

Identification of a Tumor Cell Receptor for VGVAPG, an Elastin-derived Chemotactic Peptide

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Abstract. Extracellular matrix proteins and their proteolytic products have been shown to modulate cell motility. We have found that certain tumor cells display a chemotactic response to degradation products of the matrix protein elastin, and to an elastin-derived peptide, VGVAPG. The hexapeptide VGVAPG is a particularly potent chemotaxin for lung-colonizing Lewis lung carcinoma cells (line M27), with 5 nM VGVAPG eliciting maximal chemotactic response when assayed in 48-microwell chemotaxis chambers. Binding of the elastin-derived peptide to M27 cells was studied using a tyrosinated analog (Y-VGVAPG) to allow iodination. Scatchard analysis of [¹²⁵I]Y-VGVAPG binding to viable

M27 tumor cells at both 37 and 4°C indicates the presence of a single class of high affinity binding sites. The dissociation constant obtained from these studies (2.7×10^{-9} M) is equivalent to the concentration of VGVAPG required for chemotactic activity. The receptor molecule was identified as an M_r 59,000 species by covalent cross-linking of the radiolabeled ligand to the M27 tumor cell surface and subsequent analysis of the cross-linked material by electrophoresis and size-exclusion high performance liquid chromatography. These results suggest that M27 tumor cell chemotaxis to VGVAPG is initiated by high affinity binding of the peptide to a distinct cell surface receptor.

ELASTIN, an insoluble component of the extracellular matrix is best known for its ability to confer elasticity to extensible tissues such as the lung, arteries, skin, and elastic cartilage (6, 20). Elastin and elastin sequences interact with a variety of cell types to modulate cellular behavior. Insoluble elastin, for example, has been shown to mediate cell adhesion of monocytes, fibroblasts, and tumor cells (10, 17, 26). Elastin digests and elastin-derived peptides have also been shown to be chemoattractants for monocytes and fibroblasts (24, 25). The recent observation that elastin degradation products act on calcium ion channels in monocytes, fibroblasts, and smooth muscle cells provides additional evidence for phenotypic modulation by elastin (11).

VGVAPG is a hexapeptide that is repeated multiple times in the elastin molecule (22). This elastin sequence is active as a chemoattractant for monocytes and for elastin-producing fibroblasts (25). Although VGVAPG represents a potentially important class of matrix-derived chemotactic molecules, little is known concerning its mechanism of action. Because tumor cells can adhere to elastin (17) and degrade elastin to small peptides (13, 14), we have inquired whether VGVAPG can stimulate tumor cell migration. Our results demonstrate that VGVAPG is chemotactic for certain tumor cell lines such as the M27 line of the Lewis lung carcinoma. Using a radiolabeled tyrosinated analog (Y-VGVAPG), we then studied the binding of this ligand to molecules on the M27 cell surface.

Scatchard analysis of binding and covalent cross-linking experiments indicate that the primary binding site for the elastin-derived chemotactic peptide is an 59,000- M_r species on the tumor cell surface.

Materials and Methods

Cell Culture

The M27 clone of murine Lewis lung carcinoma was selected *in vivo* from a metastatic tumor cell colony found in a mouse lung after subcutaneous inoculation of the parental 3LL Lewis lung carcinoma tumor (3). The cells were cultured as previously described (3). To ensure maintenance of the invasive metastatic phenotype, cells were periodically reisolated from lung metastases formed subsequent to subcutaneous injection of 5×10^5 cells in C57BL/6 mice. All studies were performed on cells that had been subcultured <10 times *in vitro*.

Chemoattractants

Synthetic peptides VGVAPG and Y-VGVAPG were prepared by solid phase methods (16, 21) using an automated peptide synthesizer (model 430A; Applied Biosystems Inc., Foster City, CA). Purity of the synthetic peptides was analyzed by reversed phase chromatography on a C8 column (Rainin Instruments, Woburn, MA) and was found to exceed 95% in each case.

Migration Assays

Cell migration was evaluated by a modification of the method of Boyden (2), using a 48-multiwell chemotaxis chamber (Neuro Probe, Inc., Cabin John, MD). Dilutions of the peptide to be tested were placed in lower compartments of the chamber and covered with an 8- μ m porosity, 25 \times 80 mm polycarbonate membrane filter (Nucleopore Corp., Pleasanton, CA), which

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had previously been incubated in 15 ml of PBS containing 100 µg of human fibronectin (Cooper Biomedical, Inc., Malvern, PA) to increase cellular adhesion to the filter. Fibronectin was determined in control experiments to be superior to laminin or type IV collagen as a substratum for adhesion and migration of the M27 tumor cells. Cells to be assayed were detached from culture vessels using a solution of 0.05% trypsin–0.53 mM EDTA and the trypsin was subsequently inactivated by addition of medium containing 5% FBS. The cells were washed and suspended in serum-free medium for the migration assay. Aliquots of 50 µl, containing ~5,000 cells were placed in the upper wells of the chamber, and the entire apparatus was incubated at 37°C for 3 h. After incubation, cells adhering to the upper surface of the filter were removed by scraping with a rubber spatula; cells that had migrated to the lower surface were fixed in neutral-buffered 10% formalin followed by overnight staining with 1% nuclear fast red. The filter was then rinsed and mounted between two glass slides with 90% glycerol in PBS. The total number of cells that had migrated across the filter in each micro-well was counted at 100× under bright-field illumination. Chemotactic response was defined as the number of cells which traversed the filter in response to the peptide minus the number of cells migrating in the absence of peptide.

Preparation of Radiolabeled Peptide

To allow iodination of the elastin-derived peptide, VGVAPG, a related peptide was synthesized that contained tyrosine at the amino terminus (YGVGAPG). Iodination of YGVGAPG was performed using chloramine-T according to the method of Greenwood et al. (7). The reaction product was applied to a 5-ml column of DEAE-Sephadex A-25 ion exchange resin equilibrated with 0.01 N ammonium acetate, pH 8.5. Radiolabeled YGVGAPG was eluted with 0.6 N ammonium acetate, pH 8.5, and the pH of peak fractions was neutralized.

[¹²⁵I]YGVGAPG was further purified before use in binding assays by reversed-phase HPLC in order to ensure removal of free iodine and iodinated tyrosine. The radioiodinated peptide was diluted 1:10 in 0.1% trifluoroacetic acid (TFA)¹ and the sample was then applied to a Vydac C18 HPLC column (Rainin Instrument Co., Inc., Woburn, MA) equilibrated in 0.1% TFA. The column was washed for 10 min with equilibration buffer at a flow rate of 1 ml/min and radioactivity was then eluted with a linear gradient to 90% acetonitrile, 0.1% TFA formed over a 30-min period at 1 ml/min. Approximately 88% of the radioactivity applied to the column eluted with 36% acetonitrile, conditions which elute unlabeled YGVGAPG. This material was stored at –20°C and subsequently used for binding assays. Calculation of specific activity (110 Ci/mmol) was based on the amount of radioactivity in the recovered radiolabeled peptide and its optical density at 280 nm. Approximately 70% of the peptide added to the chloramine-T iodination reaction was recovered after HPLC purification.

Measurement of Peptide Binding

Because M27 cells are easily detached from culture dishes, binding assays were performed on cell suspensions to minimize cell loss during washing steps. Initial binding experiments determined that cells disrupted with trypsin–EDTA bound [¹²⁵I]YGVGAPG equally well as did cells disrupted with EDTA alone. M27 cells were subsequently prepared for binding assays with trypsin–EDTA solution and suspended in Hank's balanced salt solution (HBSS) buffered with Hepes pre-equilibrated to 37 or 4°C. As shown in Table I, binding at 4°C showed a relatively narrow pH optimum between pH 6.5 and 7.0. Subsequent experiments were performed at pH 6.8.

M27 tumor cells were suspended in binding buffer at a cell density of 250,000 cells/ml. The binding reaction was initiated by addition of 1.0 ml cell suspension to 10 µl [¹²⁵I]YGVGAPG in microcentrifuge tubes. To control for nonspecific binding, parallel reaction tubes were incubated in the presence of 100- to 1,000-fold excess unlabeled nontyrosinated VGVAPG. Reaction tubes were incubated with rocking at the indicated temperature. Binding was terminated by centrifugation at 4,000 rpm for 10 s in an Eppendorf microfuge and washed rapidly with fresh binding buffer at 37 or 4°C. The tips of microcentrifuge tubes containing the cell pellets were snipped and transferred to counting tubes. The termination steps were completed in 1.0–1.5 min by this method, depending on the number of tubes in each experiment. [¹²⁵I]YGVGAPG associated with the cells was determined in a gamma spectrometer (model 8000; Beckman Instruments, Inc., Fullerton, CA). Specific binding was calculated as the difference between the amount of

1. *Abbreviations used in this paper:* DSS, disuccinimidyl suberate; TFA, trifluoroacetic acid.

Table I. Effect of pH on [¹²⁵I]Y-VGVAPG Binding

pH	Specific binding*
	cpm
5.5	1,227 ± 502
6.0	2,468 ± 801
6.5	4,344 ± 552
7.0	3,267 ± 722
7.5	1,106 ± 401
8.0	227 ± 163
8.5	20 ± 28

[¹²⁵I]Y-VGVAPG (300,000 cpm/ml) was incubated at 4°C with 10⁶ cells in the presence or absence of 10⁻⁶ M unlabeled VGVAPG in Hepes-buffered HBSS at the indicated pH.

* Values are mean ± SD of triplicate determinations.

[¹²⁵I]Y-VGVAPG bound in the absence (total binding) and presence (nonsaturable binding) of unlabeled VGVAPG.

Chemical Cross-linking

Radioiodinated elastin peptide was covalently bound to M27 cells using a modification of the method of Friesel et al. (5). Binding of [¹²⁵I]Y-VGVAPG to M27 cells was carried out as described above. After washing, the cells were incubated at 4°C in 1 ml HBSS, pH 6.8, containing 0.2 mM disuccinimidyl suberate (DSS; Pierce Chemical Co., Rockford, IL). DSS solution was prepared as a 20 mM stock in DMSO immediately before cross-linking. After 20 min, the reaction was terminated by addition of 20 µl of 2.0 M Tris–HCl, pH 8.0. Cells were washed once with buffer and resuspended in 25–50 µl of extraction buffer (50 mM Tris, pH 7.3, containing 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 200 mM NaCl, 1.0% [wt/vol] Triton X-100). Cells were incubated in the extraction buffer at 4°C for 30 min. Insoluble material was removed by centrifugation at 15,000 g for 5 min at 4°C. The supernatant was collected for immediate analysis or was frozen at –70°C.

Electrophoresis

SDS–PAGE was performed as described by Laemmli (15). Samples were heated to 100°C for 2 min in SDS–sample buffer and subjected to electrophoresis on 10% acrylamide slab gels using a Protean minigel apparatus (Bio-Rad Laboratories, Richmond, CA). After electrophoresis, gels were stained with 0.05% Coomassie Blue dye and subjected to autoradiography at –70°C for 1–5 d using XAR film (Eastman Kodak Co., Rochester, NY) and Lighting Plus intensifying screens (Dupont Co., Wilmington, DE). Relative intensities of bands on autoradiograms were quantified using a scanning densitometer (model GS 300; Hoefer Scientific Instruments, San Francisco, CA).

Size-exclusion Chromatography

Chromatography was performed on a Bio-Rad Laboratories TSK-250 column (300 × 7.5 mm) equilibrated with 0.2 M phosphate buffer containing 0.1 M NaCl, 5% methanol, and 0.1% SDS (Buffer A). Detergent-solubilized cross-linked material in Buffer A was applied to the column and eluted with Buffer A at a flow rate of 1 ml/min. Fractions of 0.5 ml were collected and radioactivity in each fraction was counted. Standard proteins prepared in buffer A were chromatographed and their elution volumes were determined by OD at 280 nm. The molecular size of the receptor/ligand complex was estimated by interpolation using a standard curve prepared with the following proteins: thyroglobulin, *M*, 670,000; gamma globulin, *M*, 158,000; BSA, *M*, 67,000; ovalbumin, *M*, 44,000; myoglobin, *M*, 17,000; and vitamin B-12, *M*, 1,350.

Results

Chemotactic Response to VGVAPG

The repeating elastin hexapeptide, VGVAPG, which has been shown to possess chemotactic activity for monocytes and elastin-producing fibroblasts (25), was synthesized and

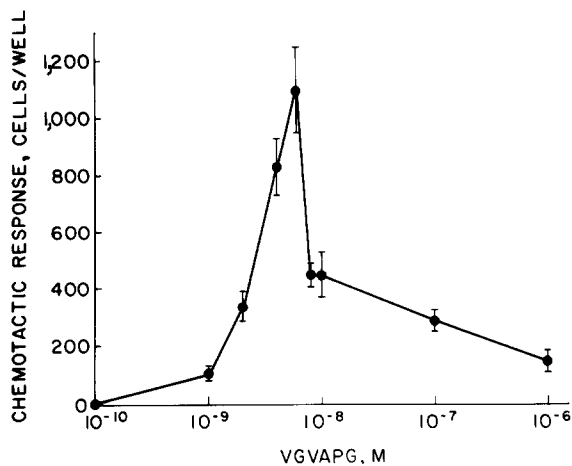


Figure 1. M27 tumor cell migration in response to an elastin-derived peptide. The indicated concentrations of synthetic hexapeptide VGVPAG were placed in lower wells and 5,000 cells were placed in the upper wells of chemotaxis chambers. Data are expressed as the number of tumor cells/well that had migrated across the filter during a 3-h incubation period (mean \pm SD, $n > 12$).

tested for chemotactic activity on an invasive, metastatic tumor cell line (Lewis lung carcinoma; line M27). When placed in the lower wells of a Boyden multiwell chemotaxis chamber, VGVPAG stimulated cell migration across the micropore filter at concentrations between 10^{-9} and 10^{-8} M (Fig. 1). The concentration of VGVPAG required to elicit half-maximal activity was $\sim 3 \times 10^{-9}$ M. The optimal VGVPAG dose for stimulation of directional M27 cell migration is in the same concentration range as the effective dose reported for monocytes and elastin-producing fibroblasts (25). A chemotactic response to the elastin-derived peptide is not a property of all tumor cells but it is not unique to the M27 Lewis lung carcinoma cells. B16 melanoma variants were also chemotactic for VGVPAG although the magnitude of the B16 response was less than that of M27 tumor cells (data not shown).

Several mammalian cell chemoattractants have been shown to bind to specific cell surface receptors on their target cell surfaces (1, 8, 19, 30). We therefore investigated whether the elastin hexapeptide, VGVPAG, might bind to a corresponding receptor on M27 tumor cells. To facilitate radiolabeling of the peptide for receptor binding studies, a second peptide was synthesized with a tyrosine residue attached to the amino terminus (Y-VGVPAG). The biological activities of the tyrosinated peptide analog before and after iodination were evaluated using the migration assay. As shown in Table II, the effective doses of VGVPAG, Y-VGVPAG, and [¹²⁵I]Y-

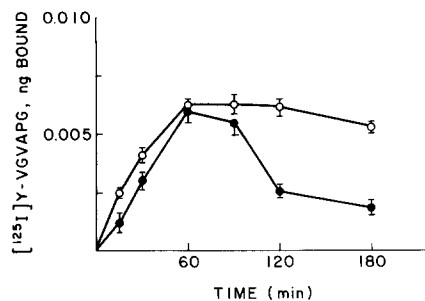


Figure 2. Time course of specific binding of [¹²⁵I]Y-VGVPAG to M27 cells at different temperatures. Tumor cells (250,000 cells/ml) were incubated with 1 ng [¹²⁵I]Y-VGVPAG in the presence or absence of excess unlabeled VGVPAG at 37°C (●) or 4°C (○). Cell-associated radioactivity was determined at the indicated times. Each point shows the mean specific binding \pm SD observed in duplicate tubes in two independent experiments.

VGVPAG for stimulation of M27 cell chemotaxis were comparable to each other.

[¹²⁵I]Y-VGVPAG Binding to M27 Tumor Cells

The binding of [¹²⁵I]Y-VGVPAG by viable M27 tumor cells was specific, saturable, and of high affinity. Binding of the radiolabeled peptide to M27 cells was effectively inhibited by excess concentrations of unlabeled VGVPAG, indicating that both tyrosinated and nontyrosinated ligands competitively interact with the same class of binding sites. [¹²⁵I]Y-VGVPAG binding was fully inhibited by pancreatic elastase digests of insoluble elastin, but was not blocked by the chemotactic peptides fMLP and C5a (data not shown).

A time course of specific binding at two incubation temperatures is shown in Fig. 2. At 4°C, steady state binding was reached by 60 min and maintained through 180 min. Binding of [¹²⁵I]Y-VGVPAG at 37°C reached a maximal level by 60 min, but longer incubation times resulted in decreased specific binding. Decreases in cell-associated radioactivity after prolonged incubation at 37°C are frequently encountered in radioligand binding assays of biologically active peptides and are thought to result from internalization and degradation of the receptor/ligand complex (4). To test for internalization of [¹²⁵I]Y-VGVPAG at 37°C, the sensitivity of cell-associated radioactivity to removal by trypsin was determined. Fig. 3 shows that radiolabeled peptide bound to M27 cells rapidly became trypsin-insensitive, suggesting that the receptor/ligand complex was being internalized. Parallel experiments performed at 4°C resulted in trypsin sensitivity of 70–80% bound radioactivity (data not shown). Comparative reversed phase HPLC analysis of unbound radioactivity after

Table II. Chemotactic Activity of VGVPAG Analogs Modified at the Amino Terminus

Peptide	Chemotactic response*			
	10 ⁻⁹ M	5 × 10 ⁻⁹ M	10 ⁻⁸ M	10 ⁻⁷ M
VGVPAG	109 \pm 25	968 \pm 150	454 \pm 75	290 \pm 28
Y-VGVPAG	135 \pm 26	832 \pm 94	412 \pm 55	347 \pm 15
[¹²⁵ I]Y-VGVPAG	159 \pm 17	777 \pm 58	429 \pm 59	348 \pm 81

* Mean number of M27 cells/well migrating in response to peptide \pm SD, $n \geq 8$.

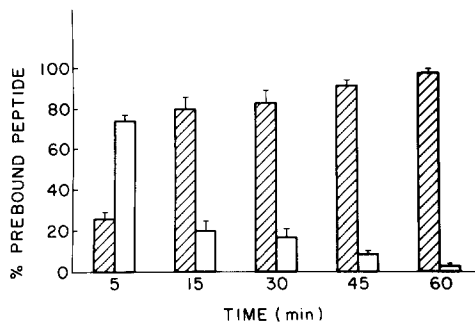


Figure 3. Internalization of bound [125 I]Y-VGVAPG by M27 cells. Cells were incubated with 1 ng/ml [125 I]Y-VGVAPG for 45 min at 37°C. Binding was terminated by removal of unbound peptide, washing, and resuspension of cells in 1 ml fresh binding buffer at 37°C. At the indicated times, cells were treated with trypsin for 10 min at 25°C and rapidly pelleted at 13,000 g in an Eppendorf microfuge. Radioactivity in the supernatant (open bars, trypsin-sensitive) and associated with cell pellet (hatched bars, trypsin-insensitive) was determined.

45 and 120 min at 37°C revealed significant extrusion of iodinated degradation products into the medium at 120 min (Fig. 4, b and c). The integrity of unbound radiolabeled ligand was remarkably stable during binding assays performed at 4°C, even after prolonged 2-h incubation periods (Fig. 4 a). Therefore, at 37°C receptor-bound ligand appears to be degraded by M27 cells subsequent to transformation to a trypsin-insensitive form. This process was inhibited during binding assays performed at 4°C.

The rates of release of cell-associated radioactivity after binding at 37 and 4°C are compared in Fig. 5. The half-time for release of radiolabeled ligand at 4°C, a temperature at which receptor-mediated endocytosis is inhibited, was short ($t_{1/2} = 11$ min) and followed first order kinetics. Release of radioactivity from M27 cells at 37°C was prolonged ($t_{1/2} = 55$ min), a result which is consistent with the ligand internalization demonstrated in Fig. 3. The off-rate at 37°C is, therefore, more representative of the rate at which the cells extrude degraded ligand, while the off-rate at 4°C more accurately defines the ligand/receptor interaction.

The concentration dependence of [125 I]Y-VGVAPG binding to M27 cells over 45 min at both 4 and 37°C is shown in Fig. 6 A. Specific binding of VGVAPG to M27 cells is saturable at concentrations in excess of 5 ng/ml. Under these conditions, nonspecific binding at 37°C was 30–45% of total bound radioactivity. Nonspecific binding at 4°C was higher, ranging from 40–60% of total bound radioactivity. Equivalent quantities of [125 I]Y-VGVAPG were specifically bound by M27 cells at each temperature, indicating the same number of available binding sites.

Scatchard analysis (Fig. 6 B) of the binding data presented in Fig. 6 A revealed that the ratio of bound vs. bound/free labeled peptide was a linear function ($r = 0.98$ at 37°C; $r = 0.99$ at 4°C), indicating a single class of noninteracting binding sites in this concentration range (23). [125 I]Y-VGVAPG binds to M27 cells with high affinity ($K_d = 2.7$ nM) as determined from the slope of the plot. After 45 min exposure to [125 I]Y-VGVAPG, each cell had a maximum binding capacity of 52,000 ligand molecules. Analysis is based on binding data from multiple experiments ($n = 6$ at 37°C; $n = 4$

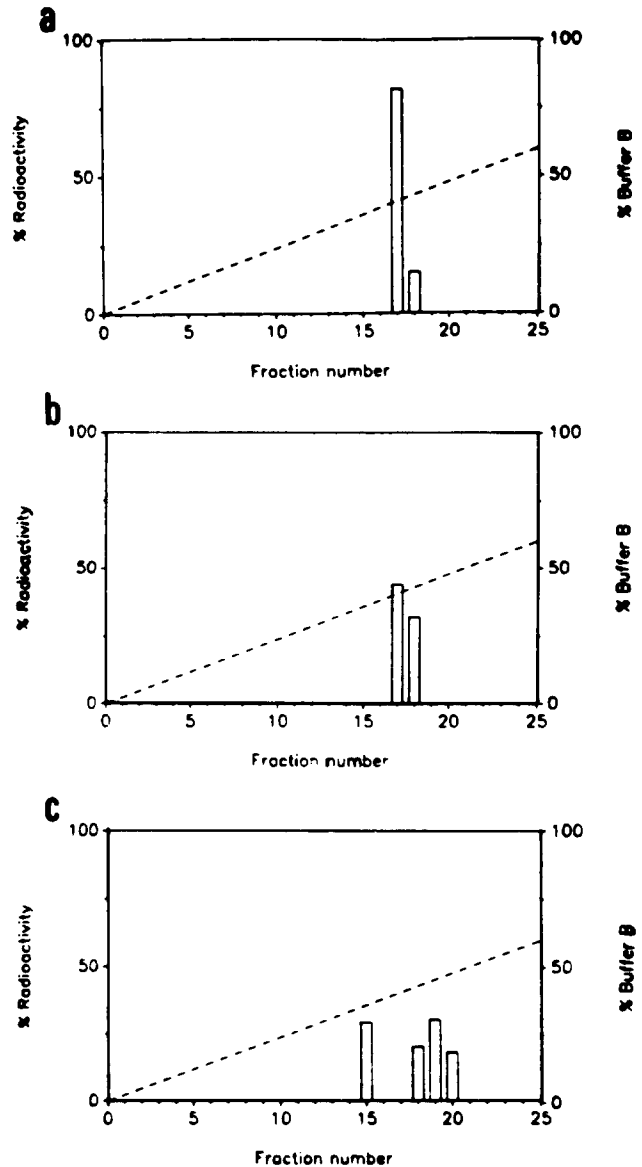


Figure 4. Reversed phase chromatography of unbound radiolabeled ligand at 4 and 37°C during binding assays. [125 I]Y-VGVAPG binding to M27 cells was allowed to proceed at (a) 4°C for 120 min, (b) 37°C for 45 min, and (c) 37°C for 120 min. Cells were centrifuged and supernatants were immediately applied to a C18 Vydac HPLC column equilibrated with 0.1% TFA. Bars, radioactivity present in each fraction eluted with a gradient of 0–80% Buffer B (0.1% TFA in 90% acetonitrile) generated over a 30-min period. 95–100% of radioactivity from parallel tubes incubated in the absence of cells at either temperature eluted in fraction 17.

at 4°C). These results firmly establish the existence of a high affinity binding site for VGVAPG on M27 cells that has the properties of a specific receptor.

Cross-linking of [125 I]Y-VGVAPG to the Cell Surface

M27 carcinoma cells contain a single membrane component that can be specifically cross-linked to [125 I]Y-VGVAPG with 200 μ M DSS. The [125 I]Y-VGVAPG cross-linked species has an M_r of 59,000, as determined by SDS-PAGE (Fig. 7). Incorporation of label into this species was not observed using

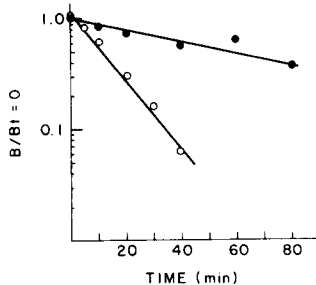


Figure 5. Rates of dissociation of [^{125}I]Y-VGVAPG bound by M27 cells at different temperatures. Cells were incubated with 1 ng/ml [^{125}I]Y-VGVAPG for 45 min at 37 (●) or 4°C (○), washed free of unbound peptide, and then resuspended in fresh binding buffer pre-equilibrated to the appropriate temperature. At the indicated times, cell-associated radioactivity was determined. Results are the means of triplicate determinations.

M27 cells that were incubated with the radioligand in the absence of DSS. Radiolabeling of the M_r 59,000 species was effectively competed in a concentration-dependent manner by excess unlabeled VGVAPG. Densitometric scans of the autoradiographic bands demonstrate inhibition of receptor labeling by excess unlabeled VGVAPG (Table III). The electrophoretic mobility of the cross-linked species was not altered

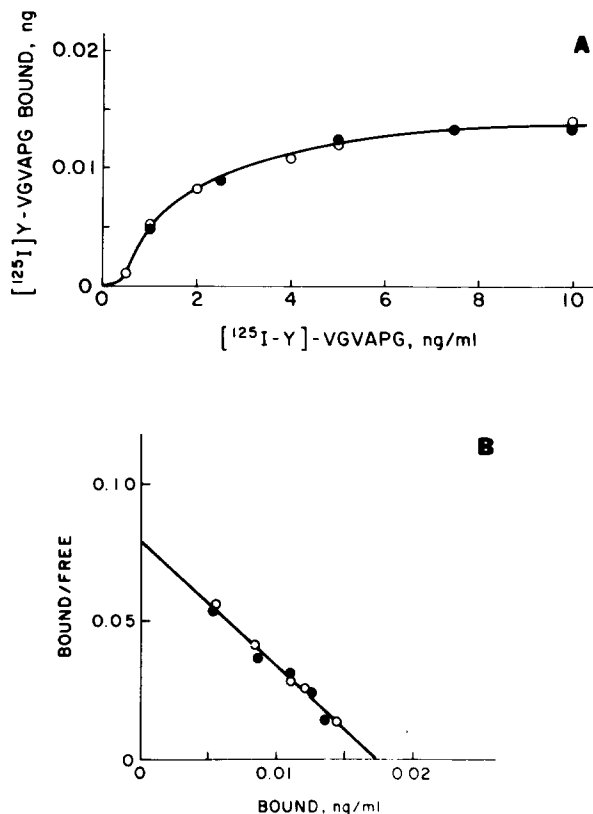


Figure 6. Concentration dependence of [^{125}I]Y-VGVAPG binding to M27 cells. Tumor cells (250,000 cells/ml) were incubated for 45 min at either 37 (●) or 4°C (○) with the indicated concentrations of [^{125}I]Y-VGVAPG in the presence or absence of excess unlabeled VGVAPG. (A) Each point represents the mean specific binding of radiolabeled peptide (SD \leq 10%, $n = 4$). (B) The ratio of [^{125}I]Y-VGVAPG specifically bound to unbound peptide was calculated from the binding data shown in Fig. 6 A and plotted as a function of specific binding.

Table III. Specific Incorporation of [^{125}I]Y-VGVAPG into M_r 59,000 species

VGVAPG Added μM	% Inhibition
0	0
2	86
10	98
50	100

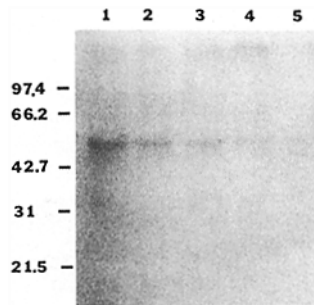
by the addition of the reducing agent, 2-mercaptoethanol, suggesting that the M27 tumor cell surface VGVAPG-binding protein is monomeric. Occasionally minor bands appeared at M_r 49,000, 37,000, and 33,000. Since detergent extracts of whole M27 cells were used for these studies, the minor bands may represent receptor molecules that were modified during internalization of the ligand/receptor complex.

[^{125}I]Y-VGVAPG binding to M27 cells at 4°C rather than 37°C before addition of the cross-linking agent resulted in the incorporation of radiolabel into only the 59,000- M_r molecule, as determined by size-exclusion chromatography (Fig. 8). An estimate of the molecular size of the cross-linked species was obtained by comparison to elution volumes of standards with known molecular sizes in a parallel run. For this analysis addition of detergent to the mobile phase was required to inhibit interaction of the radioactive ligand/receptor complex with the TSK column. The single peak of radioactivity at \sim 59,000 M_r was not observed for cells in which excess unlabeled VGVAPG was added to the binding reaction.

Discussion

The extracellular matrix protein elastin contains domains that modulate cellular behavior (10, 11, 17, 24, 26). We now demonstrate the ability of Lewis lung carcinoma cells (line M27), an invasive and metastatic tumor cell line, to respond chemotactically to VGVAPG, an elastin-derived repeat sequence. M27 cells detect soluble VGVAPG by binding of the chemotaxin to a specific high affinity cell surface receptor, which we have identified as a protein with an M_r of 59,000. We postulate that VGVAPG binding to its receptor initiates intracellular processes that result in the M27 tumor cell migratory response.

Figure 7. Cross-linking of [^{125}I]Y-VGVAPG to M27 cells and analysis of the cross-linked complex by SDS-PAGE. M27 tumor cells (5×10^5) were incubated in the presence of 5 ng/ml [^{125}I]Y-VGVAPG alone or with various concentrations of unlabeled VGVAPG. At the end of the incubation, cells were rapidly washed, cross-linked with DSS, and processed for electrophoresis. The resulting autoradiogram of a 10% (wt/vol) SDS-PAGE gel is shown. Lane 1, 0 μM VGVAPG; lane 2, 2 μM VGVAPG; lane 3, 10 μM VGVAPG; lane 4, 50 μM VGVAPG; and lane 5, 100 μM VGVAPG.



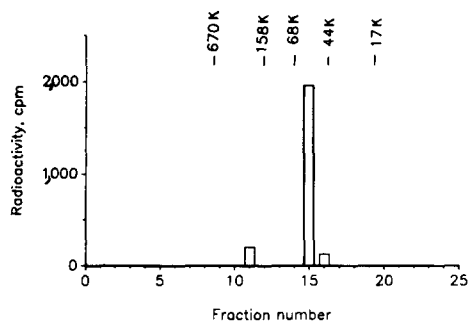


Figure 8. Size exclusion chromatography of cross-linked M27 cell extracts. [125 I]Y-VGVAPG was bound to M27 cells at 4°C for 45 min and cross-linked to the cell surface with 200 μ M DSS. Detergent extracts of M27 cells were immediately fractionated on a TSK-250 column preequilibrated with Buffer A. Bars, the radioactivity present in each fraction.

Radioligand binding assays and subsequent Scatchard analysis of specific peptide binding detected the presence of one class of high affinity binding molecules on the surface of M27 tumor cells. The affinity constant of this receptor was calculated to be 2.7×10^{-9} M, which correlates closely with the concentration range required to elicit a chemotactic response by these cells. Receptors on M27 cells bound [125 I]Y-VGVAPG with similar kinetics at 4 and 37°C. These results indicate that M27 cells present receptor at the cell surface without the requirement for mobilization of receptor to the membrane in response to initial receptor occupancy. The difference in off-rates between the two temperatures is a reflection of uptake of the ligand at 37°C, as is evidenced by internalization data. This disparity in ligand off-rates has also been encountered with receptor binding to the chemotactic peptide f-MLP (12, 29, 30).

Several other cell surface molecules have been identified which interact with the elastin molecule (9, 10). Using elastin-affinity chromatography Hornebeck et al. (10) identified a 120,000 M_r protein on the surface of mesenchymal cells, which was termed "elastonection." This protein was shown to mediate cellular adhesion to insoluble elastin. A 67-kD receptor for tropoelastin has been described (28) and identified (9), which is thought to be involved in assembly of elastin fibers from the tropoelastin precursor. Elastin-affinity chromatography on detergent-solubilized M27 cell membrane proteins resulted in VGVAPG elution of proteins migrating on electrophoresis as major bands with M_s of 67,000, 60,000, and 47,000. However, at VGVAPG concentrations used for binding studies and covalent cross-linking, only the 59,000- M_r protein was detected. It is not presently known whether this 59,000- M_r protein exhibits considerably higher affinity for VGVAPG than other potential elastin-binding proteins on the M27 cell surface or whether only the 59,000- M_r protein binds the iodinated, tyrosinated agonist. Alternatively, this receptor may be unoccupied, whereas other elastin receptors may be occupied on M27 cells.

Since only one class of binding protein for VGVAPG was detected on M27 carcinoma cells by Scatchard analysis, we postulate that the 59,000- M_r species identified by cross-linking is likely to be critical to the chemotactic response of M27 tumor cells. Chemotaxis of leukocytic cells has been shown to involve guanine nucleotide binding proteins (18, 27)

and intracellular calcium mobilization (27). It will therefore be of interest to determine whether VGVAPG binding to the 59,000- M_r receptor initiates a similar sequence of intracellular events. Elastin peptides modulate calcium ion fluxes in monocytes, fibroblasts, and smooth muscle cells (11), which also migrate in response to VGVAPG (25). It is not presently known whether the 59,000- M_r receptor for VGVAPG is expressed on these cell types. The requirement of a high affinity 59,000- M_r VGVAPG receptor for chemotaxis to the peptide is presently being evaluated on responsive and non-responsive cell types.

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