation.

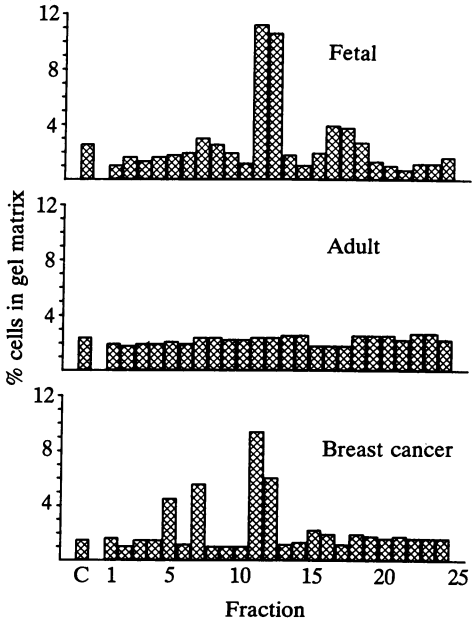

FIG. 1. Fractionation of migration stimulating activity in fibroblast CM by gel-filtration chromatography. Neat CM obtained from fetal, adult, and breast cancer patient skin fibroblasts was fractionated by gel-filtration chromatography using a Sepharose-12 column (FPLC). One-milliliter fractions were collected. The first three fractions were pooled, so that fractions two and above correspond to column retention volumes 2 ml greater than the indicated fraction number. All samples were assessed for migration stimulating activity; C, level of migration obtained in control cultures in which SF-MEM was used instead of a CM fraction.

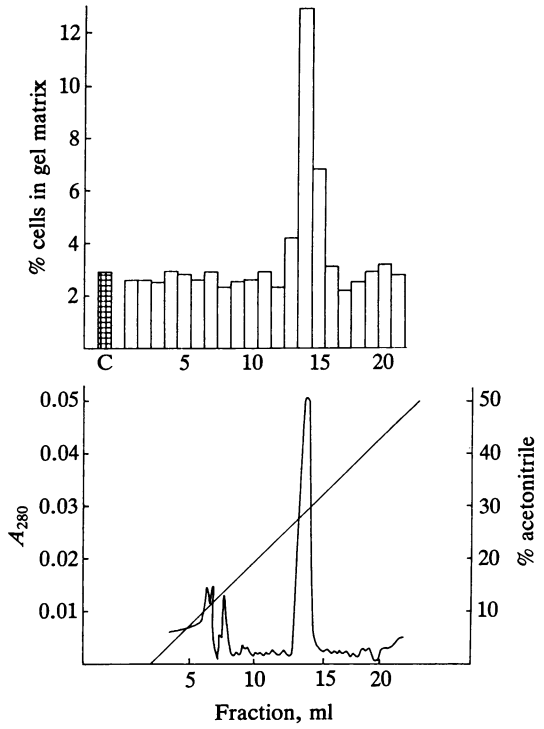

FIG. 2. Final purification step of fetal MSF by reverse-phase chromatography. Partially purified MSF obtained in the gel-filtration step of our purification protocol was fractionated by reverse-phase chromatography with a ProRPC column (FPLC). One-milliliter fractions were collected. The UV adsorption profile (*Lower*) indicates a major peak at fraction 14. Corresponding biological activity was assessed in the migration assay (*Upper*); C, level of migration in control cultures in which SF-MEM was used instead of a column fraction.

The binding of MSF to heparin provides a convenient means of separating MSF from the majority of proteins present in neat CM. A substantial number of polypeptide growth factors with heparin binding capacity have been isolated from various tissue sources (21); many of these are now recognized as belonging to two major families, of which acidic and basic fibroblast growth factors form the prototypes. It is therefore of interest to note that although fibroblast growth factor stimulates fibroblast proliferation, it has no effect on the migration of the target adult fibroblast line used in this study (unpublished observations). In contrast, MSF stimulates fibroblast migration but has no effect on cell proliferation (7).

We have previously reported that the MSF had an estimated molecular mass of 55 kDa (7). This value was based primarily on the elution position of migration stimulating activity using the Superose-12 gel-filtration column and was therefore only

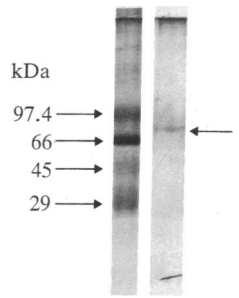

FIG. 3. PAGE of purified MSF. Purified fetal MSF was analyzed by PAGE under nonreducing conditions. A single band was observed with an estimated molecular mass of 70 kDa (arrow on right). The positions of the molecular mass standards are shown on the left.

Table 4. Amino acid analysis of purified fetal fibroblast MSF

Amino acid	Residues per 100	Amino acid	Residues per 100
Asp	7.39	Tyr	1.20
Glu	8.92	Val	4.02
Ser	11.13	Met	—
Gly	16.83	Ile	2.53
His	4.53	Leu	6.13
Arg	3.25	Phe	2.88
Thr	5.06	Lys	5.55
Ala	5.15	Cys	2.10
Pro	13.34		

The amino acid analysis of fetal fibroblast MSF was performed as described. No attempt was made to protect cysteine residues; the measured cysteic acid (Cys) may therefore represent an underestimate of cysteines.

an approximate figure. The value of 70 kDa reported in this communication is based on the relative mobility of MSF during PAGE under nonreducing conditions (Fig. 3). We have obtained somewhat variable estimates of molecular mass by this method (values between 60 and 70 kDa). Our preliminary data suggest that MSF is glycosylated and that these differences in estimated molecular mass may result from differential cleavage of saccharide chains under the acid conditions used in the final reverse-phase chromatography step.

Various other migration stimulating factors with molecular masses between 55 and 70 kDa have been described in the literature. These do not appear to be identical to MSF on the basis of several criteria, including biochemical properties and target cell specificity. For example, fetal fibroblasts produce a "scatter factor," which induces the dispersion of normal epithelial cells from tightly packed colonies (22). Using this assay system, it was found that fetal fibroblasts produce scatter factor activity while breast cancer patient fibroblasts do not (22). Migration stimulating factors have also been reported to be produced by metastatic tumor cell lines; autocrine motility factor described by Liotta *et al.* (23) is the best characterized of these. We have previously reported that autocrine motility factor and MSF differ with respect to a number of fundamental biochemical parameters, including heat stability, pH sensitivity, serum dependence of biological activity, and target cell specificity (7). The amino acid analysis of MSF (this paper) and autocrine motility factor (23) support the view that these are indeed different factors—i.e., the relatively high proline content of MSF (13.3 residues per 100) differs substantially from that of autocrine motility factor (4.7 residues per 100).

Cell migration into collagen matrices is a complex phenomenon subject to regulation at various levels (24). In this context, we have reported that MSF exerts a primary effect on the synthesis of hyaluronic acid and that this matrix macromolecule is required for the observed stimulation of cell migration (24, 25). It should therefore be emphasized that although our assay for MSF is based on its stimulation of fibroblast migration, the biologically significant effect of this factor *in vivo* may be related to alterations in the pattern of matrix synthesis and deposition. These alterations could, in turn, lead to a dysfunction in epithelial–mesenchymal interactions (26). Such a model implies that persistent fetal-like fibroblasts in the adult may play a direct role in the development of cancer. This view is in contrast to the interpretation favored in the majority of previous studies documenting the presence of aberrant fibroblasts in cancer patients; these have tended to consider such fibroblasts merely as convenient markers of a genetic lesion that assumes relevance in terms of cancer pathogenesis only when expressed in the relevant target epithelial cell population.

The persistence of fetal-like skin fibroblasts is not a specific feature of breast cancer, as we have also reported their presence in patients with other types of cancer (27). If these aberrant fibroblasts do indeed contribute in some fashion to cancer development, we may speculate that determination of MSF levels by a simplified means (possibly radioimmunoassay of serum) may provide a convenient means of screening the population for individuals at elevated risk of developing cancer. Furthermore, it may prove possible to develop new therapeutic modalities designed to induce persistent fetal-like fibroblasts in the adult to express a normal phenotype; such agents may reduce the risk of developing cancer in individuals identified as being at elevated risk to the disease, as well as inhibiting tumor progression in patients already suffering from cancer.

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