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Membrane Association of the Hyaluronate Stimulatory Factor From LX-1 Human Lung Carcinoma Cells

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LX-1 human lung carcinoma cells interact with human fibroblasts in culture to cause an increase in hyaluronate production (Knudson et al: *Proceedings of the National Academy of Sciences of the United States of America* 81:6767, 1984). It is shown here that a similar increase in hyaluronate production also occurs when membranes derived from LX-1 cells, or detergent extracts thereof, are added to cultures of the human fibroblasts. However, no stimulation occurs when membranes or extracts from fibroblasts are added to cultures of the LX-1 cells. The hyaluronate stimulatory factor present in the detergent extracts is a heat- and trypsin-sensitive protein, requires more than 12 h for its action on fibroblasts, causes an elevation in hyaluronate synthetase activity in membranes derived from the fibroblasts, and can be reconstituted into artificial lipid vesicles. Thus, it is concluded that the stimulatory factor is a membrane-bound protein present on the surface of the LX-1 cells and that it interacts with fibroblasts to induce increased hyaluronate synthesis.

Key words: tumor cell-fibroblast interaction, tumor invasion, hyaluronate synthetase, glycosaminoglycans

The extracellular matrix associated with invasive tumors is often enriched in the glycosaminoglycan (GAG) hyaluronate [1–7]. Hyaluronate is thought to contribute to the formation of hydrated pathways for cell migration during embryonic morphogenesis, tissue remodelling, and tumor invasion [8–10]. Interactions between tumor cells and normal connective tissue fibroblasts may lead to increased hyaluronate synthesis during tumor invasion since it has been observed that GAG synthesis is stimulated in cocultures of fibroblasts with several types of human tumor cells or in cultures of fibroblasts with conditioned medium from tumor cell cultures [11–15].

We have shown that the stimulation of hyaluronate synthesis in cocultures of LX-1 human lung carcinoma cells and human fibroblasts is dependent on cell-cell

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contact [11]. In the present study we show that membranes isolated from LX-1 cells, but not fibroblasts, contain a protein (termed hyaluronate stimulatory factor: HSF) which stimulates hyaluronate synthesis by fibroblasts and that this factor can be extracted in active form with deoxycholate detergent solution and then reconstituted into artificial lipid vesicles.

MATERIALS AND METHODS

Cell Cultures

Normal human fibroblasts were derived from explant cultures of adult skin or were obtained from the American Type Culture Collection (CCD28Sk). LX-1 human lung carcinoma cells were isolated by explant culture of tumor tissue grown in nude mice and obtained from Frederick Cancer Institute (Bethesda, MD). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution, incubated at 37° in humidified 5% CO_2/95% air. Experiments were done with human fibroblasts and tumor cells between the 4th and 12th passage.

Preparation of Tumor Cell Membranes and Detergent Extraction of HSF

LX-1 cells were grown in 100-mm^2 culture dishes in DMEM containing 10% FBS and antibiotics. Prior to isolation of the membranes the medium was changed twice during a 12-h period to serum-free DMEM, the medium was removed, and the tumor cells scraped from the dish in 0.05 M Tris, pH 7.05, containing 0.24 M sucrose. Membranes were prepared from these cells by the procedure of Appel et al. [16]. The suspended cells were sonicated on ice with four 15-s bursts (Heat Systems Ultrasonics sonicator, at 60 W), followed by centrifugation at 100,000*g* to pellet the membranes. The supernatant was removed and assayed for HSF activity; the pellet was resuspended in 1/20th of the original volume of either DMEM (for assay of HSF activity prior to solubilization) or DMEM containing 0.5% deoxycholate (for detergent extraction). For extraction, the pellet was stirred for 12 h at 4°C in 0.5% deoxycholate (Sigma, St. Louis, MO) followed by centrifugation at 100,000*g* to remove nonsolubilized material.

For comparison, membranes and detergent extracts thereof were prepared from fibroblast cultures under similar conditions.

Assay for Hyaluronate Stimulatory Factor (HSF) Activity

HSF activity was measured by the increase in incorporation of ^{3}H-acetate into hyaluronate on addition of the various preparations to cultures of normal fibroblasts. The fibroblasts were plated into 16-mm^2 wells (Falcon) at a density of 10^5 cells per well in 1.0 ml DMEM containing 10% FBS and antibiotics. After allowing the cells to attach for 24 h the medium was replaced twice with 1.0 ml serum-free DMEM followed by 1.0 ml DMEM containing 20 μCi/ml ^{3}H-acetate (2.3 Ci/mmol, New England Nuclear, Boston MA) and various concentrations of membrane-derived fractions from LX-1 cells or fibroblasts. All test fractions were dialyzed extensively at 4°C against several changes of DMEM, then sterilized by exposure to UV light for 20 min on ice, prior to addition to the fibroblast cultures. After 24-h incubation (except for the time-course study), duplicate cultures were processed for analysis of

cell number and ^{3}H-GAG by a modification of previous methods [11] as described below.

Analysis of ^{3}H-GAG

Medium plus cell washes were digested with 0.5 mg/ml Protease (Type XIV, Sigma) in 0.1 M Tris, pH 8.0, for 24 h at 37°C, followed by inactivation of the protease at 100°C for 10 min. Aliquots were then incubated for 12 h at 37°C with or without 30 units of testicular hyaluronidase (Type I-S, Sigma) in 0.1 M Na acetate/0.05 M NaCl, pH 5.0, or 2.5 units of *Streptomyces* hyaluronidase (Calbiochem, La Jolla, CA) in a 0.1 M Na acetate/0.05 M NaCl, pH 6.0. The former enzyme degrades hyaluronate and the chondroitin sulfates, the latter only hyaluronate. The samples were heated at 100°C for 10 min, carrier hyaluronate and chondroitin sulfate were added, and the GAG was precipitated with 10% cetylpyridinium chloride (CPC). The pellets were washed with 0.05% CPC/0.05 M NaCl, dissolved in methanol, and the radioactivity was measured. The amounts of radioactivity obtained in the untreated samples were used as a measure of incorporation into total GAG. Incorporation into specific GAG was calculated from the difference in radioactivity obtained in enzyme-treated and untreated samples. The amount of radioactivity remaining after testicular hyaluronidase digestion was less than 10% of the total CPC-precipitable counts.

Hyaluronate Synthetase Assay

Fibroblasts were grown in 175-cm flasks (12 per experiment) until nearly confluent, then the medium was changed twice to serum-free DMEM. Dialyzed deoxycholate extract of the LX-1 membranes was added to half of the fibroblast cultures (the other half served as controls) in amounts equivalent to those used routinely for demonstrating stimulation of hyaluronate synthesis—i.e., 100 μg of deoxycholate-extracted protein per 1×10^5 cells (e.g., see Fig. 3). The cultures were then incubated for either 12 or 24 h, and the media were removed. The cells were scraped from the flasks in 0.05 M Tris, pH 7.1/0.24 M sucrose, placed on ice, and sonicated (60 s at 100 W then 30 s at 100 W). The sonicate was centrifuged at 100,000*g* for 60 min, the supernatant removed, and the pellet suspended in 0.5 ml of the Tris/sucrose buffer.

Synthetase activity in these membranes was measured by the method of Appel et al. [16], in which 20-μl aliquots of membrane suspension are incubated for 2 h at 37°C in a standard incubation mixture containing UDP-[^{14}C] glucuronic acid. At the end of the incubation, triplicate samples were used for paper chromatography during which unincorporated isotope migrates and synthesized hyaluronate remains at the origin. Other samples were digested with *Streptomyces* hyaluronidase prior to chromatography. Synthesis of hyaluronate was defined as the difference in radioactivity at the origin in the untreated and hyaluronidase-treated samples. Less than 20% of the incorporated radioactivity was resistant to the hyaluronidase treatment.

RESULTS

Membrane Association of HSF

In previous experiments, cocultures of LX-1 human lung carcinoma cells and human fibroblasts were shown to produce two- to threefold greater amounts of hyaluronate than the sum of individual cultures of the LX-1 cells and fibroblasts [11].

In this study we have prepared total membrane fractions from the LX-1 cells and fibroblasts by centrifugation of cell sonicates at 100,000g and measured their effect on hyaluronate synthesis by LX-1 cells and fibroblasts in culture. Resuspended membrane fractions from LX-1 cells were added to cultures of fibroblasts and found to cause an approximately twofold stimulation in hyaluronate production by the fibroblasts (Fig. 1), an effect equivalent to that observed in the cocultures. However, the supernatant fraction from centrifugation of the LX-1 cell sonicates had no significant effect on fibroblast hyaluronate production (data not shown). Membrane fractions prepared from the fibroblasts themselves did not stimulate fibroblast hyaluronate production (Fig. 1). Also, when membrane fractions prepared from the fibroblasts were tested in cultures of LX-1 cells, they had no effect on hyaluronate synthesis by the LX-1 cells (data not shown). Thus we conclude that a) the hyaluronate stimulation previously seen in cocultures of LX-1 cells and fibroblasts is due to a factor produced by the LX-1 cells rather than the fibroblasts; and b) the HSF from LX-1 cells is membrane associated.

Extraction of Hyaluronate Stimulatory Factor (HSF) From LX-1 Cell Membranes

Several reagents were tested for their ability to extract the HSF in active form from LX-1 cell membranes. Of the those tested deoxycholate was the most effective, yielding an activity similar in potency to the membranes before extraction (Table I).

TABLE I. Extraction of HSF Activity

Reagent used for extraction	Incorporation into hyaluronate[a]	Percent of control
Membrane extracts[b]		
Control	1,156 ± 177	100
0.5% deoxycholate	2,131 ± 217	184
1.0% CHAPS	1,346 ± 52	116
1.0% octylglucoside	889 ± 79	78
1 M urea	1,019 ± 118	88
Whole cells[c]		
Control	806 ± 164	100
2.5% butanol	1,870 ± 18	232

[a]Results are expressed as incorporation of isotopic precursor into hyaluronate (see Materials and Methods). Each value is the mean ± the range of 4 measurements from 2 separate experiments.

[b]Membranes were extracted with the indicated reagents for 4–12 h at 4°C; then the extracts were dialyzed extensively against DMEM, sterilized under UV light, and added to fibroblast cultures for assay of HSF activity. The final concentration of extract in the fibroblast cultures was in each case adjusted to be equivalent to 3×10^5 cells/ml.

[c]Monolayer cultures of LX-1 cells (6×175-cm^2 flasks each with approximately 4×10^6 cells) were treated with a total volume of 6 ml of 2.5% butanol in PBS for 5 min at room temperature. The extract was dialyzed against DMEM, sterilized under UV light, and aliquots equivalent to approximately 0.2×10^5 cells were added to fibroblast cultures for assay of HSF activity. For controls the same volume of 2.5% butanol was processed in the same way and added to the fibroblast cultures.

Addition of increasing concentrations of deoxycholate extract to fibroblast cultures gave rise to increasing increments in hyaluronate production up to a maximum of 2.5-fold that of the control fibroblasts (Fig. 2) A twofold stimulation, characteristic of cocultures of equal numbers of LX-1 cells and fibroblasts, was obtained when an amount of extract equivalent to 2×10^5 LX-1 cells was added to 1×10^5 fibroblasts. When the activity of the deoxycholate extract was compared with the residual membrane after extraction, approximately 85% of total membrane activity was found in the former and 15% in the latter (Fig. 2). The deoxycholate extract had approximately twice the specific activity of the original membranes since only 50% of the total protein was extracted. Deoxycholate extracts of fibroblast membranes did not stimulate hyaluronate production by the fibroblasts but were in fact inhibitory (data not shown).

Intact monolayers of the LX-1 cells were also extracted with 2.5% butanol, a reagent which has been shown in other systems to extract proteins only from the outer leaflet of the plasma membrane of intact cells [17]. As can be seen from Table I, the butanol extracted a similar level of HSF activity to that obtained by extraction of isolated membranes with deoxycholate. Similar extraction of fibroblasts did not yield any HSF activity.

Properties of Detergent Extracted HSF

The protein nature of the deoxycholate-extracted HSF was established by measurement of its sensitivity to trypsin and to heat. Treatment of the HSF with TPCK (1-tosylamido-2-phenylethylchloro-methyl ketone) trypsin resulted in complete loss of activity (Table II). Heating at 50°C or 60°C for 10 min caused 20% loss of HSF activity and heating at 70°C or 80°C caused 60–100% loss of activity.

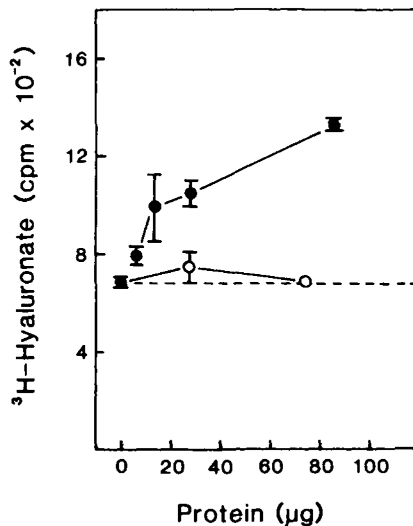

Fig. 1. Effect of LX-1 and fibroblast cell membranes on hyaluronate synthesis by fibroblasts. Various concentrations of LX-1 (●) or human fibroblast (○) membrane suspensions were added to wells containing 1×10^5 human fibroblasts. The wells were then incubated for 24 h in the presence of ^{3}H-acetate for measurement of HSF activity as described in the Methods. The results are presented as incorporation into hyaluronate in the presence (●, ○) or absence (———) of the membranes. Each value represents the mean ± range of 4 measurements from 2 separate experiments.

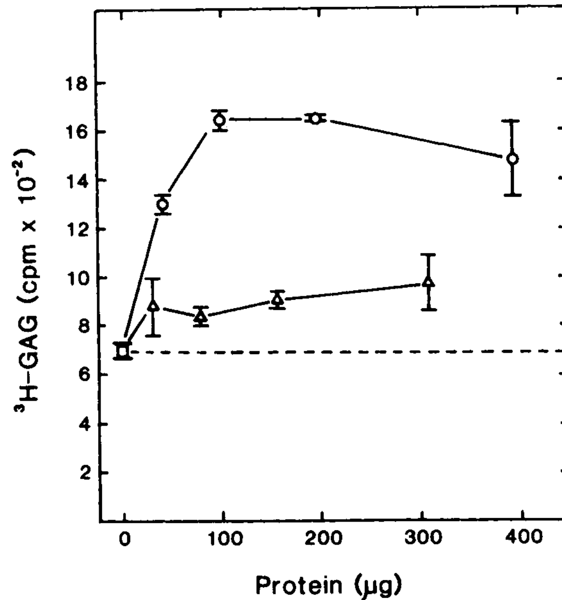

Fig. 2. Stimulation of fibroblast hyaluronate synthesis by deoxycholate extract of LX-1 membranes. Various concentrations of deoxycholate extract of LX-1 membranes (○) or a suspension of the residue from deoxycholate extraction (△) were added to wells containing 1×10^5 human fibroblasts. The wells were then incubated for 24 h in the presence of ^{3}H-acetate for measurement of HSF activity as described in Materials and Methods. Results are presented as incorporation into testicular hyaluronidase-sensitive GAG in the presence (○, △) or absence (———) of the extract or residue. Each value represents the mean ± range of 4 measurements from 2 separate experiments.

The time required for the onset of action of HSF was determined by exposure of fibroblasts to deoxycholate-extracted HSF for varying time intervals. As shown in Figure 3, hyaluronate production by cultures in the presence or absence of HSF was indistinguishable for the first 12 h of culture, but a clearly apparent difference was observed at 24 h. No increase in cell number was observed over these time intervals.

The specificity of stimulation by the HSF was determined by analyzing the nature of the GAG produced by control and stimulated fibroblasts. Table III shows that the major GAG produced in both cases was hyaluronate, that no significant increase in GAGs other than hyaluronate occurred, and that the proportion of total GAG that was hyaluronate increased from 78% to 85–89% on stimulation.

We also examined the effect of HSF on the level of hyaluronate synthetase activity in the fibroblasts. The fibroblasts were treated with the HSF extract for 12 and 24 h, then harvested, and membranes were prepared for assay of hyaluronate synthetase activity. No stimulation was seen at 12 h, but a 1.7-fold increase had occurred at 24 h (Fig. 4). Thus, a similar level and time course of increase occurred in hyaluronate production by the stimulated fibroblasts and hyaluronate synthetase activity in membranes derived from the stimulated fibroblasts.

Since HSF was derived from cell membranes, its potentially lipophilic nature was tested by attempting reconstitution of HSF into the membrane of lipid vesicles. In order to be able to monitor the formation of vesicles, ^{3}H-dipalmitoyl-phosphatidylcholine was included in the samples. Vesicles were formed by extensive dialysis

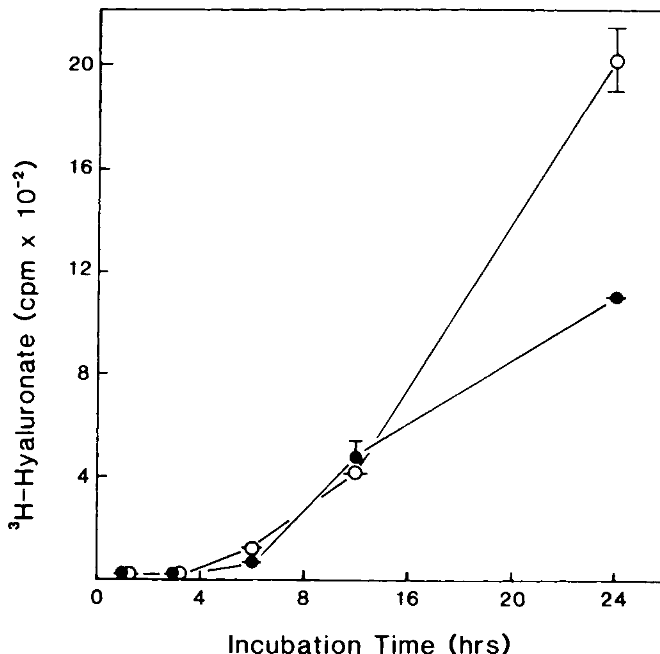

Fig. 3. Time course of stimulation of hyaluronate synthesis by deoxycholate extract of LX-1 membranes. Human fibroblasts (1×10^5 per well) were incubated for various lengths of time with (○) or without (●) a constant amount of deoxycholate extract containing 100 μg protein. Incubation was carried out in the presence of ^{3}H-acetate as described for measurement of HSF activity in Materials and Methods. The extract and isotope were added at time zero in each case. Results are presented as incorporation into hyaluronate. Each value represents the mean $\pm$ range of 4 measurements from 2 separate experiments. Cell number was determined at the 0-, 12-, and 24-h time points. No change in cell number was found over this period.

against DMEM of deoxycholate extracts containing the ^{3}H-labeled lipid, then collected by high-speed centrifugation [18]. Table IV shows that 97% of the ^{3}H-labeled lipid and 38% of the protein of the extract were recovered in the pellet containing the vesicles. The HSF activities of the pellets after resuspension and of the supernatants from centrifugation were also measured by addition to cultures of fibroblasts. The HSF activities of these two fractions were found to be approximately equal (Table IV), suggesting that approximately 50% of the HSF had become associated with the vesicles. Since the volume of the vesicle pellet, prior to suspension, was much smaller than that of the supernatant, this distribution was not due to nonspecific trapping inside the vesicles. Also the specific activity of the vesicle-bound HSF was approximately three times that of the whole detergent extract. Addition of further exogenous unlabeled lipid (2,4, or 8 mg/ml soy phosphatidylcholine plus 1 mg/ml cholic acid) did not alter the above distribution.

The reconstituted vesicles obtained above were further characterized by sucrose density centrifugation and gel filtration. When centrifuged in a step gradient of sucrose for 16 h, approximately 24% of the applied protein and greater than 90% of the ^{3}H-labeled lipid banded at the 17–30% sucrose interface (Fig. 5), characteristic of lipid vesicles formed in this way [18]. Fractions from the gradient were tested for HSF activity which was also found to be localized at the 17–30% interface (Fig. 5A).

TABLE II. Effect of Trypsin on HSF Activity

Additions to fibroblasts	Incorporation into hyaluronate	Percent of control
Control (no additions	3,966 ± 89	100
DOC extract[a]	6,927 ± 390	175
Trypsin-treated DOC extract[b]	3,577 ± 698	90
Trypsin/SBTI[c]	3,821 ± 415	96
Trypsin/SBTI plus DOC extract[d]	6,809 ± 136	172

[a]Aliquots of dialyzed deoxycholate (DOC) extract of LX-1 membrane, containing 200 μg protein in DMEM, were added to cultures of fibroblasts for measurement of HSF activity. Results are expressed as incorporation of isotopic precursor into hyaluronate (see Materials and Methods). Each value is the mean ± range of 4 measurements from 2 separate experiments.
[b]Aliquots of DOC extract containing 200 μg protein were treated with 14 μg TPCK trypsin in DMEM for 30 min at 37°C, then the trypsin was inactivated with 70 μg soybean trypsin inhibitor (SBTI) in DMEM and the mixture added to cultures for measurement of HSF activity.
[c]Concentrations of TPCK trypsin and SBTI equivalent to those listed above were added simultaneously to fibroblast cultures.
[d]Equivalent concentrations of TPCK trypsin and SBTI followed by amounts of untreated DOC extract equivalent to those listed above were added to fibroblast cultures.

TABLE III. Analysis of GAG Composition

	Total GAG[a]	Hyaluronate	Chondroitin sulfate
Control	1,491 ± 206	1,017 ± 41	143 ± 21
100 μg HSF	2,422 ± 310	1,988 ± 380	87 ± 12
200 μg HSF	2,223 ± 215	1,795 ± 208	140 ± 15

[a]Fibroblast cultures were incubated with or without aliquots of deoxycholate extract containing 100 or 200 μg protein under the conditions described in the legend of Figure 2. The media were collected and analyzed for ^{3}H-GAG as described in Materials and Methods. Each value represents the mean ± range of 4 measurements from 2 separate experiments.

The peak fractions of HSF activity from the sucrose gradient were then pooled and chromatographed on Sepharose CL-4B in the presence or absence of added detergent. As shown in Fig. 6A, greater than 90% of the protein and of the ^{3}H-lipid eluted together in the void volume in the absence of detergent, as would be expected if the protein and lipid were combined in the form of vesicles. In the presence of added deoxycholate, which would disrupt the vesicles, the lipid and protein became dissociated from one another. The lipid chromatographed in the total volume of the column, whereas the protein was recovered in the included volume (Fig. 6B). As a control, vesicles were formed by dialysis of a mixture of 2 mg/ml of phosphatidylcholine plus 1 mg/ml of BSA in 0.5% deoxycholate, then chromatographed in the presence or absence of detergent. The lipid eluted in the same fashion as shown in Figure 6A, B, whereas the BSA eluted in the included volume whether or not detergent was present. Thus the soluble protein, BSA, did not interact nonspecifically with the lipid vesicles.

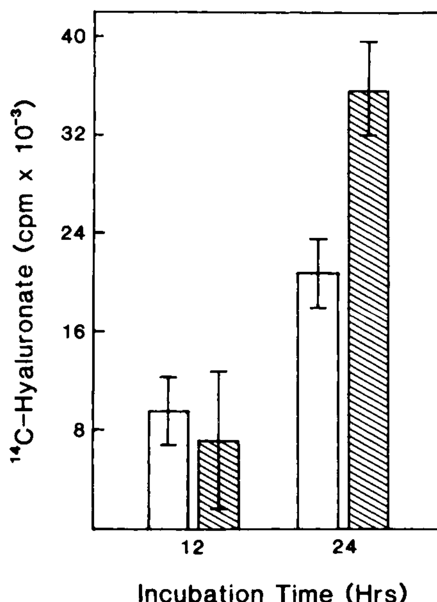

Fig. 4. Stimulation of fibroblast hyaluronate synthetase activity by deoxycholate extract of LX-1 membranes. Human fibroblasts were incubated for 12 or 24 h with (hatched bars) or without (open bars) deoxycholate extract of LX-1 membranes. The amounts of extract used were the same as those used in Figure 3 for demonstration of HSF activity, i.e., 100 μg of protein per 1×10^5 cells. Hyaluronate synthetase activity was measured in the fibroblasts as described in Materials and Methods. Each value represents the mean $\pm$ range of triplicate measurements.

TABLE IV. Reconstitution of HSF Into Lipid Vesicles*

Fraction[a]	HSF activity[b] (% of control)	Protein[c] (mg/ml)	Phospholipid[d] (cpm)
Supernatant	180	1.89	130
Pellet	170	1.18	3320

*All values represent means of duplicate experiments with all errors less than 10%.
[a]Deoxycholate extracts of LX-1 membranes were dialyzed against DMEM to remove detergent, then centrifuged at 100,000*g* to obtain the supernatant and pellet (vesicles) fractions. The pellet fraction was resuspended in DMEM, and then both fractions sterilized and added to fibroblast cultures for assay of HSF activity (see Materials and Methods).
[b]HSF activity is measured as the relative incorporation of isotopic precursor into hyaluronate in the presence or absence of the supernatant or pellet (see Materials and Methods). Controls were untreated fibroblast cultures.
[c]Protein was measured by the method of Lowry et al. [29].
[d]^{3}H-phosphatidylcholine was added to the deoxycholate extracts prior to the dialysis step to measure distribution of lipids.

DISCUSSION

The invasion of tumor cells into surrounding tissues is one of several critical events which occur during the process of metastasis [19]. Under normal circumstances, the extracellular matrix provides an effective barrier to cell movement [20,21]. However, several lines of evidence suggest that, during tumor cell invasion, this matrix barrier is altered both by enzymatic cleavage of existing matrix macromolecules, e.g., by collagenases, and by synthesis of new matrix conducive to cell

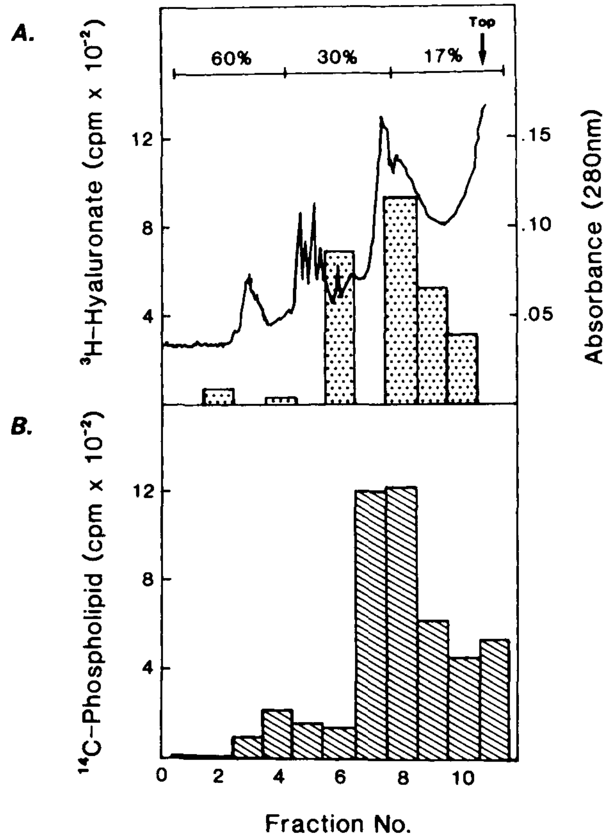

Fig. 5. Sucrose density gradient centrifugation of reconstituted lipid vesicles. Vesicles reconstituted as described in Table IV were applied in a 1-ml volume to an 11-ml step gradient comprised of equal volumes of 17%, 30%, and 60% sucrose. The tubes were then centrifuged for 16 h at 27,000 rpm (85,000*g*) at 10°C in a Beckman SW41 rotor. Fractions (0.5 ml) were collected from the bottom of the centrifuge tubes and monitored for protein by absorbance at 280 nm (line) (**A**) and for ^{3}H-phosphatidylcholine radioactivity (hatched bars) (**B**). Following extensive dialysis and sterilization, fractions were also assayed for HSF activity (dotted bars) (**A**) as described in Materials and Methods, and the results are presented as incorporation into hyaluronate.

migration, e.g., hyaluronate and proteoglycan-rich matrices [9,10,21–24]. Recently, it has been shown that the interaction of tumor cells with fibroblasts may be important in this alteration of the extracellular matrix surrounding tumors. For instance, it has been shown that fibroblasts derived from the stroma of a basal cell carcinoma produce higher levels of collagenase than control fibroblasts [25] and that several tumor cell types secrete factors which stimulate collagenase production by normal fibroblasts [26,27].

Our previous experiments have demonstrated that coculture of human tumor cell lines, derived from a lung carcinoma (LX-1), a pancreatic carcinoma (DAN) and a melanoma (TRIG), with normal human skin fibroblasts results in a substantial increase in hyaluronate production [11]. This stimulation was shown to occur only when the tumor cells and fibroblasts were in contact with one another. No HSF activity was detected in conditioned medium harvested from tumor cell or fibroblast

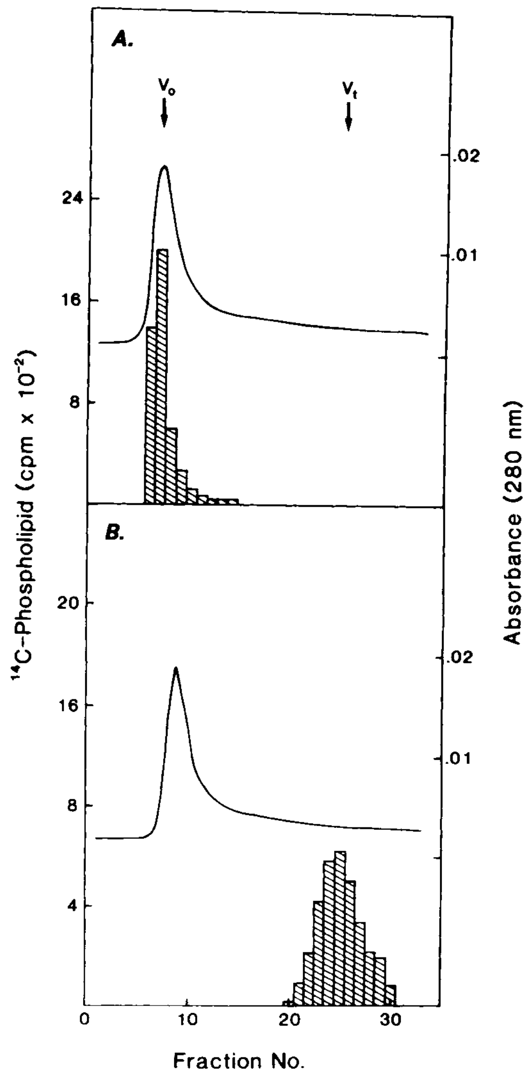

Fig. 6. Sepharose CL-4B gel filtration of reconstituted vesicles. Fractions 7 and 8 from sucrose density gradient centrifugation (Fig. 5) were pooled, dialyzed, and applied to a 0.9 × 15-cm column of Sepharose CL-4B. The column was eluted in 0.02 M Tris, pH 8.0/0.15 M NaCl either **A)** without or **B)** with the addition of 0.5% deoxycholate. Fractions (0.6 ml) were monitored for protein by absorbance at 280 nm (line) and for ^{3}H-phosphatidylcholine radioactivity (hatched bars).

cultures. Thus, it was not clear previously which cell type was reponsible for the stimulation and which produced the elevated levels of hyaluronate. The present study indicates in the case of LX-1/fibroblast cocultures that the HSF(s) is a protein, that its source is the tumor cells, and that the elevated hyaluronate production occurs in the fibroblasts. The latter is accompanied by a corresponding increase in hyaluronate synthetase activity in membranes isolated from the fibroblasts after stimulation by the tumor cell HSF.

Other investigators [12–15] have also observed stimulation of fibroblast hyaluronate and/or proteoglycan production in cocultures of a variety of human carcinoma

and melanoma cells with human fibroblasts. In some cases, factors present in tumor cell conditioned media elicited an effect similar to that observed in the cocultures [12,13]. In other cases, such as our results with lung carcinoma cells [11], cell contact was required. The nature of this dichotomy, where in some cases cell contact is necessary for stimulation and in others the stimulatory factors are secreted or shed by the tumor cells, is unknown. A similar dichotomy has also been noted for a collagenase stimulatory factor derived from murine B-16 melanoma cells [28]. In this system the presence of the collagenase stimulatory factor in conditioned medium depended on interaction of the tumor cells with fibroblasts in coculture or with matrix materials deposited by the fibroblasts. We have attempted similar experiments with LX-1 cells, but have not been able to detect HSF activity in the conditioned medium.

Our finding that hyaluronate stimulation in cocultures of LX-1 cells and fibroblasts requires cell contact has led us to investigate the association of the HSF with the tumor cell surface. The results of the present study indicate that the HSF is in fact bound to membranes isolated from LX-1 cells and that at least a portion of the HSF activity interacts with the membranes of artificial lipid vesicles. The physical nature of the interaction between HSF and the membranes is not yet clear, but our finding that the HSF can be extracted from intact cells with 2.5% butanol suggests strongly that the HSF is associated with the outer leaflet of the plasma membrane rather than spanning the membrane [17]. On the basis of the above points, we propose that the HSF(s) is a protein which is associated with the tumor cell plasma membrane, projecting externally from the cell surface to elicit stimulation of hyaluronate synthesis in adjacent fibroblasts.

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