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Purification and Characterization of a M_r ~66,000 Lung-Derived (Paracrine) Growth Factor That Preferentially Stimulates the In Vitro Proliferation of Lung-Metastasizing Tumor Cells

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In medium containing low concentrations of serum, rat 13762NF mammary adenocarcinoma cell lines and clones (MTPa and MTC; isolated from the locally growing tumor) of low metastatic potential to lung did not exhibit a growth response to lung-conditioned medium, whereas a highly metastatic cell clone isolated from a spontaneous lung metastasis (MTLn3) did. The major growth-promoting factor for MTLn3 cells from porcine and rat lung-conditioned media was isolated by using a five-step procedure (anion exchange chromatography, Affi-gel blue affinity chromatography, chromatofocusing, size exclusion chromatography, and preparative native gel electrophoresis). The lung-derived factor that stimulated the growth of highly metastatic MTLn3 cells was a glycoprotein of M_r ≈66,000 (non-reduced) or M_r ~72,000 (reduced) and possessed a pI of 6.9–7.0. It preferentially promoted the growth of lung-metastasizing tumor lines over their poorly lung-metastasizing counterparts in three tumor systems: rat 13762NF mammary adenocarcinoma, murine B16 melanoma, and murine RAW117 large-cell lymphoma. The factor's growth-stimulatory affect was inactivated by reduction or exposure to high temperature (95°C). Although the growth factor appears to be glycosylated, its molecular weight was not altered by treatment with the protein-deglycosylating agent, trifluoromethane sulfonic acid. Cleavage of the protein by cyanogen bromide resulted in the formation of five fragments. Malignant cell response to this lung-derived paracrine growth factor may be important in the successful formation of lung metastases.

Key words: tumor metastasis, glycoprotein purification, malignant cells

The ability of particular tumor cells to preferentially metastasize to certain organ sites cannot be explained by the mechanical configurations of the circulatory system or the architecture of individual organs [1,2]. Such considerations lead to the proposal that the high incidence of metastasis to certain organs was due to the unique properties of particular malignant cells and differences in host organ environments [3]. Multiple

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cellular properties are now thought to be involved in the ability of tumor cells to metastasize to distant sites, such as the ability to adhere to tissue-specific endothelial cells, invade extracellular matrix, interact with platelets or components of the coagulation system, respond to tissue-specific chemotactic and growth signals, and escape host surveillance mechanisms. These and other tumor cell properties collectively probably enable malignant cells to successfully form metastases in certain organs [4–7].

One property of malignant cells that probably aids their colonization of particular organ sites is their ability to respond to growth factor(s) produced by or present in the target organ. In performing experiments to study this, we found that only the most metastatic sublines of murine B16 melanoma [8], murine RAW117 large-cell lymphoma [9], and rat 13762NF mammary carcinoma [10] exhibited high growth rates in medium containing low concentrations of serum and conditioned by the target organ tissue for metastatic colonization. Others have also found that lung-conditioned medium [11,12] or lung extracts [13] preferentially enhanced the growth of lung-metastasizing cell lines. Enhancement of the growth of metastatic cells was also observed when such cells were in contact with cells from the target organ [14]. Here we report on the purification and some of the properties of a growth factor from porcine and rat lung-conditioned medium that differentially stimulates the growth of lung-metastasizing tumor cells.

MATERIALS AND METHODS

Cells

Rat 13762NF mammary adenocarcinoma cell lines of low spontaneous metastatic potential were derived from the mammary fat pad tumor (line MTPa, clone MTC) and of high metastatic potential from a spontaneous lung metastasis (clone MTLn3) [15]. Murine B16 melanoma sublines of low (B16-F1) and high (B16-F10) lung colonization potentials were selected as described by Fidler [16]. Murine RAW117 large-cell lymphoma sublines of low (RAW117-P) and high (RAW117-H10) liver colonization potential and high (RAW117-L17) lung colonization potential were derived as described elsewhere [17]. Cells were grown in the appropriate medium, alpha-modified minimal essential medium (αMEM, GIBCO, Grand Island, NY) for 13762NF lines, a 1:1 (v/v) mixture of Dulbecco-modified Eagle's medium and Ham's F12 medium (DMEM:F12, GIBCO) for B16 lines, or 25 mM HEPES-buffered (pH 7.5) high glucose DMEM for RAW117 lines, supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO_2. All cell lines were negative for *Mycoplasmas* and other infections.

LCM Preparation

Lungs were obtained from freshly killed animals. When rats were used, the animals were anesthetized with Metofane (Pittman-Moore, Washington Crossing, NJ) and perfused with 25 mM HEPES, 0.15 M NaCl, 0.38% (w/v) sodium citrate, pH 7.5. Lungs were minced into 2–3 mm^3 pieces, filtered through a 4-mm mesh screen, and collected on a 150-μm mesh nylon screen. The tissue pieces were then washed with 25 mM HEPES, 0.15 M NaCl (pH 7.5), and placed in a 1:1 mixture of DME:F12 medium containing 100 mg/l penicillin, 100 mg/l streptomycin, and 0.25 mg/l amphotericin B at a medium:tissue ratio of 4:1 (v/v). These suspensions were placed in roller bottles and

rotated at 37°C for 24–48 hr in a 5% CO_2 atmosphere [10]. Medium was collected, centrifuged at 5000*g* for 30 min, filtered through a 1-μm filter, and made 10 mM in potassium phosphate (pH 7.0), 0.05% (w/v) in sodium azide, 100 μM in phenylmethylsulfonyl fluoride (PMSF; Sigma, St. Louis, MO), 10 μM in α-p-tosyl-lysine chloromethylketone (TLCK; Sigma), and 2 mM in EDTA.

Growth Assays

Fractions to be tested for growth-stimulating activity were made 1 mg/l in bovine serum albumin, dialyzed against 25 mM HEPES, pH 7.5, for 18–24 hr, and then made equimolar in medium components by the addition of 1/9 volume of 10 × medium, and were filter sterilized. Assays for activity were performed by plating MTLn3 cells into 96-well microtiter plates at a density of 2,000 cells per well in αMEM containing 1% (v/v) FBS. After a 24-hr incubation, all wells were washed with 200 μl αMEM and drained, and 100 μl αMEM containing 0.3% (v/v) FBS was added. Conditioned medium or purified medium fractions (25 μl) were placed into test wells, and the corresponding negative control wells received 25 μl of growth factor solvent that was dialyzed and treated in parallel with the test fractions. After 4 days incubation, the assays were terminated and cell numbers were quantitated by using a colorimetric crystal violet staining procedure [18]. Medium was removed and all wells were washed with 0.5–1.0 ml phosphate-buffered saline (PBS). The cells were then fixed with 50 μl per well 5% (v/v) glutaraldehyde (Fisher, Houston, TX) for 30 min. The fixative was removed, and the cells were stained with 50 μl 0.1% (w/v) crystal violet (Sigma) for 30 min. The wells were then washed with 1–2 ml super-Q-purified water and dried, and the fixed, stained cells were solubilized with 50 μl 1% (w/v) sodium dodecyl sulfate (SDS). To determine cell number the absorbance of each well was read at 590 nm on a Titertek plate reader.

Purification of Growth Activity

Conditioned medium was concentrated to 200–300 ml by using a pressurized Amicon device equipped with a YM-10 filter. The concentrate was dialyzed against 25 mM ethanolamine, pH 9.5, and applied to a 2.5 × 25 cm QAE-sepharose column equilibrated with the same buffer. Bound components were eluted with a linear 350 ml 0.0–0.3 M NaCl gradient followed by a 150 ml gradient of 0.3–0.6 M NaCl. Five-milliliter fractions were collected at a flow rate of 0.5 ml/min. The active material from the anion exchange step was dialyzed against 25 mM NaH_2PO_4 at pH 7.0, and applied to a 2.5 × 20 cm Affi-gel Blue column equilibrated with the same buffer. Bound components were eluted with a 200 ml 0.0–0.5 M NaCl gradient. Five-milliliter fractions were collected at a flow rate of 0.5 ml/min. Active fractions were dialyzed against 25 mM Tris at pH 8.5 and were applied to a 1 × 10 cm Polybuffer Exchanger 94 column equilibrated with the same buffer. Bound proteins were eluted with 150 ml Polybuffer 9-6 adjusted to pH 5 with HCl. Fractions (3 ml) were collected at a flow rate of 0.5 ml per min. The active fractions were pooled, concentrated using an Amicon YM-10 membrane to 1.5 ml total volume, and applied to a 2.5 × 50 cm Bio-Gel P-200 column equilibrated and run with 25 mM Tris buffer, pH 8.5, containing 0.2 M NaCl. Fractions (2 ml) were collected at a flow rate of 12 ml per hr.

The final purification step was separation by preparative native gel electrophoresis. The system used was based on the procedures of Chrambach and Jovin [19]. The acrylamide solution contained 0.37 M Tris, 0.17 M HCl, 9.75% (w/v) acrylamide,

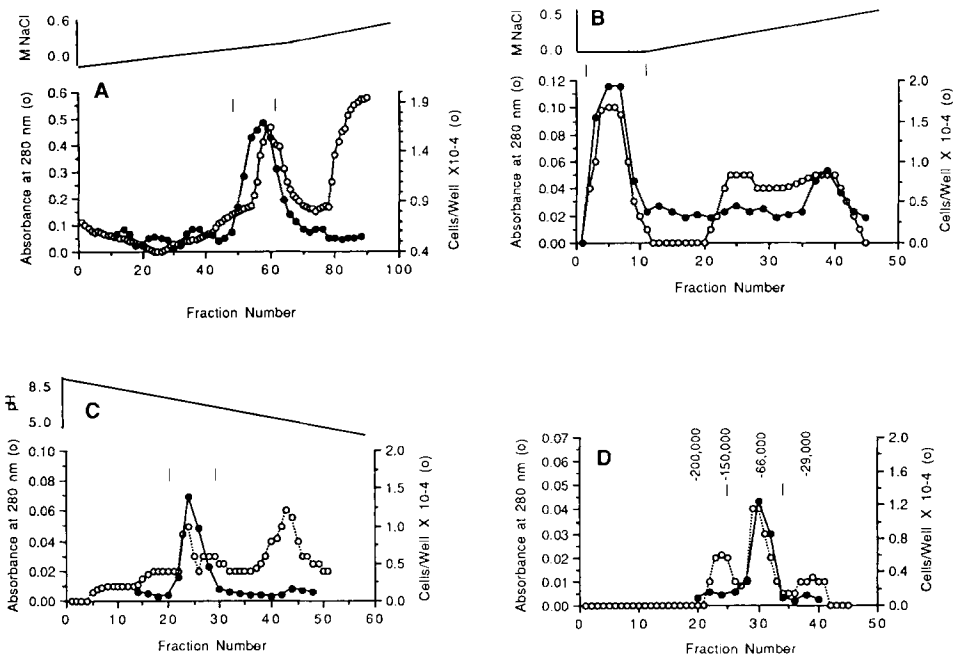

Fig. 1. Chomatograms from the first four purification steps of lung-derived growth factor from porcine LCM. In all cases, crystal violet assay A_{590} values were converted into cell numbers (see Fig. 4 for standard curve), to render the graphs more readily interpretable. All procedures were performed as described in Materials and Methods. **A:** Anion exchange chromatography of crude porcine LCM on QAE-sepharose. **B:** Affi-gel blue affinity chromatography of active material from the anion exchange step. **C:** Chromatofocusing of the active pool of material from the affi-gel blue step. **D:** Gel filtration of the active pool of material from the chromatofocusing step. Numbers at the top of D indicate the running positions of molecular weight standards. Molecular weight standards were: β-amylase ($M_r \approx 200{,}000$), alcohol dehydrogenase ($M_r \approx 150{,}000$), bovine serum albumin ($M_r \approx 66{,}000$), and carbonic anhydrase ($M_r \approx 29{,}000$).

0.25% (w/v) *bis* acrylamide, 0.1% (w/v) CHAPS (3-[(3-cholidamido-propyl)dimethyl-ammonio]-1-propane sulfonate; Sigma). The anolyte composition was 20 mM Tris, 0.01 N HCl; the catholyte was 0.02 M glycine, 0.01 N KOH. Gels were polymerized in a 16 × 16 × 0.15 cm (LKB, Gaithersburg, MD) model 2001 apparatus by using a TEMED/ammonium persulfate catalyst, and were pre-electrophoresed at 50 mA constant current prior to the application of a test sample. The active material from the size exclusion chromatography step was concentrated to 2 ml and dialyzed against 0.37 M Tris, 0.17 M HCl, containing 10% (v/v) glycerol, 2% (w/v) CHAPS, and 0.01% (w/v) bromophenol blue. This sample was applied to the polyacrylamide gel made as described above and electrophoresed at 50 mA constant current until the marker dye was 1 cm from the bottom of the glass plates. A 0.5-cm-wide strip was removed from one edge of the gel, stained with Coomassie blue, destained, and aligned with the remainder of the gel. Based on the appearance of the stained segment, the intact gel was sliced horizontally into 10 portions. Each slice was placed into 25 ml of 25 mM Tris, pH 8.5, containing 0.02% sodium azide, homogenized, and stirred for 24 hr. These suspensions were centrifuged at 2,000*g* for 15 min, and resulting supernatants were assayed for activity. The active fraction was stored in the Tris buffer containing 10% w/v sorbitol at

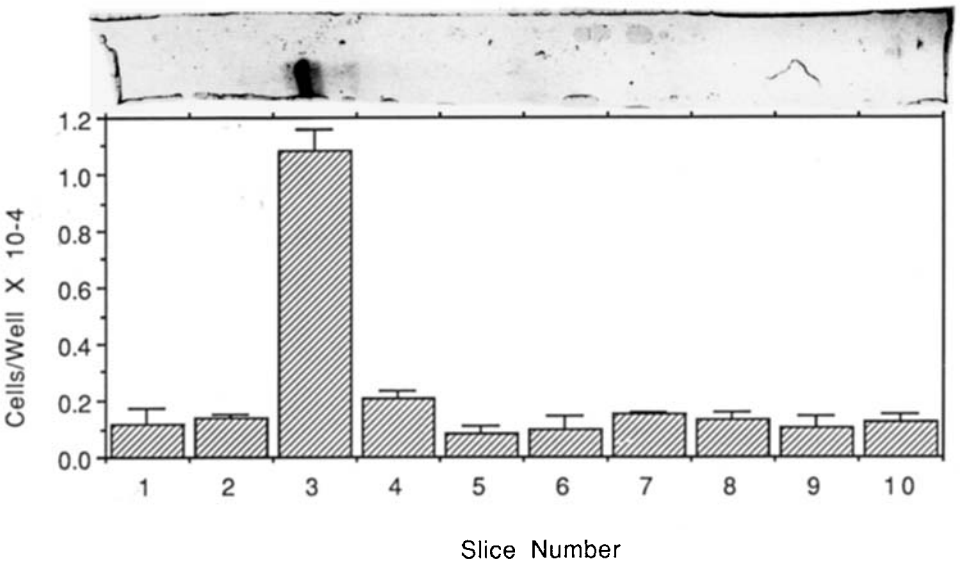

Fig. 2. Native gel electrophoresis of porcine lung-derived growth factor. The active pool from the gel filtration step was equilibrated in native electrophoresis gel buffer and separated by preparative native electrophoresis (see Materials and Methods). The appearance of the Coomassie blue–stained gel strip is shown at the top. Bars (mean ± SD from four replicates) represent the results from assaying gel strip homogenate supernatants on MTLn3 cells.

−70°C. For biochemical characterization studies, the material was dialyzed against water, lyophilized, and treated. For addition to cell culture, the factor was dialyzed and filter sterilized as described in the growth assays section. Protein determinations were performed by using the Pierce Chemical (Rockford, IL) Coomassie blue dye binding assay.

Dithiothreitol and Temperature Stability

Porcine lung-derived growth factor was incubated at 25°C for 1 hr with 100 mM dithiothreitol (DTT). For temperature stability studies, porcine factor was incubated at 56°C or 95°C for 1 hr. All treated preparations were then assayed as usual on MTLn3 cells. Five growth-stimulating-activity (gsa) units of each treated sample of lung-derived growth factor were added into each well.

Trifluoromethane Sulfonic Acid (TFMS) Exposure

The procedure used was based on that of Sojar and Bahl [20]. To 10 μg porcine lung-derived growth factor was added 20 μl TFMS. This mixture was incubated at 25°C for 1 hr and placed in a dry ice–ethanol bath, and 100 μl cold 60% pyridine was slowly added. Additional H_2O was added to clear the precipitate; the reaction contents were dialyzed against water, lypholized, resuspended in SDS treatment solution, and electrophoresed according to Laemmli [21].

Cyanogen Bromide Cleavage

The procedure used was based on that of Nikodem and Fresco [22]. To 50 μg porcine lung-derived growth factor (in 1 ml 0.1N HCl) was added 20 μl 2 g/ml CnBr (in

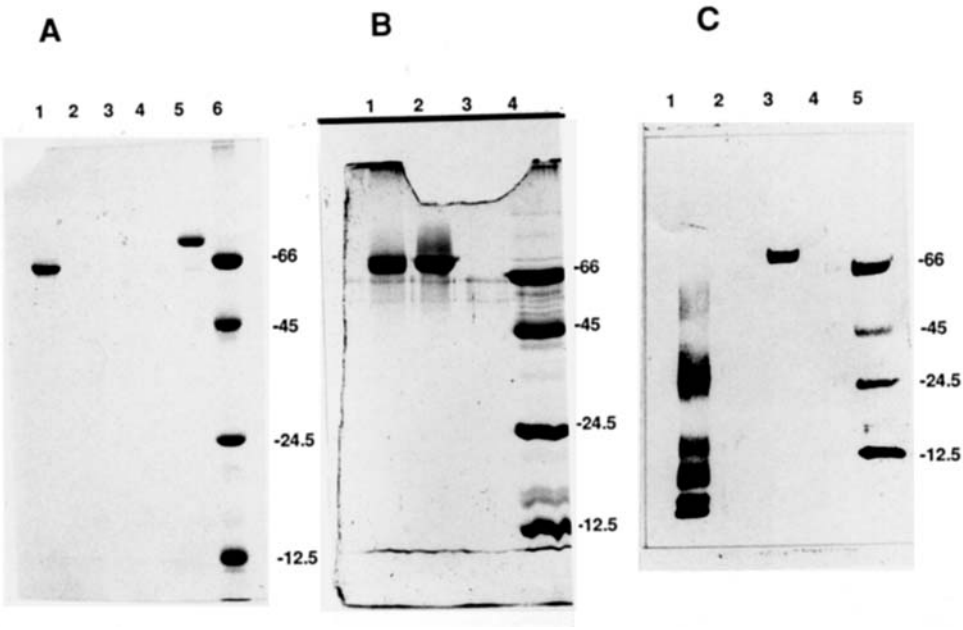

Fig. 3. Various electrophoretic analyses of porcine lung-derived growth factor. **A:** SDS-PAGE of purified lung-derived growth factor. The material was analyzed by silver-stained SDS-PAGE performed according to Laemmli [21] on a 12% (w/v) acrylamide gel. **Lane 1:** 1 μg unreduced lung-derived growth factor. **Lane 5:** 1 μg reduced lung-derived growth factor. **Lane 6:** M_r standards. Increased or decreased amounts of lung-derived growth factor did not reveal multiple bands. **Lanes 2–4** contained no samples. Molecular weight standards ($\times 10^{-3}$) are indicated to the right of each panel and were: bovine serum albumin ($M_r \approx 66{,}000$), ovalbumin ($M_r \approx 45{,}000$), chymotrypsinogen ($M_r \approx 24{,}500$), and ribonuclease ($M_r \approx 12{,}500$). **B:** Electrophoretic migration pattern of intact and TFMS-treated porcine lung-derived growth factor. The protein was treated as described in Materials and Methods and electrophoresed on a 12% acrylamide gel run at 20 mA constant current under reducing conditions according to Laemmli [21]. Five micrograms intact (**lane 2**) and 2 μg treated material (**lane 1**) were applied to the gel. Proteins were visualized by silver staining. Standards (**lane 4**) were identical to those in A. **C:** Electrophoretic migration pattern of intact and CnBr-treated porcine lung-derived growth factor. Five micrograms intact (**lane 3**) and 50 μg cleaved lung-derived growth factor (**lane 1**) were loaded onto a 5–15% acrylamide gradient gel formulated according to system 11 (gel buffer pH = 7.55) of Chrambach and Jovin [19]. The gel was run at 40 mA constant current. Both samples were treated with 1% (w/v) SDS in 1 × gel buffer at 95°C for 5 min and were reduced with mercaptoethanol. Separated proteins were blotted onto PVDF according to Moos et al. [23] and were stained with Coomassie blue. Standards were identical to those in A.

acetonitrile). This mixture was incubated at 56°C for 4 hr, lypholized, resuspended in SDS-PAGE treatment solution (1 × gel buffer containing 1% [w/v] SDS and 2% [v/v] mercaptoethanol), heated at 95°C for 5 min, and electrophoresed by using system 11 (gel buffer pH = 7.55) proposed by Chrambach and Jovin [19]. Separated samples were blotted onto a PVDF (polyvinylidene difluoride; Millpore, Bedford, MA) membrane [23] and visualized with Coomassie blue. Blotted samples were applied directly to an Applied Biosystems (Foster City, CA) model 477A protein sequencer equipped with an Applied Biosystems model 120A analyzer. Derivitization, Edman degradation, reverse-phase HPLC separation, and detection of derivitized amino acids from the N-terminus of blotted polypeptides were performed according to manufacturer's procedures.

RESULTS

All of the growth-stimulating activity (gsa) for MTLn3 cells from porcine lung-conditioned medium adhered to QAE-sepharose at pH 9.5. The gsa activity eluted at approximately 0.15–0.2 M NaCl (Fig. 1A) whereas most protein eluted at higher salt concentrations. Activity pooled from the anion exchange step did not bind to Affi-gel blue but most protein did (Fig. 1B). Further fractionation was accomplished by chromatofocusing (growth activity eluted at a pH of 6.8–7.0; Fig. 1C) and by gel filtration (gsa migrated as a species of M_r ~66,000; Fig. 1D). Final purification was performed by native gel electrophoresis. In this step, gsa migrated as the major band of protein, with an R_f of 0.2 (Fig. 2). Sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) analysis of the lung-derived growth factor recovered from the native gel procedure demonstrated a single band of M_r ~66,000, (Fig. 3A) which correlated well with its elution volume from the Bio-Gel P-200 column (Fig. 1D). Disulfide-linked subunits were not detected but interchain disulfide bonds appeared to be present because the M_r increased slightly after reduction with mercaptoethanol. Based on the chromatofocusing results, the approximate pI of the factor was 6.9–7.0 [24]. Material from rat and porcine sources behaved identically through all chromatographic steps, and the final purified rat and porcine factors exhibited the same M_r and other properties.

The crystal violet assay used to estimate cell number was linear with up to 3.5×10^4 cells per well (Fig. 4). A linear increase in absorbance at 590 nm (and in cell number) was seen after 4 days when increasing amounts of crude rat LCM (Fig. 5A) or pure rat lung-derived growth factor (Fig. 5B) were added into MTLn3 cultures. To determine the effectiveness of the purification scheme, aliquots of the pooled fractions from the chromatographic steps were assayed simultaneously with MTLn3 indicator cells. The specific activity obtained after each purification step was determined based on the ability to increase cell numbers compared to the negative control. One unit of activity was defined as that amount required to produce an absorbance double that of the negative

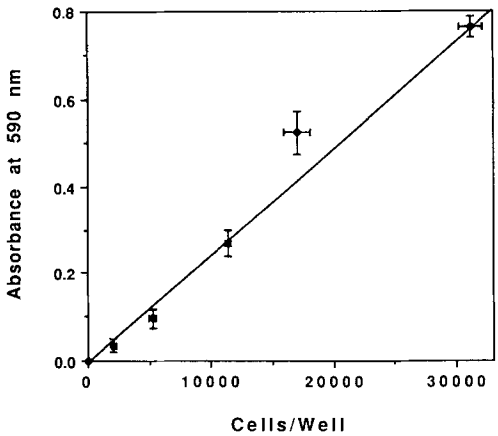

Fig. 4. Demonstration of the linearity of the crystal violet cell quantitation assay. Increasing numbers of MTLn3 cells were plated in each row of a 96-well plate. Two days later, cell numbers in four wells of each row were enumerated by Coulter Counter. The remaining four wells were quantitated by the crystal violet assay. Data points represent the mean ± SD of the four measurements.

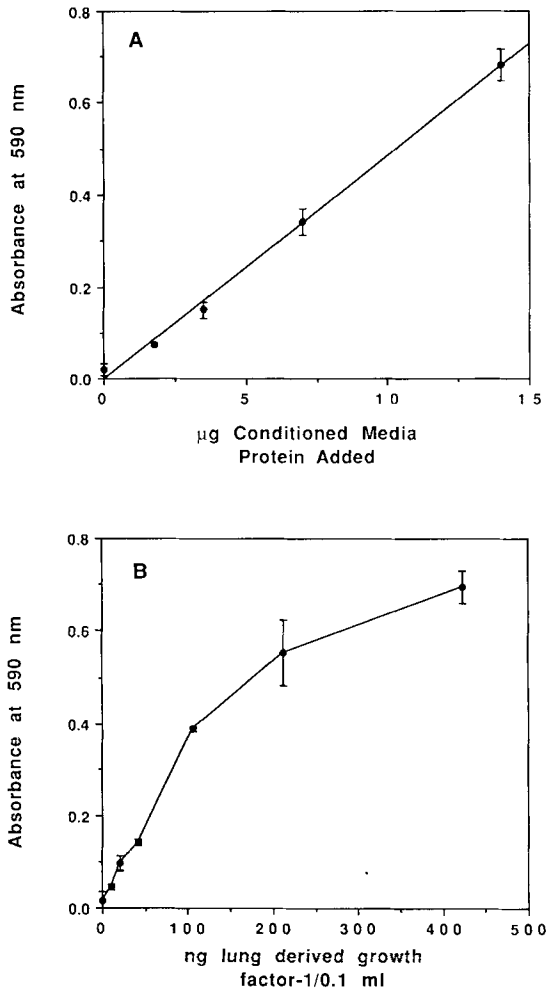

Fig. 5. Effects of LCM on MTLn3 cell growth. **A:** Increasing amounts of rat LCM were added into MTLn3 cultures, and a linear increase in cell number was seen with the amount of LCM added. Data points represent the mean ± SD of four replicates. **B:** Results obtained when increasing amounts of pure rat lung-derived growth factor were added into MTLn3 cultures.

control. Results from the purification of porcine lung-derived growth factor are shown in Table I. A 184.3-fold increase in specific activity was achieved.

The activity of the lung-derived growth factor was completely abolished after exposure to 95°C or 100 mM DTT. No loss in activity was observed in the 56°C-treated sample (Fig. 6).

Approximately 40% of the growth activity from the LCM preparations bound to Concanavalin A–agarose. The Concanavalin A–bound and –unbound components appeared identical when further fractionated and analyzed by SDS-PAGE. Such disparate results in lectin-binding properties probably reflect differences in glycosylation. Because of this, lectin-affinity chromatography was eliminated from the purification scheme. This procedure demonstrated, however, that some growth factor molecules were

TABLE I. Results From the Purification of Porcine Lung-Derived Growth Factor*

Fraction	Total protein (μg)	Units/μg protein	Total units	% recovery	Fold purification
LCM	158,315	1.02	161,302		
QAE sepharose	31,663	2.44	77,257	47.9	2.4
Affi-gel blue	5,497	14.5	79,707	49.4	14.2
Chromato-focusing	475	166.6	79,135	49.0	163.3
Bio-gel P-200	330	109.0	35,970	22.2	106.9
Preparative PAGE	102	188.0	19,176	11.9	184.3

*Aliquots of various chromatography samples were assayed together on the same set of MTLn3 cells.

glycosylated. The carbohydrate content of the growth factor is apparently low, since after exposure to TFMS, porcine lung-derived growth factor migrated in a Laemmli reducing SDS-PAGE gel similar to untreated protein (Fig. 3B).

Using an Applied Biosystems gas phase sequenator we failed repeatedly to detect a derivitized amino acid signal from intact porcine factor, indicating that the factor possessed a blocked amino terminus. Cleavage of porcine lung-derived growth factor at methionine residues with cyanogen bromide resulted in the formation of polypeptides of $M_r \approx 27{,}000$, $\approx 17{,}000$, $\approx 14{,}000$, $\approx 12{,}000$, and $\approx 10{,}500$ (Fig. 3C). The factor was extremely labile to CnBr, because intact protein could not be visualized by SDS-PAGE analysis after CnBr treatment. Cleavage in the presence of 0.1 N HCl alone was negligible (data not shown). Only the $M_r \approx 17{,}000$ cleavage product produced significant signals when subjected to amino acid sequencing. Sequence results from this polypeptide were: Gly-Leu-Leu-Tyr-Asn-Lys-Ile-Asn.

The spectrum of activity of the porcine lung-derived growth factor was partially established by testing its effect on various cell lines. The procedures used were identical to those described for MTLn3 cells; 5 gsa units of porcine growth factor were added to cultures of human MCF-7 breast adenocarcinoma cells; rat MTPa and MTC (poorly lung metastatic) and MTLn3 (highly lung metastatic) mammary adenocarcinoma cells; murine RAW117-P (poorly metastatic), RAW117-H10 (highly liver-colonizing), and RAW117-L17 (highly liver- and lung-colonizing) large cell lymphoma cells; murine B16-F1 (poorly lung-colonizing) and B16-F10 (highly lung-colonizing) melanoma cells; and normal rat kidney (NRK) fibroblasts. In the metastatic systems studied (13762NF, RAW117, and B16), lung-derived growth factor preferentially enhanced the growth of highly lung-metastasizing lines (MTLn3; RAW117 L-17, B16-F10) compared to their poorly lung-metastasizing counterparts (MTC or MTPa; RAW117-P or -H10; B16-F1) (Fig. 7). There was a growth-promoting effect on MCF-7 cells, a human pleural effusion-derived breast carcinoma cell line known to produce metastases in nude mice under the proper hormonal conditions [25,26]. There were negligible growth effects of lung-derived growth factor on NRK cells (Fig. 7) and rat lung-derived endothelial cells (data not shown).

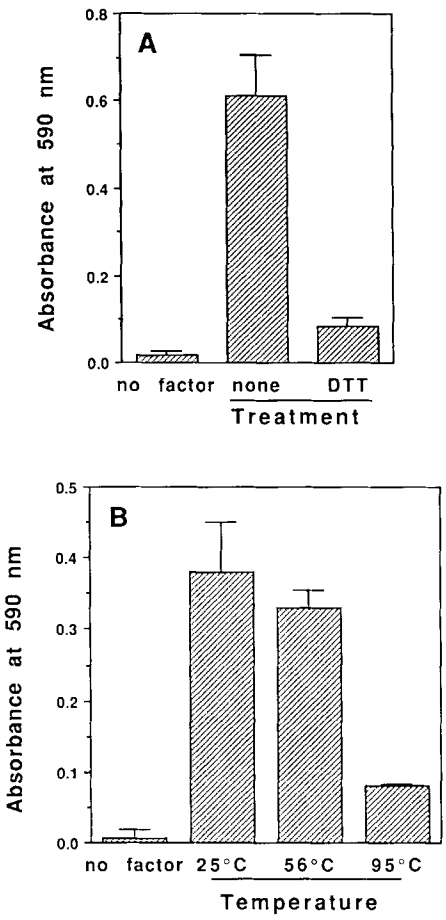

Fig. 6. Effects of DTT and temperature on porcine lung-derived growth factor. **A:** Results obtained when porcine lung-derived growth factor was exposed to DTT. **B:** Results obtained when porcine lung-derived growth factor was exposed to various temperatures. The factor was treated as described in Material and Methods; 5 gsa units of treated or untreated material were added into MTLn3 cultures. Relative cell numbers were quantitated 4 days later by using the crystal violet procedure. Bars represent the mean ± SD from four replicates.

We found similarity in some properties of lung-derived growth factor to certain bone marrow cell colony-stimulating factors [27]. We found, however, that MTLn3 cells do not exhibit a growth response to 100 U/ml murine GM-CSF. Also, porcine lung-derived growth factor did not stimulate the growth of human bone marrow cells (P.G.C. and G.L.N., unpublished data).

A number of growth factors are characterized by their ability to adhere to immobilized heparin [28]. We found that lung-derived growth factor bound to heparin-agarose only weakly at a pH of 8.5 (complete elution at <0.02 M NaCl), and there was no binding observed at pH 7.0. The results indicate a weak anion-exchange interaction between the growth factor and heparin, and the strong heparin interaction characteristic of certain other growth factors was not observed.

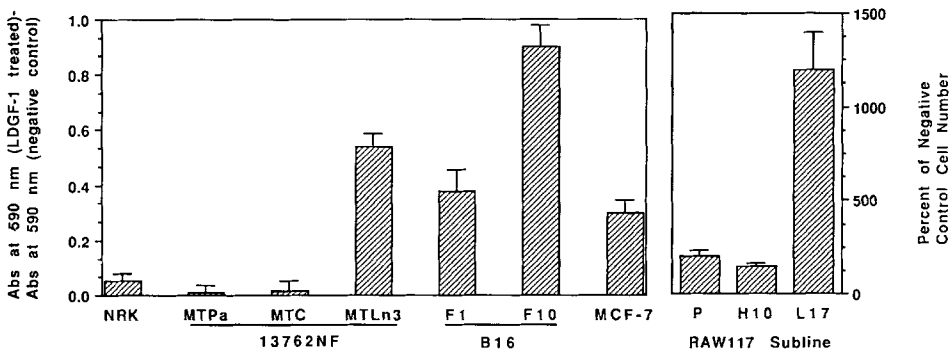

Fig. 7. Growth-stimulating properties of lung-derived growth factor on various cell lines. Cells were plated at a density of 2,000 cells per well in 96-well plates by using 100 μl medium containing 1% (v/v) FBS. One day later the medium was replaced with 100 μl medium containing 0.3% (v/v) FBS, and 25 μl of porcine lung-derived growth factor (≈5 gsa units) or 25 μl negative control solution was added. Four days later, the relative cell numbers in all wells were quantitated by using the crystal violet assay or by Coulter counter. Bars represent the mean ± SD of four replicates.

DISCUSSION

The response of tumor cells to specific growth factors is considered important to their in vivo properties [29]. In addition to the growth factors provided by blood and its cells, apparently new tissue- or organ-derived growth factors or growth inhibitors for tumor cells have also been found. Liver [30], kidney [31], uterus [32], and pituitary [32] have been found to contain such factors, and these could be important in determining the survival and growth of malignant cells at these sites.

We have isolated a growth factor from lung tissue and have found that the mitogenic action of this glycoprotein is thus far restricted to tumor cells that metastasize to the lung [33]. This lends credence to the hypothesis that one property that metastatic tumor cells possess is the ability to respond to local paracrine growth factors that are present in the target organ for metastasis.

Other lung-derived mitogenic activities for tumor cells have been only partially purified. Growth-stimulating factors of M_r ~50,000–70,000 and growth-inhibiting factors of M_r ~12,000–20,000 and ~3,000–5,000 have been partially isolated from lung tissue by Szaniawska et al. [34]. Yamori et al. [13] reported that the murine colon carcinoma growth-promoting activity from lung extracts possessed an M_r ~95,000, as determined by gel filtration. These growth factors are apparently different from the lung-derived growth factor we have purified. The relatively high molecular weight of lung-derived factor isolated here sets it apart from most known growth factors [29]. Although high-M_r forms of several known growth factors have been reported [35–37], none of these is comparable to the lung-derived factor reported here.

In many animal tumor systems, the highly malignant cells metastasize preferentially to the lung, so we chose this organ to search for a metastatic cell growth factor. We have not yet determined if the lung-derived growth factor we purified is found in other organs, but a preliminary investigation into the mitogenic activity for MTLn3 cells from liver-conditioned medium indicated that detectable concentrations of the M_r ~66,000 lung-derived growth factor cannot be found in our assays. If the lung-associated factor we have isolated exists at disparate concentrations in various organs, then the ability of

certain malignant cells to respond to the factor might be an important metastatic characteristic of such cells. The ability to respond to an organ-derived growth factor, however, would only be one of several important properties required for metastasis formation in that organ. For example, lung-metastasizing cells may also require adhesion to lung microvessel endothelial cells [6,7,17,38] and subendothelial matrix [6,7,38].

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