The Relationship between Motility Factor Receptor Internalization and the Lung Colonization Capacity of Murine Melanoma Cells

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ABSTRACT

The in vitro motility of B16-F1 melanoma cells is enhanced by incubation with a monoclonal antibody against gp78, previously characterized as a motility factor receptor. This antibody was used to study the relationship between motility stimulation in vitro and metastatic ability in vivo in the B16-F1 and K-1735 murine melanoma systems. While both high- and low-metastatic variants exhibited enhanced in vitro motility in response to the anti-gp78 monoclonal antibody, only the high-metastatic cells exhibited an increased metastatic ability. Surface immunofluorescence of low-metastatic cells was distributed more diffusely compared to a highly localized patching of gp78 on high-metastatic cells, suggesting that the directed endocytosis of gp78 to form a single leading edge is related to the metastatic ability of a cell, while fluorescence-activated cell sorter analysis revealed decreased gp78 surface expression in high-metastatic clones. Priming of cells by preventing internalization of gp78-antibody complexes by pertussis toxin resulted in a marked enhancement of pulmonary metastases by the treated cells which was directly correlated with decreased surface expression of gp78 following washout of pertussis toxin. These results suggest that cell motility induced by motility factor receptor occupancy may play a role in the process of metastasis and that the ligand-receptor complex internalization from the cell surface is involved in control of cell kinesis during metastasis.

INTRODUCTION

Tumor cell motility has been suggested as a key event of the metastatic cascade, and correlations have been demonstrated between cell motility and spontaneous and experimental metastatic potential of various tumor cells (for a review see Refs. 1 and 2). Several factors have been implicated as modulating agents for cell motility (3). Recently two types of motility factors, designated scatter factor (4, 5) and AMF (6), have been identified. It has also been suggested that growth factors including fibroblast growth factor (7) and insulin-like growth factor (8) may affect cell motility. A monoclonal antibody, SLOW-1, was reported to inhibit the locomotion of cells by an indirect signaling mechanism, through a specific cell surface receptor involved in motile control (9). We have recently demonstrated that a mAb against gp78 expressed in B16-F1 cells (10), this cell variant was used as a positive control. B16-F10 and B16-F10Lr6 cell lines were derived from B16-F1 melanoma. B16-F10 is a high-metastatic line and was selected in vivo (14, 15). B16-F10Lr6 is a low-metastatic line and was selected in vitro for resistance to lysis by lymphocytes (15). The K-1735 melanoma was developed in a C3H mouse that has been treated with a short course of exposure to UV radiation followed by chronic painting of the skin with croton oil (16). K-1735-M1 and K-1735-C1–11 cells were derived from the parent melanoma tumor as high- and low-metastatic variants, respectively. The C1–11 cloned cell line was obtained by cloning of the fifth in vitro passage of the parent cells (17). The M1 cell line was derived from pulmonary metastases after i.v. inoculation of the parent tumor cells (18).

The cells were grown in monolayer on plastic in Dulbecco's modified Eagle's minimal essential medium supplemented with glutamine, nonessential amino acids, vitamins, antibiotics, and 10% heat-inactivated fetal bovine serum. To ensure reproducibility, all experiments were done with the same batch of fetal bovine serum (control number 44N3085; GIBCO). The cells were maintained at 37°C in a humidified atmosphere of 5% CO2/95% air. To ensure reproducibility, all experiments were performed with cultures grown for no longer than 6 weeks after recovery from frozen stocks. Cell monolayers that had attained semiconfluence were used for preparing single-cell suspensions. Cultures were obtained by plating 2 × 103 cells/90-mm dish and culturing for 3 days at 37°C. Cells were harvested byoverlaying with a thin layer of 2 mM EDTA for 2 min at 37°C followed by gentle pipeting to form single-cell suspensions. Viability was recorded following a trypan blue exclusion test.

Phagokinetic Tracks. Uniform carpets of gold particles were prepared on coverslips coated with bovine serum albumin, as described previously (19, 20). Colloidal gold-coated coverslips were placed in 35-mm tissue culture dishes containing 2 ml complete minimal essential medium supplemented with glutamine, nonessential amino acids, vitamins, antibiotics, and 10% heat-inactivated fetal bovine serum, with or without 3F3A mAb ascites fluid at a concentration of 25 μl/ml, and then 2000 cells were added to each plate. After 24 h, phagokinetic tracks were visualized using dark-field illumination in a Nikon inverted microscope at ×200. The area cleared of gold particles by at least 30 cells was measured, and the standard error was calculated.

Experimental Pulmonary Metastasis. Cells grown as a monolayer were incubated for 18 h at 37°C in the presence or absence of 25 μl/ml 3F3A mAb. In the experiments with PT, cells were treated with 500 ng/ml PT for 60 min prior to mAb exposure. The cells were washed 3 times with cold PBS to wash away nonbound mAb prior to injection into mice. Syngeneic mice (C57BL/6J mice for B16 melanoma, C3H/HeJ mice for K-1735 melanoma; The Jackson Laboratory) were inoculated in the tail vein with 5 × 105 cells in 0.2 ml of PBS. After 17 days...
The control cells were incubated identically without the 3F3A mAb. After 30 min at 0°C, the cells were washed twice with PBS, and then FITC-conjugated anti-rat antibody (Zymed; 1:10) was used as the secondary antibody. After 30 min at 0°C, the cells were washed twice in PBS, and cell surface fluorescence was analyzed using a FACS (FACStar; Becton Dickinson, Mountain View, CA). A scatter window was set to eliminate dead cells and cell debris. The frequency and fluorescence profiles of the stained cells were determined using a laser output of 125 mV.

Immunofluorescence. The surface immunofluorescence of gp78 was performed by fixing cells with 3.5% paraformaldehyde in PBS for 8 min at 23°C. The cells were washed 3 times with PBS and incubated for 30 min at 23°C with a 1:10 dilution of the 3F3A mAb in PBS. The cells were then washed with PBS and labeled at 23°C with FITC-conjugated anti-rat antibody (Zymed). After 30 min the cells were washed extensively with PBS.

Double labeling of gp78 with a major lysosomal associated membrane glycoprotein, LAMP-1 (21), was performed as described (11). Briefly, cells were fixed and permeabilized by immersion of the cover slide in precooled (—80°C) methanol for 30 min at —20°C. Double labeling of gp78 with LAMP-1 was performed by the sequential incubation of permeabilized cells with anti-P2B/LAMP-1 rabbit antiserum (a gift from Dr. Jim Dennis) and FITC anti-rabbit IgG (Zymed) followed by the 3F3A mAb and tetramethylrhodamine isothiocyanate anti-rat IgG (Zymed). The coverslips bearing the stained cells were mounted in 90% glycerol in PBS and observed under a Nikon microscope.

Protein Gel Electrophoresis and Blotting. Immunoblots were performed as previously described (10). Briefly, cells grown in a monolayer were harvested, washed twice in PBS, suspended at 5 x 10^6 cells/ml in 0.5% Nonidet P-40 in PBS containing 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride for 30 min on ice, and clarified by centrifugation. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 8% polyacrylamide slab gels according to the method of Laemmli (22) and electrotransferred to nitrocellulose filters. Nitrocellulose filters were quenched overnight in PBS containing 15% skim milk and 0.2% NaNO_3 (quench solution). The filters were then incubated with the 3F3A mAb ascites diluted 1:400 in the quench solution for 1 h. The filters were washed 5 times for 10 min in the quench solution and then incubated for 1 h in the quench solution with 125I-sheep anti-rat antibody (1 μCi/10 ml; Amersham). The filters were then washed twice for 15 min in the quench solution and then twice more in the quench solution containing 0.1% Tween 20. The filters were dried and autoradiographed.

RESULTS

Locomotor Activity Stimulated by the Anti-gp78 mAb of High- and Low-Metastatic Cell Variants. The binding of the anti-gp78 mAb to gp78 mimics the effect of AMF by enhancing the locomotor activity of the treated cells (10). This observation raised the question of whether motility stimulation by the anti-gp78 mAb may be related to the metastatic potential of the cell. High- and low-metastatic cell variants from two murine melanoma cell systems were plated on gold particle-coated substrate and were examined in dark-field optics 24 h later (Fig. 1). The average area of the particle-clear zone in the low-metastatic B16-F10Lr^ cells was 5.7 μm²/h, while the area of the tracks formed by the high-metastatic B16-F10 cells was 10.2 μm²/h.

Similarly, the K-1735 high-metastatic variant (M1) exhibited a 4.0-fold higher basal locomotor activity than its low-metastatic counterpart (Cl-11) (Fig. 1B) as has been previously described (10). Treatment of all four cell lines with the anti-gp78 mAb stimulated their motility by approximately 2-fold, as previously reported for B16-F1 melanoma cells (10), with no significant differences between the high- and low-metastatic clones of either melanoma all system (Fig. 1). The effect of the anti-gp78 mAb appears to be specific because other mAbs, anti-lectin, anti-H^2, anti-transferrin receptor, and rat IgM have no significant effect on cell migration.

Lung Colonizing Response to Anti-gp78 mAb. To examine whether the lung colonization ability of the melanoma variants could also be enhanced, cells were incubated with or without the anti-gp78 mAb prior to injection into mice. As shown in Table 1, preincubation with the mAb resulted in a 2.5-fold increase in the lung colonizing capacity of the high-metastatic cell variants of either tumor system, whereas there was a negligible effect on the colonization of the low-metastatic cells. The stimulation of the lung colonizing ability of the high-metastatic cell variants by the anti-gp78 mAb corroborates the previous findings in which polyclonal anti-gp78 antibodies were demonstrated to enhance the metastatic ability of B16-F1 melanoma cells (12).
motility factor receptor endocytosis and metastasis

Table 1 Effect of the anti-gp78 mAb on lung colonization*

<table>
<thead>
<tr>
<th>Cell line</th>
<th>In vitro pretreatment of 3F3A</th>
<th>No. of lung colonies/mouse Median (range)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>B16 melanoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F10</td>
<td>–</td>
<td>8, 13, 16, 19, 20, 28, 29, 37, 42, 62</td>
<td>24 (8–62)</td>
</tr>
<tr>
<td>+</td>
<td>8, 27, 32, 37, 63, 64, 78, 79, 103</td>
<td>63 (8–103)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>F10Lr6</td>
<td>–</td>
<td>0, 1, 1, 2, 3, 4, 4, 8</td>
<td>2 (0–8)</td>
</tr>
<tr>
<td>+</td>
<td>0, 0, 1, 1, 1, 2, 2, 3</td>
<td>1 (0–3)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>K-1735 melanoma</td>
<td>M1</td>
<td>–</td>
<td>24, 37, 57, 90</td>
</tr>
<tr>
<td>+</td>
<td>59, 95, 111, 112, 132</td>
<td>111 (59–132)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cl-11</td>
<td>–</td>
<td>0, 0, 0, 0, 0, 0</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>+</td>
<td>0, 0, 0, 0, 0, 0</td>
<td>0 (0–0)</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>B16 melanoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F10</td>
<td>–</td>
<td>15, 17, 18, 21, 26, 30, 31, 36</td>
<td>23 (15–36)</td>
</tr>
<tr>
<td>+</td>
<td>32, 44, 50, 52, 66, 80, 88, 96, 141</td>
<td>66 (32–141)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>F10Lr6</td>
<td>–</td>
<td>0, 1, 2, 4, 5, 7, 9, 14</td>
<td>5 (0–14)</td>
</tr>
<tr>
<td>+</td>
<td>0, 4, 6, 7, 8, 10, 17</td>
<td>7 (0–17)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>K-1735 melanoma</td>
<td>M1</td>
<td>–</td>
<td>56, 66, 93, 93, 103, 114</td>
</tr>
<tr>
<td>+</td>
<td>97, 107, 188, 189, 190, 205</td>
<td>188 (97–205)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cl-11</td>
<td>–</td>
<td>0, 0, 0, 0, 0, 0</td>
<td>0 (0–0)</td>
</tr>
</tbody>
</table>

* Cells grown in complete minimal essential medium were incubated at 37°C in the presence or absence of the 3F3A mAb at a concentration of 25 µl/ml for 18 h prior to i.v. injection of 5 x 10⁴ cells into mice.

* Probability of no difference from the group without treatment of the 3F3A mAb (two-tailed Mann-Whitney U test).

Table 2 Effect of the anti-gp78 mAb with pertussis toxin on lung colonization*

<table>
<thead>
<tr>
<th>In vitro pretreatment</th>
<th>No. of lung colonies/mouse Median (range)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Control</td>
<td>0, 2, 4, 9, 13, 13, 13, 25</td>
</tr>
<tr>
<td>3F3A</td>
<td>3, 5, 9, 15, 17, 21, 23, 24</td>
<td>16 (3–24)</td>
</tr>
<tr>
<td>3F3A + PT</td>
<td>8, 28, 41, 43, 46, 64, 89, 120</td>
<td>46 (8–120)</td>
</tr>
<tr>
<td>PT</td>
<td>0, 0, 2, 4, 10, 14, 38</td>
<td>7 (0–38)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Control</td>
<td>0, 0, 0, 0, 0, 1, 2</td>
</tr>
<tr>
<td>3F3A</td>
<td>0, 1, 2, 3, 3, 3, 4, 7</td>
<td>3 (0–7)</td>
</tr>
<tr>
<td>3F3A + PT</td>
<td>6, 9, 18, 19, 20, 29, 32, 54</td>
<td>20 (6–54)</td>
</tr>
<tr>
<td>PT</td>
<td>0, 0, 0, 0, 0, 1, 3, 4</td>
<td>0 (0–4)</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>Control</td>
<td>3, 9, 19, 38, 42, 42, 50, 57, 79</td>
</tr>
<tr>
<td>3F3A</td>
<td>21, 29, 30, 36, 38, 42, 60, 61, 74, 88</td>
<td>40 (21–81)</td>
</tr>
<tr>
<td>3F3A + PT</td>
<td>34, 46, 51, 52, 60, 121, 132, 134, 141</td>
<td>90 (34–220)</td>
</tr>
<tr>
<td>PT</td>
<td>188, 220</td>
<td>34 (13–160)</td>
</tr>
</tbody>
</table>

* Mice were given i.v. injections of 5 x 10⁴ B16-F1 cells.

* Probability of no difference from the group with treatment of neither the 3F3A mAb nor PT (two-tailed Mann-Whitney U test).

* Cells grown in complete minimal essential medium were incubated at 37°C in the presence of the 3F3A mAb at a concentration of 25 µl/ml for 18 h prior to i.v. injection.

* Cells were treated with 500 ng/ml PT 60 min prior to interaction of the cells with the 3F3A mAb.

metastases induced by the anti-gp78 mAb, B16-F1 cells were pretreated with 500 ng/ml PT for 60 min and then treated overnight with PT and the anti-gp78 antibody. Mice were inoculated with cells preincubated (18 h) with PT only, with antibody only, or with PT followed by antibody. In three separate experiments, PT alone had no effect on pulmonary metastasis (Table 2). The anti-gp78 mAb alone exhibited varying effects on B16-F1 colonization ability, perhaps reflecting its median metastatic potential between the nonresponsive B16-F10Lr6 and the high-metastatic B16-F1 cell lines. However, cells pretreated with PT and incubated overnight in the presence of both PT and the anti-gp78 mAb exhibited significant increases in the level of lung colonization (Table 2).

Cell Surface Distribution of gp78. gp78 is localized to the leading and trailing edges of motile B16-F1 melanoma cells and BALB/c-3T3/A31 fibroblasts (10). In order to examine the quantitative expression gp78 on the cell surface, cells were immunofluorescently labeled and analyzed by FACS. As seen

Fig. 2. Cell surface staining with the 3F3A mAb by flow cytofluorometry of B16-F10 cells (Ia), B16-F10Lr6 cells (Ib), M1 cells (Ic), or Cl-11 cells (Ii). Cells were labeled with the 3F3A mAb at 4°C, washed with fluorescein-labeled rabbit anti-rat antibody, and analyzed in the FACStar (Becton Dickinson). A scatter window was set to eliminate dead cells and cell debris.

* R. Nabi, H. Watanabe, and A. Raz, unpublished results.
in Fig. 2, in both tumor systems the high-metastatic variants were labeled less than their low-metastatic counterparts. It should be noted that this difference is not due to nonspecific bindings of FITC-conjugated anti-rat antibody, since the binding patterns of anti-rat antibody to both high- and low-metastatic cells without the first mAb were undistinguishable. These results from the B16 and K-1735 melanoma systems were further corroborated by direct microscopic visualization. Approximately 80% of B16-F10Lr6 cells and 75% of C1-11 cells (low-metastatic variants) had multiple surface domains stained by the anti-gp78 mAb (Fig. 3, A and C) while most of the high-metastatic cell variants (approximately 90% of F10 and 75% of M1 cells) manifested a single labeled domain along the edge of the cells (Fig. 3, B and D). Since the coordinate extension of a single leading edge is crucial to the directed locomotion of a cell (23), the multiple surface domains on the low-metastatic variants may reflect the extension of multiple leading edges resulting in a random rather than a directional motility in response to the anti-gp78 mAb. Such an altered motility pattern would not be detectable in the two-dimensional phagokinetic track assay but would be evident in the metastatic response of the cells (Fig. 1; Table 1).

Intracellular Distribution of gp78. In A31 fibroblasts, gp78 is localized intracellularly to elongated beaded vesicles which colocalize with LAMP-1, a lysosomal glycoprotein aligned with microtubules, and we have proposed that gp78 is translocated within such tubular vesicles. To examine whether the colocalization of the intracellular gp78 with lysosomes is also present in melanoma cells, cells were immunofluorescently double-labeled with the anti-gp78 mAb and antibodies to the lysosomal antigens LAMP-1. As seen in Fig. 4, gp78 and LAMP-1 exhibit partial codistribution identifying gp78 as a lysosomal glycoprotein. There were no differences among the four melanoma cell lines in the distribution of intracellular gp78 and LAMP-1. Colocalization of the proteins was detected primarily in the larger vesicular structures. The anti-gp78 mAb, however, also labeled other smaller vesicles which were not labeled by anti-LAMP-1 antibodies and may represent endosomes, as seen in fibroblasts.

Decrease in Surface Expression of gp78 1 h after Removal of PT. The unexpected increase in the lung colonizing ability of cells treated with both PT and the anti-gp78 antibody leads us to believe that PT inhibition of G protein may be blocking the internalization of gp78 required for motility stimulation. Such
MOTILITY FACTOR RECEPTOR ENDOCYTOSIS AND METASTASIS

Fig. 4. Double indirect immunofluorescent labeling for gp78 (A) and LAMP-1 (B). Cl-11 cells were fixed and permeabilized prior to treatment with anti-P2B/LAMP-1 antisera and fluorescein goat anti-rabbit IgG followed by anti-gp78 and rhodamine goat anti-rat IgG. No cross-reactivity of the two secondary antibodies to cells labeled with either of the two primary antibodies was detected. The three other cell lines showed the same distribution and colocalization as the Cl-11 cells.

treated cells would not endocytose gp78 in overnight culture and would be injected into the mice with anti-gp78 antibody still on the cell surface. Placement of the cells in a PT free environment, either during washing of the cells with cold PBS or following injection, would allow the recruitment to the cell membrane of newly synthesized G protein which had not been inhibited by PT. Following inoculation into the mouse, internalization at 37°C of the gp78-antibody complex would occur with the resultant motility stimulus.

To determine whether such a hypothesis could explain the synergistic effect of the anti-gp78 mAb and PT on lung colonization, we analyzed the surface distribution of gp78 on cells treated with PT. B16-F1 cells were cultured in the presence of 500 ng/ml PT for 18 h and then allowed to recover in PT free medium for 1 h. PT treatment did not affect the cell surface expression of gp78 compared to that of untreated cells (data not shown). However, replacement of the media for 1 h with regular media lacking PT resulted in the decreased cell surface expression of gp78 both quantitatively by FACS analysis (Fig. 5A) and qualitatively by surface immunofluorescence (Fig. 5, B

Fig. 5. Internalization of gp78 in B16-F1 melanoma cells following PT treatment and removal. Indirect immunofluorescent labeling of cell surface in the presence of PT for 18 h (B) and 1 h after washout of PT (C). Down-regulation was shown quantitatively in FACS analysis (A). Surface expression of gp78 was analyzed by FACS (A) and indirect surface immunofluorescence (B and C) following treatment of cells with PT for 18 h (A and B) and 1 h after washout of PT (A and C). Gp78 internalization following PT washout was demonstrated both quantitatively (A) and qualitatively (C). × 1800.
and C). PT treatment of cells incubated with anti-gp78 mAb may serve to prime the cells, preventing receptor activation and internalization, so that only following removal of the PT can the motility stimulus of the anti-gp78 mAb be transmitted to the cell.

### DISCUSSION

Local invasion of the host stroma by cells from the primary tumor is the first step in the metastatic process. Mechanisms such as generation of mechanical pressure, the release of protease, and an increased motility of tumor cells are believed to play an important role in this process (24, 25). After dispersal through vessels, tumor cells arrest in the capillary bed of distant organs and extravasate into the organ parenchyma. The attainment of an extravascular position is believed to involve an active locomotion similar to that responsible for the initial invasion into the blood vessels (1, 2, 24, 25). Therefore, locomotion of tumor cells and its regulation is thought to be crucial for metastasis. This was supported by results obtained in a previous study on the motility of variants derived from K-1735 melanoma, in which a direct correlation was found between locomotion in vitro and lung colonization in vivo (20). In a different study a Fourier analysis revealed that cell motility, especially pseudopodal extension, correlates with metastatic potential in several cell lines of Dunning R3327 rat prostate adenocarcinoma with different metastatic capacities (26). It should also be pointed out that several tumor cells were reported to grow in an intravascular location at the site of arrest and extravasate by breaking the enclosing vessel (28, 29).

The mechanisms by which tumor cells regulate locomotor activity during metastasis are not clear, and it has been suggested that during locomotion cells either use internal programs or respond to signals from the environment. Several factors including growth factors have been implicated as signal modulating agents for cell motility (8). Human synovial cells produce a $M_0$, 13,000 stimulant for polymorphonuclear locomotion (27). Attnip et al. (30) have shown that both chemotactic and chemokinetic movements of MTLn3 rat mammary adenocarcinoma cells are stimulated by the MTLn3 derived cytokine ($M_0$, 53,000) and that there have been such properties of cytokines may represent a phenotypic difference between high- and low-metastatic variants (30). Previous studies have indicated that extracellular proteins, i.e., collagen, laminin, and fibronectin, and their degradative products are chemotactic for some tumor cells (31, 32).

We have recently demonstrated that anti-gp78 mAb stimulates in vitro cell migration (10, 11). gp78 was identified as a possible receptor for motility factor because the binding of the anti-gp78 mAb to the cell surface mimics the binding of a motility factor stimulating cell locomotion (10) and shows a direct binding competition between AMF and anti-gp78 to gp78. In the present study, to determine the relationship between motility factor receptor activation and metastatic capacity, we have used the anti-gp78 mAb as a motility stimulus and examined the motility response in vitro and metastatic response in vivo in cell pairs exhibiting low- or high-metastatic potential. In both the B16 and K-1735 melanoma systems the high metastatic cell variants exhibited a higher basal locomotor activity in the absence of an external stimulus. Preincubation of cells with the anti-gp78 resulted in a significant increase in the lung colonizing capacity of high-metastatic variants, whereas there was no significant response to the mAb in the low-metastatic variants. The in vitro motility assay of area clearing does not measure directional movement. While we detected an increased in vitro motility of the low-metastatic variants, this may reflect the multifaceted extension of many leading edges and random movement and not the directional extension of a single leading edge. The finding that low-metastatic variants, which seem to show multidirectional leading edge extension, did not respond to the anti-gp78 mAb in lung colonizing capacity indicates that metastasis requires a directional motility stimulation.

This hypothesis is supported by studies of the surface expression of gp78 on the low- and high-metastatic variants. FACS analysis showed that the high-metastatic variants express less gp78 on their surface than the low-metastatic variants in both melanoma systems. Immunofluorescent examination revealed that there is a distinct pattern of distribution of gp78 on the cell surface; low-metastatic variants had multiple domains that were stained diffusely, while most of the high-metastatic cells exhibited a single polarized densely labeled area (Fig. 3). Such a distribution of gp78 on the cell surface corresponds closely to the suggestion by Bretscher (33) and Bergmann et al. (34) that on a stationary cell the internalized membrane is returned at random to the cell surface, whereas on a motile cell it is transported through the cell to the leading edge. The diffuse surface labeling of low-metastatic variants may reflect the extension of multiple leading edges with a resultant decrease in net forward movement. Indeed, we have shown that gp78 may be translocated from the internalized surface (endocytosis) to the leading edge (exocytosis) through lysosomal compartments. Guirguis et al. (35) reported that the formation of pseudopodal protrusions is stimulated by an AMF. Partin et al. (26) showed that pseudopodal extension correlates with metastatic potential in several cell lines of Dunning R3327 rat model of prostatic cancer with different metastatic propensities. Internalized membrane with gp78 may serve as a main source of membrane for extension of the leading edge, enabling the more dynamic phenomena of cell motility such as membrane ruffling, pseudopodial extension, and translation to occur actively at the leading edge in high-metastatic variants.

PT irreversibly blocks activity of guanine nucleotide-binding proteins (G proteins) essential for signal transducing (36, 37) and the action on second messengers (38). A PT sensitive pathway is involved in motility stimulated by both AMF (38) and anti-gp78 mAb (10). We have shown here that PT treatment combined with the 3F3A mAb enhances pulmonary metastasis formation following i.v. inoculation. The enhancement is not due to the direct effect of PT, since PT alone has no effect on the production of pulmonary metastatic nodules; furthermore, PT at a higher concentration (1 ¡ìg/ml) was reported to inhibit liver metastasis (39). It is more likely that PT exerts an enhancing action on the external motility stimulators which make cells motile and metastatic. The receptor-mediated motile response is independent of adenylate cyclase and requires direct participation of a G protein (40). In the present study, we show that internalization of gp78 occurs when B16-F1 cells are placed in PT free media for 1 h after pretreatment with PT for 18 h. The increase in pulmonary metastasis following treatment with both anti-gp78 mAb and PT (Table 2) could result from a similar process. In the presence of PT the 3F3A mAb binds to surface-exposed gp78 but is not internalized to the extent that the motility induction process of the treated cells is
expression of membrane surface mediator(s) of cell motility like gp78 would therefore play a role in the development of stimulating factor(s) during the metastatic cascade, and in turn produce increased pulmonary metastases. Resulting in the timely stimulation of cell motility which would express newly synthesized functional G protein at the cell membrane and its relation to decreases in the expression of proteolytic enzymes and low-metastatic murine neoplastic cells. Cancer Res., 44: 811-824, 1984.


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