Foetal and cancer patient fibroblasts produce an autocrine migration-stimulating factor not made by normal adult cells

SETH L. SCHOR[1,*], ANA M. SCHOR[2], ANN MARIE GREY[1] and GRAHAM RUSHTON[2]

[1]*Department of Cell and Structural Biology, Coupland III Building, University of Manchester, Manchester M13 9PL, England*
[2]*CRC Department of Medical Oncology, Christie Hospital, Wilmslow Road, Manchester M20 9BX, England*

* Author for correspondence

Summary

We have previously reported that (1) the migration of foetal and adult fibroblasts into three-dimensional collagen matrices is differentially affected by cell density, and (2) skin fibroblasts from cancer patients commonly display a foetal-like mode of migratory behaviour. Data presented here indicate that differences in the migration of these cell types are particularly apparent in cultures plated at high density (i.e. at cell confluence); under these conditions, foetal fibroblasts and the foetal-like fibroblasts of cancer patients migrate into the three-dimensional collagen matrix to a significantly greater extent than do normal adult cells. In this initial study concerned with the biochemical basis of these observations, we report that medium conditioned by either foetal or cancer patient fibroblasts stimulates the migration of confluent adult cells. This stimulation of migration is specific to confluent cells, as the migration of subconfluent adult fibroblasts is unaffected by these conditioned media. Gel filtration chromatography of foetal fibroblast-conditioned medium indicates that migration-stimulating activity is recovered in a single peak with an apparent molecular mass in the range of 50–60 ($\times 10^3$). The active migration stimulating factor (MSF) in both foetal and cancer patient fibroblast-conditioned media appears to be a protein stable at acid pH, but inactivated by heat, alkaline pH and reductive alkylation. MSF produced by foetal and cancer patient fibroblasts is presumably responsible for the characteristically elevated levels of migration displayed by these cells in confluent culture, thereby suggesting an autocrine mode of action for this factor. Stimulation of adult cell migration by MSF requires the presence of either serum or platelet-poor plasma and is not observed in serum-free medium. MSF does not appear to affect either the proliferation or morphology of normal adult cells under any of the culture conditions examined.

Key words: fibroblasts, migration, chemotaxis, cancer patient skin fibroblasts, growth factor.

Introduction

In spite of their widespread tissue distribution and relative ease with which they are cultured, fibroblasts remain a rather poorly defined group of cells commonly identified *in vitro* on the basis of such non-specific attributes as spindle-shaped morphology. Recent evidence has served to underscore the fact that fibroblasts are in fact a highly heterogeneous family of cells displaying both developmental and site-specific differences in such fundamental aspects of cell behaviour as proliferation, response to growth factors and matrix biosynthesis (Schor & Schor, 1987*a*). This heterogeneity in fibroblast phenotype is presumably of fundamental importance to the normal functioning of connective tissues, including their inductive interactions with neighbouring epithelia.

When dermal fibroblasts are plated on the surface of three-dimensional gels of type I collagen fibres, they begin to migrate down into the underlying gel matrix within 24 h. This aspect of fibroblast migratory behaviour (i.e. from the gel surface to the interior) is influenced by a number of experimental parameters, including cell density on the gel surface (Schor *et al.* 1982). The effect of cell density on fibroblast migration may be expressed in quantitative terms by a function we have defined as the 'cell density migration index' or

CDMI (Schor *et al.* 1985*a*). Using this experimental approach, we have previously reported that (1) foetal and normal adult skin fibroblasts may be distinguished on the basis of the distinctive CDMI values they express (Schor *et al.* 1985*a*), and (2) ostensibly normal skin fibroblasts from patients with cancer commonly express CDMI values falling within the foetal range (Durning *et al.* 1984; Schor *et al.* 1985*b*, 1986; Haggie *et al.* 1987). On the basis of these findings, we put forward an hypothesis suggesting that the dysfunction in normal epithelial–mesenchymal interactions caused by the persistence of foetal-like fibroblasts in the adult leads to an increased susceptibility to the development of cancer (Schor *et al.* 1987).

The CDMI is specifically a measure of the effects of cell density on migration. This differential effect of cell density may be mediated by a number of mechanisms, including: (1) cell-induced alterations in the orientation and/or packing density of collagen fibres in the gel (Grinnell & Lamke, 1984); (2) density-dependent changes in the deposition of specific matrix macromolecules (e.g. fibronectin) known to affect cell migration (Mautner & Hynes, 1977; Schor *et al.* 1981); (3) social interactions between cells (Abercrombie, 1970); and (4) the secretion of a soluble migration-stimulating or -inhibiting factor. These different possibilities have been discussed in a recent review (Schor & Schor, 1987*b*).

The objective of the present study is to learn more about the biochemical basis of the different CDMI values displayed by foetal, normal adult and cancer patient skin fibroblasts. Our data suggest that foetal fibroblasts and the foetal-like fibroblasts of cancer patients secrete an autocrine migration-stimulating factor not produced by normal adult cells.

Materials and methods

Cells and culture conditions

Fibroblast lines were established from explant cultures, as described by Ham (1980). FSF37 cells were derived from a foreskin biopsy of a 6-year-old child. Further details regarding the normal adult and foetal skin fibroblasts used in this study have been published (Schor *et al.* 1985*a*); similar data regarding the breast cancer patient skin fibroblasts (BSF) were presented by Haggie *et al.* (1987).

Stock cultures of skin fibroblasts were grown in 90 mm plastic tissue culture dishes in MEM growth medium (Gibo-BioCult, Scotland) supplemented with 15 % aseptic newborn calf serum, glutamine, non-essential amino acids, sodium pyruvate, penicillin and streptomycin (Schor & Court, 1979). Cultures were passaged at a split ratio of 1:5 approximately once a week when confluence was achieved.

Where indicated, bovine plasma and serum were prepared as described by Vogel *et al.* (1978).

Collection and treatment of conditioned media

Confluent stock cultures were washed five times with serum-free growth medium (SF-MEM) and then incubated with 5 ml of SF-MEM for 72 h at 37°C in a humidified gassed incubator. The resultant conditioned medium (CM) was then collected, passed through a 0·22 μm Millipore filter to remove cellular debris and stored at −70°C until required.

Where indicated, samples of CM were treated as follows: (1) *dialysis*: against 100× volume of SF-MEM for 24 h at 4°C; (2) *trypsinization*: 2·5 % solution of crystalline trypsin (Sigma Chemical Co., T-0134) in calcium- and magnesium-free Dulbecco's phosphate-buffered saline (PBS) added to give a final concentration of 0·25 % trypsin; after incubation for 10 min at 37°C, the trypsin was inactivated by addition of soybean trypsin inhibitor (Sigma Chemical Co., T-9003); (3) *heated*: to either 60°C or 100°C for 10 min; (4) *pH stability*: CM adjusted to either pH 2 with 1 M-HCl or pH 10 with 1 M-NaOH, incubated for 60 min at 4°C and then readjusted to pH 7·2; (5) *reductive alkylation*: CM treated with 20 mM-mercaptoethanol for 24 h at room temperature, followed by addition of iodoacetic acid (18 μg ml^{-1}) and incubation at 4°C for 1 h; the pH was then adjusted to 7·5 with solid Tris-base and the treated sample dialysed against SF-MEM for 24 h.

Samples of foetal fibroblast (FS6) CM were concentrated tenfold using an Amicon filtration cell fitted with a YM5 membrane (molecular weight cut off 5000×10^3). Neat or concentrated CM was fractionated by gel filtration chromatography using a Pharmacia fast protein liquid chromatography system (FPLC^{tm}). A 200 μl sample of CM was applied to a Superose 12 column and fractionated at a flow rate of 0·3 ml min^{-1} in 20 mM-Tris·HCl buffer (pH 7·4) containing 0·15 M-NaCl. Fractions (1 ml) were collected and then dialysed against SF-MEM for 48 h at 4°C. The column was calibrated by running a mixture of molecular weight standards (β-amylase, 200×10^3; alcohol dehydrogenase, 150×10^3; bovine serum albumin, 66×10^3; carbonic anhydrase, 29×10^3; cytochrome *c*, $12{\cdot}4\times10^3$).

Cell growth and migration assay

Cell proliferation was measured as described (Schor, 1980). Cells growing on 35 mm plastic tissue culture dishes were brought into a single-cell suspension by treatment with 0·05 % trypsin in PBS containing 2 mM-ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA). Cell number was measured with a Coulter electronic particle counter.

Type I collagen was extracted from rat tail tendons in 3 % acetic acid, dialysed for 2 days against distilled water and used to make 2 ml collagen gels in 35 mm plastic tissue culture dishes as described (Schor & Court, 1979). For the migration assays, collagen gels were overlaid with 1 ml of either SF-MEM or CM. Fibroblasts growing in stock culture were trypsinized and resuspended in growth medium containing 20 % serum. This cell suspension was then used to prepare two plating inocula (high and low density, respectively). The cell count in the high-density inoculum was adjusted so that plating 1 ml gave $2{\cdot}5\times10^4$ cells cm^{-2} on the gel surface; the count in the low-density inoculum was similarly adjusted to give 10^3 cells cm^{-2}. Fibroblasts attached and were fully

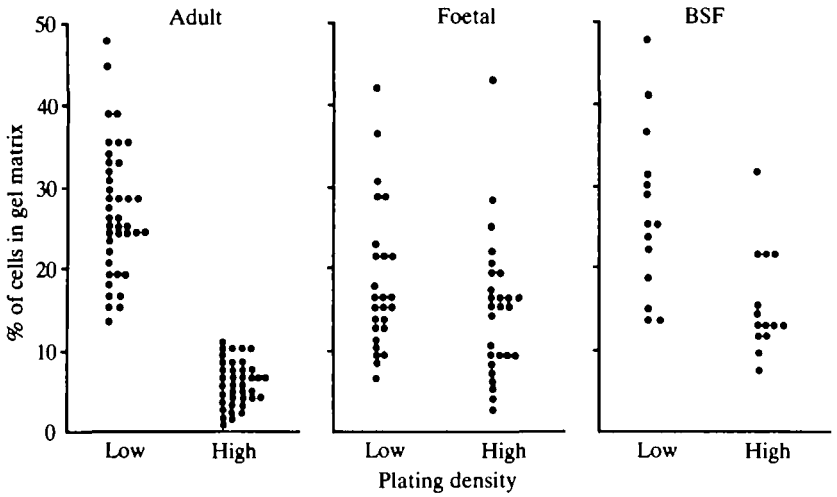

Fig. 1. Scatter diagram of migration values of normal adult, foetal and breast cancer patient (BSF) skin fibroblasts plated at high and low cell densities.

spread on the gel surface within 2 h after plating; cells in the low-density cultures were sparsely distributed on the gel, whilst cells in the high-density cultures formed a confluent layer immediately upon spreading. Considering the 2 ml volume of the hydrated collagen gel, this procedure gives final concentrations of 5 % serum and 25 % CM in the standard migration assay. Replicate high- and low-density cultures were incubated for 4 days and the number of cells on the gel surface and within the three-dimensional collagen matrix was then determined by microscopic observation of living cultures using phase-contrast optics (Schor, 1980). Data are expressed as the percentage of cells found within the gel matrix. A minimum of 1000 and 1500 cells were counted to determine each percentage point for cells plated at low density and high density, respectively.

CDMI determinations

The cell density migration index or CDMI may be used to express the effects of cell density on fibroblast migration in quantitative terms (Schor *et al.* 1985*a*). The CDMI is defined as follows:

$$\text{CDMI} = \log\left[\frac{\text{migration low density}}{\text{migration high density}}\right],$$

where 'migration low density' is the percentage of cells within the gel matrix in low-density cultures and 'migration high density' is the corresponding value for high-density cultures. On the basis of results obtained with a large number of well-characterized normal adult, foreskin, foetal and overtly transformed cell lines, we have previously defined four ranges of CDMI values (Schor *et al.* 1985*a*); i.e. the transformed range (T) with CDMI values less than −0·4, the transformed/foetal range (T/F) with CDMI values between −0·4 and 0, the foetal/normal range (F/N) with CDMI values between 0 and +0·4 and the normal range (N) with CDMI values greater than +0·4. These four ranges were empirically derived so that greater than 90 % of normal adult and foreskin fibroblasts had CDMI values falling in the N range, whilst greater than 90 % of foetal cells had CDMI in the F/N and T/F ranges. For clarity in this paper, the N range is renamed A (for normal adult), whilst the T/F and F/N ranges are collectively referred to as the F range (for foetal).

Results

The differential effect of cell density on the migration of adult, foetal and breast cancer patient skin fibroblasts

Fibroblasts were plated on the surface of collagen gel substrata at 10^3 cells cm^{-2} (low density) and $2{\cdot}5\times10^4$ cells cm^{-2} (high density) and cell migration into the gel was then measured as described in Materials and methods. Data obtained with 40 lines of adult skin fibroblasts, 26 lines of foetal skin fibroblasts and 15 lines of breast cancer patient skin fibroblasts (BSF) are presented in Fig. 1. The migration of adult skin fibroblasts was inversely proportional to cell density, with median values of 24·6 % cells within the gel in low-density cultures and 5·9 % in high-density cultures. In contrast, the migration of the foetal cells was not significantly influenced by cell density, with median values of 16·5 % and 15·8 % in the low- and high-density cultures, respectively. Corresponding data for the BSF cells gave median values of 25·3 % (low-density cultures) and 13·2 % (high-density cultures). These results indicate that foetal and BSF fibroblasts in high-density culture migrate to a significantly greater extent than normal adult cells ($P < 0{\cdot}001$ in both cases, Student's t-test).

CDMI values for the individual cell lines were calculated from these results: 36/40 (90 %) of the adult fibroblast lines gave CDMI values falling in the A range, 24/26 (92 %) of the foetal fibroblasts were in the F range and 13/15 (87 %) of the BSF cells were in the F range.

Effects of conditioned media on cell migration

The possible involvement of a cell-produced soluble factor in determining the characteristic migratory behaviour of the adult, foetal and BSF fibroblasts was investigated in the following fashion. Foreskin (FSF37) and foetal (FS6) fibroblasts were plated onto

Table 1. *The effects of conditioned medium on fibroblast migration*

Target cell	CM	% Of cells within gel matrix		CDMI
		Low density	High density	
Adult (FSF37)	None	20·8	2·0	+1·02 (A)
	FS6	18·6	12·7	+0·16 (F)
	FSF37	27·8	3·9	+1·14 (A)
	BSF11	19·7	13·9	+0·15 (F)
Foetal (FS6)	None	15·3	14·8	+0·01 (F)
	FS6	11·1	10·5	+0·02 (F)
	FSF37	15·9	12·6	+0·10 (F)
	BSF11	14·8	15·0	−0·01 (F)

Conditioned media (CM) were prepared by incubating confluent cultures of the different fibroblast lines in serum-free growth medium for 3 days. The migration assays were carried out in the presence of 5 % serum and 25 % CM. The migration data obtained in high- and low-density cultures were used to calculate the respective CDMI values; A, normal adult range and F, foetal range.

replicate collagen gels at both high and low densities in growth medium containing a final concentration of 5 % serum, with or without 25 % conditioned medium (CM) from foreskin, foetal or BSF skin fibroblasts. The percentage of cells present within the collagen matrix was measured after 4 days of incubation. Data presented in a previous study (Schor *et al.* 1985*a*) indicated that foreskin fibroblasts (obtained from donors with ages ranging between 2 and 8 years) were indistinguishable from normal adult cells (both male and female, obtained from a variety of anatomical sites) in terms of their migratory behaviour on collagen gels. As can be seen in Table 1, the FSF37 foreskin fibroblasts displayed a characteristically adult-like migratory response to cell density in control cultures incubated in the absence of CM; under these conditions, 20·8 % of the cells were within the gel matrix in low-density cultures compared to only 2·0 % in high-density cultures, with a resultant CDMI of +1·02 (A range). The presence of foetal CM had no significant effect on FSF37 cell migration in low-density cultures (18·6 %), but resulted in an approximately sixfold stimulation of migration in high-density cultures (12·7 %); these cells now displayed a characteristically foetal-like pattern of migration, with a calculated CDMI of +0·16 (F range). BSF CM had a similar effect to that of foetal CM on the migration of FSF37 cells, giving values of 19·7 % and 13·9 % cells in the gel matrix in low- and high-density cultures, respectively (calculated CDMI of +0·15 in F range). The presence of FSF37 CM had no effect on the migration of FSF37 cells at either high or low cell densities.

In the reciprocal experiment, FS6 foetal fibroblasts displayed characteristically foetal-like migratory behaviour under all culture conditions. None of the CMs examined had a significant effect on cell migration at either high or low cell density. It should be noted that FSF37 fibroblast CM did not inhibit the migration of foetal fibroblasts in high-density culture. The migration of BSF cells at both high and low cell densities was similarly unaffected by all of the CMs examined (data not presented).

The effect of CM produced by a number of other normal adult, foetal and BSF skin fibroblast lines on the migration of FSF37 cells in high-density culture are presented in Table 2. None of the adult CMs had any demonstrable effect on cell migration. In contrast, CMs produced by all of the foetal and BSF lines induced a significant stimulation of migration. None of these CMs affected the already high levels of migration of FSF37 cells in low-density cultures (data not presented).

Initial characterization of the foetal and BSF migration-stimulating factor

The above results suggest that foetal and BSF skin fibroblasts produce a soluble factor that stimulates the migration of adult fibroblasts in high-density culture, but has no effect on cell migration in low-density culture. Data concerned with the initial characterization of the active migration-stimulating factor (MSF) in foetal and BSF CM are presented in Table 3. Foetal and BSF CMs were exposed to a variety of treatments and their ability to stimulate the migration of FSF37 fibroblasts in high-density culture was then examined. The results indicate that the migration-stimulating activity in both foetal and BSF CM is non-dialysable, acid-stable and inactivated by heat, trypsin, exposure to pH 10 and alkylation reduction. These data are consistent with MSF being a protein containing disulphide bonds, required for its activity. The migration of FSF37 cells in low-density culture was not affected by CMs treated by any of the above procedures.

Dose–response data for the stimulation of FSF37 fibroblast migration by foetal and BSF CMs are presented in Fig. 2. There was an approximately linear response to CM concentrations between 1 and 25 %, after which point a plateau level of stimulation was

Table 2. *The effects of different fibroblast conditioned media on the migration of FSF37 cells*

Source of CM	% of FSF37 cells in gel	Relative stimulation
Control		
No CM	2·2	
Foetal		
FS6	13·1	5·9
FS2	12·9	5·8
FS8	15·9	7·2
FS13	13·4	6·0
FS10	17·9	8·1
FS12	9·2	4·2
		Mean = 6·2 ± 1·3
Adult		
FSF37	1·2	0·6
FSF36	2·1	0·9
NSF28	2·9	1·3
NSF30	2·4	1·1
NSF111	2·2	1·0
NSF130	2·3	1·1
		Mean = 1·0 ± 0·2
Breast cancer patient		
BSF7	8·7	3·9
BSF11 PS	12·6	5·7
BSF30	10·1	4·6
BSF31 SE	10·7	4·8
BSF37 EWT	6·7	3·1
		Mean = 4·4 ± 1·0

Data are presented concerning the effects of conditioned media produced by different foetal, normal adult and breast cancer patient skin fibroblasts on the migration of a target foreskin fibroblast line (FSF37) in high-density culture. The mean ± S.D. for each class of fibroblast (foetal, adult and cancer patient) are also given.

achieved. Both foetal and BSF CMs gave identical results. The maximal stimulation of FSF37 migration (13·3 %) is within the range of that displayed by the foetal and BSF cells themselves in high-density culture (see Fig. 1).

Gel filtration chromatography of foetal fibroblast CM

Neat CM produced by FS6 foetal fibroblasts was fractionated by gel filtration chromatography as described in Materials and methods. Individual fractions were then tested for their ability to stimulate the migration of FSF37 fibroblasts plated at high cell density in our standard assay system. As can be seen in Fig. 3, a single peak of migration-stimulating activity was recovered in fractions 11 and 12, corresponding to an apparent molecular weight in the range of 50–60 ($\times 10^3$). Identical results were obtained following the fractionation of 10-fold concentrated FS6 CM (data not shown).

Table 3. *Sensitivity of foetal and BSF CM to different treatments*

	% of FSF37 fibroblasts in gel matrix		
	Foetal CM		
	Low density	High density	BSF CM High density
Control (i.e. no CM)	23·7	1·7	1·7
Treatments			
None	22·7	13·3	12·6
56°C	21·8	4·8	3·3
100°C	22·8	1·7	1·5
Trypsin	20·6	0·5	3·0
Dialysis	25·1	12·4	10·2
pH 2	19·7	8·6	16·6
pH 10	22·2	1·9	5·5
Alkylation/reduction	24·8	2·9	3·2

CM obtained from foetal (FS6) and breast cancer patient (BSF11) fibroblasts were exposed to the various treatments indicated in the Table and then used in a standard migration assay, using FSF37 fibroblasts as the target cell line. The effects of foetal CM were ascertained at both high and low plating densities, while BSF CM was examined only in high-density culture.

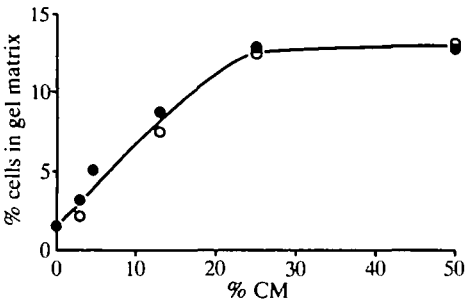

Fig. 2. Dose–response data concerning the effects of foetal (○) and BSF (●)-conditioned medium on the migration of a foreskin fibroblast target line (FSF37) plated at high density.

The effects of foetal and BSF CM on fibroblast proliferation

The effects of foetal and BSF CM on the proliferation of foetal (FS6) and adult (FSF37) fibroblasts are shown in Fig. 4. Cells were plated in 35 mm plastic tissue culture dishes at 2×10^4 cells per dish in medium containing 5 % serum and 25 % of the indicated CM. The foetal fibroblasts achieved a higher saturation density ($4{\cdot}9\times10^5$ cells/dish) than the adult cells ($1{\cdot}4\times10^5$ cells/dish). Neither foetal nor BSF CM had any effect on the proliferation of these cells. The growth of FSF37 cells on or within three-dimensional collagen gel substrata was similarly unaffected by both foetal and BSF CM (data not shown).

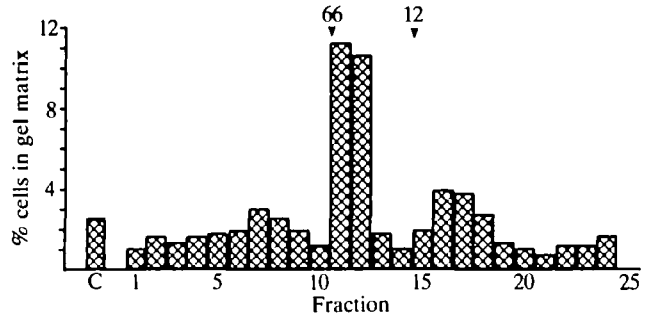

Fig. 3. Foetal fibroblast (FS6) CM was fractionated by gel filtration chromatography and the collected samples were then tested for their ability to stimulate the migration of FSF37 target cells. As in the standard migration assay, the FS6 CM fractions were tested at a final concentration of 25 % in medium containing 5 % serum. The first bar (labelled C) is the level of FSF37 migration achieved in control cultures in which SF-MEM was used instead of the FS6 CM fractions. (M_r values are given, $\times 10^{-3}$.)

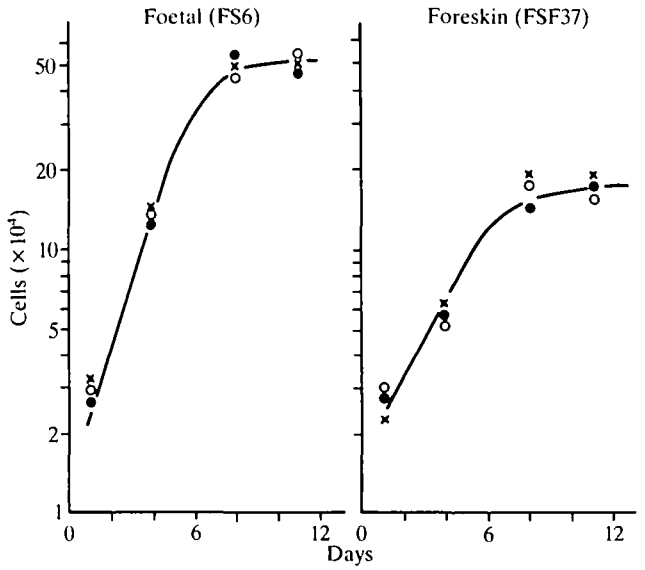

Fig. 4. The effects of foetal and BSF CM on the proliferation of foetal (FS6) and foreskin (FSF37) fibroblasts. Cells were plated in 35 mm plastic tissue culture dishes at 2×10^4 cells per dish in medium containing 5 % serum (×), or this medium supplemented with 25 % foetal (○) or BSF (●) CM. The foetal fibroblasts achieved a higher plateau cell density ($4{\cdot}9\times10^5$) than the adult cells ($1{\cdot}4\times10^5$). Neither foetal nor BSF CM affected cell proliferation.

The stimulation of adult fibroblast migration by MSF is dependent upon serum or plasma

Cell migration is known to be affected by a number of well-characterized soluble growth factors; e.g. platelet-derived growth factor, PDGF (Bernstein *et al.* 1982), and epidermal growth factor, EGF (Blay & Brown, 1985). These factors are present in different quantities in serum and plasma. With this rationale in mind, we examined the effects of serum and plasma on the ability of foetal and breast cancer patient fibroblast-conditioned medium to stimulate the migration of FSF37 target foreskin cells.

Table 4. *The effects of serum and plasma on the stimulation of fibroblast migration by foetal and breast cancer patient fibroblast-conditioned media*

	% of cells in gel matrix		
Conditioned medium	Serum-free	Plasma	Serum
None	4·6	2·3	4·9
Foetal	3·9	8·4	10·8
Breast cancer	4·6	9·4	11·1

FSF37 foreskin fibroblasts were plated onto collagen gels at high density in the presence and absence of foetal (FS6) and breast cancer patient (BSF11) fibroblast-conditioned media in (1) serum-free medium, (2) medium containing 5 % bovine plasma and (3) medium containing 5 % bovine serum. The percentage of cells within the gel matrix was measured after 4 days of incubation.

In the experiment presented in Table 4, cells were plated at high density in the presence or absence of the different conditioned media in (1) serum-free medium, (2) medium supplemented with 5 % bovine plasma and (3) medium supplemented with 5 % bovine serum. Our results indicate that cell migration in the absence of conditioned medium occurs to the same extent in serum-free medium and medium supplemented with either serum or plasma. These results are consistent with previous reports indicating that the migration of several cell types, including human skin fibroblasts and melanoma cells, into collagen gels is not dependent upon the presence of serum (Schor *et al.* 1981). In contrast, stimulation of FSF37 migration in high-density cultures by either foetal or breast cancer patient-conditioned medium was only observed in the presence of serum or plasma; cells cultured in serum-free medium were completely unresponsive to these conditioned media.

Discussion

The data presented here indicate that: (1) foetal and BSF fibroblasts migrate to a significantly greater extent in high-density culture than do normal adult cells; (2) foetal and BSF fibroblasts produce a soluble factor (MSF) that stimulates the migration of normal adult cells in high-density culture (with a consequent expression of CDMI values falling in the foetal range); (3) foetal and BSF CM do not affect the migration of adult cells in low-density culture; and (4) adult fibroblast CM does not inhibit cell migration. The differential effect of the factor on the migration of FSF37 cells

at high and low densities is consistent with the potential role of this factor in determining the distinctive CDMI values displayed by the different classes of fibroblasts.

The migration-stimulating activity in foetal and BSF CM displayed the same sensitivity to various treatments (trypsinization, dialysis, heat, pH change, reductive alkylation). The similarity of foetal and BSF migration-stimulating activity is again indicated by the identical dose–response curves of the respective CMs. Further characterization and purification of the respective factors will reveal whether foetal and breast cancer patient fibroblast MSF are in fact identical. Gel filtration chromatography of foetal fibroblast CM indicates that MSF has an apparent molecular mass in the range of 50–60 ($\times 10^3$). Identical results have been obtained for MSF produced by other foetal and breast cancer patient fibroblasts (Grey *et al.* unpublished); these data will be published with further details regarding the purification and biochemical characterization of MSF.

For various purposes (including the purification of MSF), it will be convenient to express migration-stimulating activity in terms of units ml^{-1} CM or units μg^{-1} protein. We have therefore defined a unit of activity as that required to produce a threefold stimulation in the migration of a target adult fibroblast in high-density culture. Examination of the dose–response data presented in Fig. 2 indicates that a migration level corresponding to one unit of migration-stimulating activity is achieved at a concentration of 10% CM. Since both foetal and BSF CM contain approximately 50 μg protein ml^{-1}, we calculate that these CMs contain in the region of 10 units per ml or 0·2 unit μg^{-1} protein.

Neither foetal nor BSF CMs stimulated adult cell proliferation; these results indicate that the ability of MSF to stimulate fibroblast migration is not dependent upon the induction of cell proliferation. In this regard, recent data suggest that the stimulation of adult fibroblast migration by MSF is a secondary consequence of its primary effect upon the deposition of matrix macromolecules, in particular hyaluronic acid (Schor & Schor, 1987*b*; Schor *et al.* unpublished data). This finding is in keeping with the growing body of evidence indicating that many soluble 'growth' factors, e.g. transforming growth factor-beta, exert a primary effect upon matrix synthesis (Ignotz & Massague, 1986).

The results presented in Table 4 suggest that the mechanism by which the MSF stimulates cell migration is dependent upon the concerted action of other factors present in either serum or plasma. These results would appear to exclude a dependence upon PDGF and other platelet-derived factors since these are not present in platelet-poor plasma. It should also be noted that in contrast to this absolute dependence upon serum or plasma for MSF action, it is nonetheless produced by both foetal and breast cancer patient fibroblasts under serum-free conditions, i.e. our standard method for obtaining conditioned medium.

Migration-stimulating factors produced by a variety of cell types and displaying a diversity of target cell specificities have recently been described. It is of interest to note that many of these factors have apparent molecular masses in the range of 50–60 ($\times 10^3$) (Stoker & Pennyman, 1986; Liotta *et al.* 1986; Stoker *et al.* 1987; Atnip *et al.* 1987). A number of lines of evidence, however, suggest that they are not identical with fibroblast MSF. For example, foetal fibroblasts produce a scatter factor that induces the dispersion of normal epithelial cells from their characteristically tightly packed colonies (Stoker & Pennyman, 1986; Stoker *et al.* 1987). Using this assay system, it was found that FS6 foetal fibroblast CM contained measurable scatter factor activity, whilst BSF11 breast cancer patient fibroblast-conditioned medium was negative (Stoker *et al.* 1987, and personal communication). These results suggest that the epithelial scatter factor described by Stoker (present *only* in foetal fibroblast CM) and the fibroblast migration-stimulating factor discussed here (present in *both* foetal and BSF CM) may not be the same biochemical entity. In addition, scatter factor acts in a paracrine fashion, since it is produced by foetal fibroblasts, but exerts its biological activity on normal epithelial cells; in contrast, the migration-stimulating factor described here is assayed by virtue of its effect upon target adult fibroblasts. The elevated levels of migration displayed by foetal and BSF fibroblasts in high-density culture most probably result from the constitutive production of MSF by these cells, thereby suggesting an autocrine mode of action for this factor.

Migration-stimulating factors have also been reported to be produced by metastatic tumour cell lines. Autocrine motility factor (AMF) described by Liotta *et al.* (1986) is perhaps the best characterized. Our preliminary data suggest that AMF is not the same molecule as fibroblast MSF; significant differences between the two factors include: (1) heat stability (MSF inactivated by heating to 60°C, whilst AMF is not); (2) pH sensitivity (MSF stable at acid pH and labile at alkaline pH, whilst AMF labile at acid pH and stable at alkaline pH); (3) dependence upon serum or plasma (MSF requires the presence of either serum or plasma for activity, whilst AMF is assayed in serum-free medium); and (4) target cell specificity (MSF stimulates the migration of non-producing normal adult target cells, whilst AMF stimulates the migration of producing metastatic tumour cell lines, but not normal or non-metastatic cell lines).

It should also be emphasized that the activities of these various migration-stimulating factors are assayed under quite different conditions; MSF is identified by

its stimulation of fibroblast migration into three-dimensional collagen gels during an extended incubation period of 4 days, scatter factor by its dispersion of epithelial cells growing on plastic dishes and AMF by its effect upon cell migration into a Nucleopore filter during an incubation period of only 4 h. Such fundamental differences in the assay procedure must be borne in mind when comparing different factors that affect such a complex behavioural attribute as cell migration. In spite of these important caveats, the apparent similarity in molecular weight displayed by these various factors (50–60 ($\times 10^3$)) raises the intriguing possibility that they may nonetheless be members of a related family of molecules.

We have previously discussed the possibility that the presence of foetal-like fibroblasts in the adult increases susceptibility to the development of cancer by virtue of the resultant dysfunction in normal epithelial–mesenchymal interactions (Schor *et al.* 1987). In the present study we have demonstrated that the foetal-like fibroblasts of cancer patients secrete a migration-stimulating factor with similar biochemical properties to that produced by *bone fide* foetal cells. A more detailed consideration of the foetal-like fibroblasts of cancer patients and their potential involvement in disease pathogenesis is presented in the accompanying paper.

This work has been funded by a grant from the Cancer Research Campaign.

References

Abercrombie, M. (1970). Contact inhibition in tissue culture. *Nature, Lond.* **279**, 442–444.

Atnip, K. D., Carter, L. M., Nicolson, G. L. & Dabbous, M. K. (1987). Chemotactic response of rat mammary adenocarcinoma cell clones to tumor-derived cytokines. *Biochem. biophys. Res. Commun.* **146**, 996–1002.

Bernstein, L. R., Antoniades, H. & Zetter, B. R. (1982). Migration of cultured vascular cells in response to plasma and platelet-derived factors. *J. Cell Sci.* **56**, 71–82.

Blay, J. & Brown, K. (1985). Epidermal growth factor promotes the chemotactic migration of cultured rat intestinal epithelial cells. *J. cell. Physiol.* **124**, 107–112.

Durning, P., Schor, S. L. & Sellwood, R. A. S. (1984). Fibroblasts from patients with breast cancer show abnormal migratory behaviour *in vitro*. *Lancet* **i**, 890–892.

Grinnell, F. & Lamke, C. R. (1984). Reorganization of hydrated collagen lattices by human skin fibroblasts. *J. Cell Sci.* **66**, 51–63.

Haggie, J., Howell, A., Sellwood, R. A., Birch, J. M. & Schor, S. L. (1987). Fibroblasts from relatives of patients with hereditary breast cancer show fetal-like behaviour *in vitro*. *The Lancet* **i**, 1455–1457.

Ham, R. G. (1980). Dermal fibroblasts. In *Methods in Cell Physiology*, vol. 21 (ed. C. Harris, B. F. Trump & G. Stoner), pp. 255–276. New York: Academic Press.

Ignotz, R. A. & Massague, J. (1986). Transforming growth factor beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J. biol. Chem.* **261**, 4337–4345.

Liotta, L. A., Mandler, R., Murano, G., Katz, D. A., Gordon, R. K., Chiang, P. K. & Schiffmann, E. (1986). Tumor cell autocrine motility factor. *Proc. natn. Acad. Sci. U.S.A.* **83**, 3302–3306.

Mautner, V. & Hynes, R. O. (1977). Surface distribution of LETS protein in relation to the cytoskeleton of normal and transformed fibroblasts. *J. Cell Biol.* **75**, 743–768.

Schor, S. L. (1980). Cell proliferation and migration within three-dimensional collagen gels. *J. Cell Sci.* **41**, 159–175.

Schor, S. L. & Court, J. (1979). Different mechanisms involved in the attachment of cells to native and denatured collagen. *J. Cell Sci.* **38**, 267–281.

Schor, S. L. & Schor, A. M. (1987*a*). Clonal heterogeneity in fibroblast phenotype: implications for the control of epithelial–mesenchymal interactions. *BioEssays* **7**, 200–204.

Schor, S. L. & Schor, A. M. (1987*b*). Foetal-to-adult transitions in fibroblast phenotype: their possible relevance to the pathogenesis of cancer. In *Cell Behaviour: Shape, Adhesion and Mobility* (ed. J. Heaysman, A. Middleton & F. Watt). *J. Cell Sci. Suppl.* *8*, 165–180.

Schor, S. L., Schor, A. M. & Bazill, G. W. (1981). The effects of fibronectin on the migration of human foreskin fibroblasts and Syrian hamster melanoma cells into three-dimensional gels of native collagen fibres. *J. Cell Sci.* **48**, 301–314.

Schor, S. L., Schor, A. M., Howell, A. & Crowther, D. (1987). Hypothesis: Persistent expression of fetal phenotypic characteristics by fibroblasts is associated with an increased susceptibility to neoplastic disease. *Expl Cell Biol.* **55**, 11–17.

Schor, S. L., Schor, A. M., Durning, P. & Rushton, G. (1985*b*). Skin fibroblasts obtained from cancer patients display foetal-like behaviour on collagen gels. *J. Cell Sci.* **73**, 235–244.

Schor, S. L., Haggie, J. A., Durning, P., Howell, A., Smith, L., Sellwood, R. A. S. & Crowther, D. (1986). Occurrence of a fetal fibroblast phenotype in familial breast cancer. *Int. J. Cancer* **37**, 831–836.

Schor, S. L., Schor, A. M., Rushton, G. & Smith, L. (1985*a*). Adult, foetal and transformed fibroblasts display different migratory phenotypes on collagen gels: Evidence for an isoformic transition during foetal development. *J. Cell Sci.* **73**, 221–234.

Schor, S. L., Schor, A. M., Winn, B. & Rushton, G. (1982). The use of three-dimensional collagen gels for the study of tumour cell invasion *in vitro*: Experimental parameters influencing cell migration into the gel matrix. *Int. J. Cancer* **29**, 57–62.

Stoker, M., Gherardi, E., Pennyman, M. & Gray, J. (1987). Scatter factor is a fibroblast-derived modulator of

epithelial cell mobility. *Nature, Lond.* **327**, 239–242.

STOKER, M. & PENNYMAN, M. (1986). An epithelial scatter factor released by embryo fibroblasts. *J. Cell Sci.* **77**, 209–224.

VOGEL, A. E., RAINES, B., KARIYA, B., RIVEST, M. & ROSS, R. (1978). Coordinate control of 3T3 cell proliferation by platelet derived growth factor and plasma components. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2810–2814.

(*Received 7 January 1988 – Accepted 18 March 1988*)