Regulation of Kinesin Expression and Type IV Collagenase Secretion in Invasive Human Prostate PC-3 Tumor Sublines

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ABSTRACT

PC-3 human prostatic tumor sublines have been previously isolated which exhibit striking differences in their invasive and metastatic phenotypes. This work has been extended here to measure and compare the levels of kinesin, a microtubule-dependent translocator molecule, in the PC-3 sublines. Western blots, slot blots, radiolabeling, and immunoprecipitation analysis showed that kinesin was expressed in the highly invasive and metastatic sublines at levels which were elevated above the base-line levels observed in the parent PC-3 cells. In comparison, kinesin was not expressed in detectable amounts in the noninvasive cell lines. The conditioned medium of the metastatic PC-3 sublines contained a heat- and trypsin-sensitive protein which exhibited a dosage-dependent capacity to stimulate increased kinesin expression, type IV collagenase secretion, and invasion of Matrigel by the metastatic sublines. The noninvasive sublines failed to secrete a similar stimulatory factor(s) or respond to the conditioned medium of metastatic sublines. Various growth factors and cytokines tested (platelet-derived growth factor, epidermal growth factor, insulin-like growth factor, formylmethylionineleciphenylalanine) had no significant effect on either kinesin expression or protease secretion and invasion. Pertussis toxin blocked the stimulatory effects of the conditioned medium, but other agents known to interfere with adenylate cyclase pathways (i.e., cholera toxin, forskolin, 8-bromo-cyclic AMP) failed to block stimulation. The data show for the first time that kinesin, protease secretion, and the resulting invasion process may be regulated in a coordinated manner by an autocrine factor(s) which activates G-protein-dependent processes.

INTRODUCTION

The molecular processes involved in tumor cell invasion and metastases are partially understood largely as a result of in vitro studies. Chemotactic assays using modified Boyden chambers have indicated that metastatic tumor cells secrete proteases and solubilize the basement membrane macromolecules during the penetration of basement membrane material (1-11). Laminin (9-11), fibronectin (11, 12), and type IV collagen (5, 13) have each been shown to stimulate type IV collagenase synthesis and secretion, suggesting that cellular interactions with specific macromolecules might directly influence invasion of the basement membrane (5, 9-13). Liotta et al. (14) further discovered that metastatic tumor cells secrete and respond to a pertussis toxin-sensitive autocrine motility factor with a molecular weight of about 55,000 (15, 16), indicating a guanine nucleotide-binding protein might somehow mediate motility-dependent events during invasion. We found that the conditioned medium of either A2058 cells or DU 145 human prostate cells stimulated both type IV collagenase secretion and invasion of Matrigel by DU 145 prostatic tumor cells (5). The studies suggested that malignant cells might uniquely express autocrine factors which regulate protease secretion, invasion, and related metastatic processes.

Unfortunately, the above studies were carried out by utilizing cultures which contained a heterogeneous mixture of phenotypically distinct cells. We hypothesized that a better understanding of the requirements for invasion might be achieved if the subpopulations participating in these processes were isolated and characterized. Consequently, in the past 5 years we have subcloned noninvasive, invasive, and metastatic PC-3 human prostatic tumor cells based on a selective ability (a) to migrate across Matrigel barriers in Boyden chambers, and (b) to metastasize to select target organs in severe combined immune deficient or scid mice (17). Following repeated selection steps utilizing the Boyden chambers, we have established totally noninvasive and highly invasive (>9%) PC-3 sublines. The PC-3 sublines did not appear to differ in their ability to attach to plastic, mouse type IV collagen, or Matrigel. However, Boyden chamber chemotactic assays revealed that the noninvasive, nonmetastatic sublines were unable to embed or migrate across Matrigel barriers, whereas the invasive metastatic sublines readily embedded in the Matrigel and exhibited a high invasive activity. Likewise, following tail vein injections in scid mice, the parent cells and the noninvasive sublines failed to metastasize, while the invasive sublines readily metastasized to a variety of tissues (i.e., lungs, liver, brain, vertebrae, rib, cheek, knee). We have subsequently established four highly invasive “organ targeting” sublines which preferentially metastasize with about 80% efficiency to four different tissues, including the lumbar vertebrae (PC-3 ML), the rib (PC-3 MR), the cheek (PC-3 MC), and the knee (PC-3 MK).

An important finding from our initial studies of the PC-3 sublines was that a direct correlation existed between the degree of demonstrated invasive activity in vitro and the metastatic potential in scid mice (17). This result was consistent with earlier reports showing that nonmetastatic and metastatic cell lines typically differ in their relative invasive activity (1-3, 5, 15, 16, 18).

We anticipated that the noninvasive (i.e., nonmetastatic) and metastatic PC-3 sublines might also differ substantially in the extent of protease secretion demonstrated (i.e., collagenase secretion) or in other related processes which might be up-regulated by tumor cells during invasion of the basement membrane. One possible enzyme of interest was kinesin, a microtubule-based ATPase which powers macromolecule and organelle transport (i.e., vesicles) to the plasma membrane surface (19, 20). In two earlier studies, we reported that kinesin was abundant in DU 145 cells (21) and that the transport of collagenase required the maintenance of intact microtubule networks (5). Unfortunately, given the heterogeneous nature of the cell cultures we were unable to demonstrate if kinesin synthesis was important for collagenase secretion. We have postulated, however, that kinesin and collagenase synthesis might be coregulated by autocrine factors released by the metastatic cells, since the DU 145 cells secreted collagenase in response to factors present in the conditioned medium.

In this paper, we have examined the simultaneous effects of...

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conditioned medium on three distinct properties of the PC-3 sublines, including: (a) the levels of kinesin expression; (b) type IV collagenase secretion; and (c) the invasive activity of PC-3 sublines. We found that up-regulation of kinesin synthesis and type IV collagenase secretion strongly correlated with the highly invasive behavior of the tumor sublines immediately following stimulation with conditioned medium from the metastatic sublines. Pertussis toxin simultaneously blocked the stimulatory effects of the conditioned medium on each of these processes, implicating a receptor-controlled G-protein-dependent second messenger pathway.

MATERIALS AND METHODS

Cell Cultures. Human prostatic DU 145 cells, LNCaP, and PC-3 (22) were obtained from the American Type Culture Collection and put in culture by using methods described previously (5). Using methods of Chua and Chua (23), MEF were prepared from scid mouse embryos (17) at day 10 and passed 5 times to grow sufficient cells for the experiments. The PC-3 sublines were isolated, cultured, and passed according to methods previously described (5). For the experiments described here, we have plated the sublines in dishes coated with 1% mouse type IV collagen (Sigma Chemical Co., St. Louis, MO) since all the sublines readily attached to it. The PC-3 cells were maintained in DMEM supplemented with 10% fetal calf serum and transferred to serum-free DMEM plus 1% bovine serum albumin for all the experiments. All the cell lines were stored in liquid nitrogen at passage 3, and when needed, were grown up and used at passage 5 following isolation in order to reduce the heterogeneity in cellular phenotype accompanying prolonged passage in culture. The cell lines tested include the PC-3 parent; the N.L., and I. sublines successively selected 1, 2, and 3 times by using modified Boyden chambers (6.5-mm Transwell chambers with 8-μm pore filters; Costar Corp., Cambridge, MA); and the 6-times selected PC-3 cell sublines which preferentially metastasized to the lumbar vertebrae (PC-3 ML); the rib cartilage (PC-3 BA); and the trabecular bone of the right front femur (PC-3 MK). Unless stated otherwise, the same batch of each subline (i.e., the PC-3 ML cells) at passage 5 were used in all the experiments, and for preparation of the conditioned medium.

Conditioned Medium Preparation. Falcon dishes were coated with 1% mouse type IV collagen according to published methods (5). The cells at passage 5 were plated at about 80% confluence in ten 150-mm dishes. After 6 h, the cells present in the bottom and top chambers of each study were harvested with 0.08 M sodium citrate, pH 7.2, and pooled, washed 3 times with PIPES buffer (containing 0.05 M PIPES in PBS, pH 7.2) using centrifugation at 3,000 × g for 10 min, the cells were homogenized at 4°C in 2 ml PIPES buffer, the whole cell extract was centrifuged 200,000 × g for 1 h, and the supernatant was removed. Radiolabeled kinesin in the supernatant and type IV collagenase in the medium were immunoprecipitated for scintillation counting (see below).

The cell invasion studies were carried out according to methods of Wang and Stearns (5). In brief, S radiolabeled cells were removed from the Petri dishes with 0.08 M sodium citrate, pH 8.0, washed twice, and plated at 2 × 10^5 cells/ml on Matrigel-coated 8-μm pore membrane filters (Nuncopore, Inc., Washington, DC) in 48-well Boyden chambers. Triplicate wells were used for each cell line per experiment. Pertussis toxin or other agents tested (i.e., cycloheximide) were added to both compartments of the Boyden chambers. To initiate invasion, aliquots of different conditioned medium (10 mg/ml final concentration) were added to the medium in the bottom half of the bicameral chambers. For the drug studies fresh drug was added to this medium. After 6 h, the cells present in the bottom and top chambers of each well were harvested with 0.1 ml of 0.8 M sodium citrate, pH 8.0, and solubilized in 20 ml Aquafuor for scintillation counting. Invasion was calculated as a percentage of cells (i.e., scintillation counts) in the bottom chamber versus that seeded in the top chamber.

Isolation of Kinesin and Immunoprecipitation Assays. About 2 × 10^6 cells were plated at about 80% confluence in ten 150-mm dishes. After 4 h, the attached cells were washed with methionine-free medium and the cells were labeled overnight for 18 h with 15 μCi/ml (Table 1) or 20 μCi/ml (Tables 2–6; Figs. 6–8) translated[S]methionine-cysteine (NEN) in methionine-free medium. The labeled cells were washed twice with serum-free DMEM and 10 ml of serum-free medium were added to each 150-mm dish for the appropriate time. In experimental studies with conditioned medium, about 1-ml aliquots of CM from the PC-3 sublines were added to the 10 ml serum-free medium at a final concentration indicated. After 6 h, the medium was removed from the cells for immunoprecipitation assays of type IV collagenase and the cells were collected and homogenized for measurements of kinesin and tubulin.

To isolate the “kinesin microtubules” (Figs. 1 and 2), microtubule assembly was induced by incubation of the high-speed supernatants at 37°C for 1 h in the presence of 1 μM taxol (D. Sussen, National Cancer Institute-NIH). Kinesin binding to the microtubules was then induced with 10 mM 5'-adenylylimidodiphosphate (Sigma), a nonhydrolyzable ATP analogue, the samples were incubated at 37°C for 1 h, and the kinesin microtubules were removed by centrifugation at 100,000 × g for 1 h through a 10% sucrose cushion (19). The pellet was resolubilized in 0.2 ml SDS sample buffer for SDS-PAGE and Western blotting.

Kinesin was immunoprecipitated from the high-speed supernatants of cells with affinity-purified kinesin monoclonal antibodies (21). Type IV collagenase was immunoprecipitated from the medium of the same cells utilizing affinity-purified immunospecific polyclonal antibodies, which recognized a M_4 72,000 type IV collagenase (5). Following 2 h at 37°C the antibody-antigen conjugates were removed from the crude supernatants or medium by centrifugation at 100,000 × g through a 0.5-m1 volume, 10% sucrose cushion for 1 h at 37°C. The process was repeated after resuspension of the pellet in 1 ml PBS and the final pellets were resuspended in 200 μl PBS.

In some studies the antibody-antigen complexes were linked to protein A-Sepharose beads (Sigma) and the complex was centrifuged (100,000 × g for 1 h at 22°C) through a 0.5-m1 volume. 10% sucrose cushion. The pellet was washed twice with PBS, pH 7.2, by centrifugation at 100,000 × g through a 10% sucrose cushion and the final pellet was resuspended in 200 μl PIPES buffer for scintillation counting. Aliquots were added to 20 ml Aqufluor fluid (Fisher Scientific, Inc., Philadelphia, PA), and samples were counted by using a Beckman SL-9000 scintillation counter. The counts were averaged from triplicate samples.
measurements per experiment of 25-μl aliquots. Each experiments was repeated at least 3 times and these data were averaged ± SD.

Immunoassays. Slot blots were carried out according to the immunoperoxidase reaction procedure (24), utilizing kinesin (21) and β-tubulin antibodies (Amersham, Arlington Heights, IL). Secondary antibodies (Cappel, Inc.) were used at 1:200 dilutions and development was for 10 min. All blots were done with identical reagents under similar conditions.

SDS-PAGE was by the methods of Laemmli (25). Western blots were by methods of Towbin et al. (26) and protein measurements were by procedures of Lowry et al. (27). Fluorography with an intensifying screen was carried out according to the methods described by Laskey (28) by using 35S-labeled protein. A Beckman Coulter counter was used to determine the number of cells per ml.

Reagents. Pertussis toxin and cholera toxin were from List Biological Laboratories, Inc. (Campbell, CA). Cycloheximide, epidermal growth factor, platelet-derived growth factor, insulin-like growth factor, formylmethionineleucinephenylalanine, forskolin, 8-Br cAMP, trypsin, and soybean trypsin inhibitor were from Sigma Chemical Co. (St. Louis, MO). The 2′,5′-dideoxyadenosine was from Pharmacia P-L Biochemicals (Piscataway, NJ).

RESULTS

Western blotting, slot blots, and immunoprecipitation assays were used to determine if the different PC-3 sublines expressed detectable differences in the M, 116,000 protein. Western blots showed that kinesin monoclonal antibodies (21) specifically recognized a M, 116,000 kinesin protein which was isolated in association with microtubules from crude whole cell extracts of the PC-3 parent cells (Fig. 1). Western blots further revealed that substantial differences existed in the amounts of the M, 116,000 kinesin present by the different PC-3 sublines at passage 5 in culture (Fig. 2). Two different 3 x N.I. sublines tested failed to express any detectable kinesin (Fig. 2, Lanes 1 and 2). In comparison, the 1 x N.I. cells expressed some kinesin (Fig. 2, Lane 3) and the 4 different metastatic sublines expressed easily detected amounts of kinesin (Fig. 2, Lanes 4 to 7). A faint band at M, 96,000 was occasionally stained in the latter preparations but it was also labeled in samples which were preabsorbed with kinesin antibodies (Fig. 2, Lane 8), indicating nonspecific binding might account for the labeling.

Slot blots (Fig. 3) confirmed the Western blot results. The blots showed that there was virtually no detectable kinesin present in the 3 x N.I. sublines at passage 5 (Fig. 3, A1). In comparison, the 3 x I. sublines at passage 5 (Fig. 3, A3) and the organ targeted PC-3 ML, PC-3 MR, PC-3 MK, PC-3 MC sublines at passage 5 (Fig. 3, C1–4) all expressed substantial amounts of kinesin at levels well above that expressed by the parent PC-3 cell line (Fig. 3, B1). Several heterogeneous cell lines, the parent PC-3 cells, DU 145 human prostatic cells, LNCaP human prostatic cells, and MEF cells all expressed some detectable kinesin (Fig. 3, B1–4), but always at levels below that the organ targeted PC-3 sublines. Fig. 3 further showed that differences in the levels of kinesin were most striking among the noninvasive and invasive PC-3 cultures at passage 5 (compare Fig. 3, A1 with Fig. 3, A3). This difference appeared to be subject to "phenotypic drift" with increased cell passage, however. By passage 10, both the 3 x N.I. and 3 x I. cells expressed kinesin at somewhat variable levels closer to that of the PC-3 parent (compare Fig. 3, A2 with Fig. 3, A4).

Quantitative immunoprecipitation methods were used to measure and compare the relative amount of kinesin present in whole cell extracts of [35S]methionine translated PC-3 sublines at passage 5 (Table 1). All the kinesin present in the crude high-speed supernatants was immunoprecipitated by using excess antibody. That is, more kinesin could not be removed from the kinesin-depleted supernatants with the addition of antibody. Scintillation counts of equivalent aliquots of the precipitates showed that the untreated 1 x I., 2 x I., 3 x I. sublines, and the PC-3 ML, PC-3 MR, PC-3 MK, and PC-3 MC sublines all expressed significantly more kinesin (6- to 30-fold more) than the PC-3 parent line (Table 1). In contrast, the 1 x N.I., 2 x N.I., and 3 x N.I. sublines failed to express kinesin in amounts significantly above background levels.

Stimulatory Effects of Conditioned Medium. Slot blot analysis showed that exposure to PC-3 ML conditioned medium (10 mg/ml for 6 h) increased the kinesin levels noticeably in the invasive (3 x I.) and the metastatic PC-3 ML sublines, but not in the 3 x N.I. cells (Fig. 4). Note that the slots in Fig. 4 were loaded with reduced amounts of protein (i.e., 2.5 μg/slot as compared to 10 μg/slot in Fig. 3). No kinesin was detected in the whole cell extracts of the 3 x N.I. cells (Fig. 4, A1) or the 3 x I. cells (Fig. 4, A2), and a modest signal was observed in the extracts of the PC-3 ML cells (Fig. 4, A3). Following stimulation of these same cells with the PC-3 ML conditioned medium, we observed that there was still no signal for the 3 x N.I. cells (Fig. 4, B1), a modest signal for 3 x I. cells (Fig. 4, B2), and a...
REGULATION OF KINESIN IN HUMAN PROSTATE TUMOR CELLS

Fig. 3. Slot blots with excess kinesin antibody (0.1 μg/ml) comparing the amounts of kinesin present in crude whole cell extracts from the PC-3 lines (10 μg protein loaded per well). A, (Band 1) 3 x N.I.; passage 5; (Band 2) 3 x N.I., passage 10; (Band 3) 3 x L.; passage 5; and (Band 4) 3 x L., passage 10. B, (Band 1) PC-3 parent; (Band 2) DU 145; (Band 3) LNCaP; and (Band 4) MEF. C, (Band 1) PC-3 ML; (Band 2) PC-3 MR; (Band 3) PC-3 MK; and (Band 4) PC-3 MC cells at passage 5.

Table 1 Immunoprecipitation measurements of the 32S-kinesin levels in whole cell extracts (cpm × 105)

<table>
<thead>
<tr>
<th>PC-3 subline</th>
<th>Untreated cells</th>
<th>CM</th>
<th>3 x N.I.</th>
<th>3 x I.</th>
<th>PC-3 ML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td>0.22 ± 0.11</td>
<td>0.28 ± 0.15</td>
<td>0.56 ± 0.12</td>
<td>0.61 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>1 x I.</td>
<td>1.30 ± 0.23</td>
<td>1.35 ± 0.12</td>
<td>5.01 ± 0.15</td>
<td>5.11 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>2 x I.</td>
<td>2.50 ± 0.40</td>
<td>2.81 ± 0.14</td>
<td>18.69 ± 0.21</td>
<td>19.00 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>3 x I.</td>
<td>3.28 ± 0.21</td>
<td>3.00 ± 0.15</td>
<td>29.33 ± 0.13</td>
<td>39.40 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>ML</td>
<td>5.22 ± 0.10</td>
<td>4.98 ± 0.32</td>
<td>38.11 ± 0.19</td>
<td>46.28 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>4.10 ± 0.20</td>
<td>3.91 ± 0.21</td>
<td>34.15 ± 0.22</td>
<td>42.11 ± 0.40</td>
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</tr>
<tr>
<td>MK</td>
<td>3.00 ± 0.10</td>
<td>3.15 ± 0.15</td>
<td>39.00 ± 0.16</td>
<td>49.48 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>6.21 ± 0.15</td>
<td>4.02 ± 0.09</td>
<td>38.00 ± 0.22</td>
<td>49.46 ± 0.24</td>
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</tr>
<tr>
<td>1 x N.I.</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.02</td>
<td>0.20 ± 0.00</td>
<td>0.21 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>2 x N.I.</td>
<td>0.01 ± 0.02</td>
<td>0.00 ± 0.01</td>
<td>0.05 ± 0.03</td>
<td>0.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>3 x N.I.</td>
<td>0.01 ± 0.01</td>
<td>0.00 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.00 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

very strong signal for the PC-3 ML cells (Fig. 4, B3). Note that a signal was not detected for the 3 x N.I. sublines even when 20 and 40 μg of protein were loaded on the slot (data not shown).

In contrast to the marked differences in kinesin, absolutely no difference was observed in the intensity of β-tubulin labeling in samples taken from either the 3 stimulated cell lines (Fig. 4, C) or the unstimulated cells (not shown).

Utilizing quantitative immunoprecipitation techniques, we measured and compared the relative stimulatory effects of conditioned medium from the 3 x N.I., the 3 x I., and the PC-3 ML cells on kinesin expression in the different sublines (Table 1). The data consistently showed that the CM from the 3 x N.I. cells did not stimulate any significant shift in kinesin in any of the PC-3 sublines tested. In contrast, the CM of either the 3 x I. cells or the PC-3 ML cells both induced striking increases in the kinesin levels measured in the invasive and metastatic sublines. The overall basal levels and the magnitude of response were greater in the 4 metastatic sublines than in the noninvasive cells. The approximate rank order of response was PC-3 ML, PC-3 MC, PC-3 MK, PC-3 MR > 3 x I. > 2 x L. > 1 x I. > PC-3 parent. The noninvasive sublines uniformly failed to respond to the CM from either the 3 x I. or the PC-3 ML sublines.

In general, we found that the PC-3 ML CM has a greater stimulatory effect than the 3 x I. CM. Three different batches of CM from two different PC-3 ML sublines produced identical results. Likewise, CM from the PC-3 MK, PC-3 MR, and PC-3 MC sublines had an effect similar to that of the PC-3 ML CM (data not shown).

SDS-PAGE of the immunoprecipitates confirmed that the differences in scintillation counts reported in Table 1 reflected actual differences in amounts of kinesin protein removed from the whole cell extracts (Fig. 5). For example, at a high antibody titer (0.15 μg/ml) very little of the M, 116,000 protein was found in immunoprecipitates taken from the untreated PC-3 parent cells (Fig. 5, Lane 1). Fig. 5, Lanes 2 to 6, showed that with increased dilution of the antibody concentration over a range of 0.1 to 0.01 μg/ml, a prominent M, 116,000 protein was consistently removed from extracts of stimulated PC-3 ML cells. More importantly, there was a corresponding reduction in the amount of the M, 116,000 kinesin precipitated. The amounts of contaminating protein were also reduced, indicating some of the counts in Table 1 probably arise from the proteins nonspecifically binding the antibody-antigen complexes (i.e., Fig. 5, see Lane 1). The scintillation counts obtained for each of the samples were directly proportional to the antibody dilution tested (see Fig. 5 legend). These measurements were in close agreement with the results reported in Table 1 for the CM-activated PC-3 parent and PC-3 ML cells.

In some studies, we further used protein A-Sepharose beads and centrifugation to affinity purify IgG-kinesin complexes from the immunoprecipitates of the 3 x L.3 x N.I., and ML sublines exposed to PC-3 ML CM. The immunoprecipitation counts obtained (average from 3 experiments ± SD) were very low for the 3 x N.I. cells (0.0 ± 0.1 ± 10^4 cpm), but relatively high for the 3 x I. (18.0 ± 0.1 ± 10^4 cpm) and ML cells (45.0 ± 0.0 ± 10^4 cpm)

Fig. 4. Slot blots of crude whole cell extracts (2.5 μg protein/well) showing the relative effect of PC-3 ML conditioned medium on the extent of kinesin expression in the (Band 1) 3 x N.I.; (Band 2) 3 x I.; and (Band 3) PC-3 ML sublines. The panels show (A) prior to and (B and C) following exposure to 10 mg/ml PC-3 ML conditioned medium for 6 h. C shows that the β-tubulin levels were similar in the all three PC-3 sublines.

Fig. 5. SDS-PAGE (7.5% gel) of kinesin immunoprecipitated from the crude cell extract (10 μg total protein of the (Lane 1) untreated PC-3 parent; and (Lanes 2–6) PC-3 ML cells. The final antibody concentrations tested were (Lane 1) 0.15; (Lane 2) 0.10; (Lane 3) 0.075; (Lane 4) 0.05; (Lane 5) 0.025; and (Lane 6) 0.01 μg/ml. The cells (Lanes 2–6) were exposed to 10 mg/ml PC-3 ML conditioned medium for 6 h prior to harvesting. Scintillation counts of the samples in Fig. 5 gave values of (Lane 1) 0.59 ± 0.10; (Lane 2) 15.91 ± 0.41; (Lane 3) 15.50 ± 0.22; (Lane 4) 10.11 ± 0.20; (Lane 5) 5.16 ± 0.10; (Lane 6) 2.91 ± 0.20 × 10^4 cpm. Ordinate, M, 55,000 is the IgG antibody.

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we interpret these data to mean that type IV collagenase antibody specifically recognized and removed type IV collagenase from the medium of the PC-3 sublines.

Coordinate Studies of Kinesin, Collagenase IV, and Invasion. The studies were extended to determine if a correlation existed with respect to the degree of kinesin expression, type IV collagenase secretion, and the invasive activity of the different PC-3 sublines (Fig. 7). Fig. 7 demonstrated the relative stimulatory effect of the conditioned medium from 3 x N.I. cells, the PC-3 parent, and the PC-3 ML (tested at 10 mg/ml for 6 h). The immunoprecipitation data in Fig. 7A showed that the kinesin levels remained at relatively low levels (basal levels) in all seven sublines exposed to either 3 x N.I. CM or the PC-3 parent CM. In comparison, the PC-3 ML CM stimulated almost a 10-fold increase in the level of kinesin over the normal basal levels observed in the invasive and metastatic sublines. The PC-3 ML CM did not induce a significant increase in kinesin in the 3 x N.I. subline or the parent cells. The data agreed with results reported in Tables 1 and 2. The counts recorded for the 3 x N.I. CM were similar to those reported in Table 2 and further affirmed that the immunoprecipitation assays reflected true differences in the actual kinesin levels expressed.

Using the same cells and conditioned medium, we extended the studies in Table 1 and examined the dosage-dependent effects of PC-3 ML CM on the 3 x N.I. subline and the PC-3 ML subline (Table 2). The data showed that kinesin levels in 3 x N.I. cells did not increase significantly above background levels (i.e., 0.01 x 10^6 cpm) in response to a range of concentrations of PC-3 ML conditioned medium tested (0.5 to 30 mg/ml tested). Neither did the β-tubulin levels fluctuate noticeably. In comparison, low concentrations (0.5 and 1 mg/ml) of PC-3 ML CM had no detectable effect on kinesin levels in the PC-3 ML cells. However, 2.0 and 4.0 mg/ml PC-3 ML CM stimulated a 3.5- and 7-fold increase, respectively, in the kinesin levels in PC-3 ML cells. A plateauing effect (i.e., at ~ 6 x 10^6 cpm) was observed in the presence of 10 to 30 mg/ml PC-3 ML CM, where a 10- to 12-fold increase over basal levels was recorded. There was absolutely no change in the levels of β-tubulin protein recorded, in the presence of 0.5 to 30 mg/ml PC-3 ML CM or 3 x N.I. CM, respectively.

Characterization of Type IV Collagenase Antibodies. Polyclonal antibodies were raised against a type IV collagenase isolated from the medium of DU 145 human prostatic tumor cells (5). Fluorography showed that the antibodies immunoprecipitated a M, 72,000 protein which was secreted in the medium of PC-3 ML, PC-3 MC, PC-3 MK, and PC-3 MR cells (Fig. 6A). The immunoprecipitate consisted primarily of the M, 72,000 protein plus a variable number of minor bands. By comparison, immunoprecipitates from the parent PC-3 cells contained reduced amounts of the M, 72,000 protein and virtually no detectable protein at M, 72,000 was removed from the medium of 3 x N.I. cells. Western blots confirmed that the type IV collagenase antibodies specifically recognized a M, 72,000 protein present in the medium and immunoprecipitate of the PC-3 ML cells (Fig. 6A, Lanes 7 and 8). In Fig. 6B, fluorography further showed that when increased amounts of collagenase antibody (0.025 to 0.25 μg/ml) were added to fixed amounts of PC-3 ML medium (10 mg/ml) the resulting immunoprecipitates contained proportionately increased amounts of the M, 72,000 proteinase. Some minor bands contaminated these precipitates but the amounts did not increase significantly in proportion to the amount of antibody used. Scintillation counts from aliquots of the preparations gave values of (a) 1 x 10^5, (b) 6 x 10^6, (c) 2 x 10^7 cpm, respectively. Taken together,

### Table 2 Dosage-dependent effect of PC-3 ML CM on kinesin levels in the 3 x N.I. cells and the PC-3 ML subline

<table>
<thead>
<tr>
<th>PC-3 ML CM (mg/ml)</th>
<th>Kinesin level (cpm × 10^6)</th>
<th>Tubulin level (cpm × 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>0.5</td>
<td>0.0 ± 0.0</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>5.0 ± 0.1</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>2.0</td>
<td>17.0 ± 0.2</td>
<td>7.2 ± 0.5</td>
</tr>
<tr>
<td>4.0</td>
<td>30.0 ± 0.6</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>5.0</td>
<td>48.0 ± 1.5</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>10.0</td>
<td>56.0 ± 1.4</td>
<td>7.2 ± 0.5</td>
</tr>
<tr>
<td>15.0</td>
<td>61.0 ± 1.3</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>20.0</td>
<td>59.0 ± 1.7</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>25.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>30.0</td>
<td>63.0 ± 1.8</td>
<td>7.1 ± 0.1</td>
</tr>
</tbody>
</table>

* ND, not done.

Average ± SD.

### Notes
- **Regulation of Kinesin in Human Prostate Tumor Cells**

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Fig. 7. Immunoprecipitation measurements (A) of kinesin in whole cell extracts and (B) type IV collagenase from the medium of the same cell lines. C shows measurements of the percentage of invasion. The cell lines tested include (Column 1) parent PC-3; (Column 2) 3 x N.I.; (Column 3) 3 x 1.1; (Column 4) PC-3 ML; (Column 5) PC-3 MR; (Column 6) PC-3 KM; and (Column 7) PC-3 MC. The cells were independently exposed to CM (10 mg/ml for 6 h) from 3 different cell lines, including the 3 x N.I. (■), PC-3 parent (□), and PC-3 ML (□). Both antibodies were used at a final concentration of 0.1 µg/ml. The data were averaged from 3 experiments ± 1 SD.

Table 3 Kinesin levels (cpm x 10^4)

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>3 x N.I.</th>
<th>PC-3 ML</th>
<th>NIH-3T3</th>
<th>MEFs</th>
<th>DU 145</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.05</td>
<td>6.0</td>
<td>1.0</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>3 x N.I. CM</td>
<td>0.06</td>
<td>5.0</td>
<td>1.1</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>PC-3 ML CM</td>
<td>0.04</td>
<td>47.0</td>
<td>0.9</td>
<td>1.6</td>
<td>22.2</td>
</tr>
<tr>
<td>NIH-3T3 CM</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MEFs CM</td>
<td>0.05</td>
<td>5.5</td>
<td>1.2</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>DU 145 CM</td>
<td>0.04</td>
<td>4.0</td>
<td>1.1</td>
<td>1.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 4 Collagenase levels (cpm x 10^5)

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>3 x N.I.</th>
<th>PC-3 ML</th>
<th>NIH-3T3</th>
<th>MEFs</th>
<th>DU 145</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.8</td>
<td>1.1</td>
<td>0.1</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>3 x N.I. CM</td>
<td>0.7</td>
<td>1.2</td>
<td>0.1</td>
<td>0.3</td>
<td>1.1</td>
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<tr>
<td>PC-3 ML CM</td>
<td>0.9</td>
<td>86.0</td>
<td>0.4</td>
<td>0.5</td>
<td>10.0</td>
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<tr>
<td>NIH-3T3 CM</td>
<td>1.0</td>
<td>0.9</td>
<td>0.2</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>MEFs CM</td>
<td>0.6</td>
<td>1.0</td>
<td>0.3</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>DU 145 CM</td>
<td>1.0</td>
<td>2.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 5 Percentage of invasion (%)

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>3 x N.I.</th>
<th>PC-3 ML</th>
<th>NIH-3T3</th>
<th>MEFs</th>
<th>DU 145</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3 x N.I. CM</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PC-3 ML CM</td>
<td>0.1</td>
<td>6.8</td>
<td>0.3</td>
<td>0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>NIH-3T3 CM</td>
<td>0.2</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>MEFs CM</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DU 145 CM</td>
<td>0.0</td>
<td>1.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

N.I. cells (i.e., cell lines 2) were at background levels of 2 x 10^2, 3 x 10^2, and 6 x 10^2 cpm in the presence of CM from the 3 x N.I., PC-3 parent, and PC-3 ML sublines, respectively.

In Fig. 7B, the medium from the above experiments was collected for immunoprecipitation measurements of the 35S-radiolabeled type IV collagenase released in the medium. The data revealed that the collagenase levels were not boosted above basal levels in any of the cell lines in response to the 3 x N.I. CM. Likewise, there was only a small response to the parent PC-3 CM. However, depending on the subline tested, the PC-3 ML CM stimulated 30- to 100-fold increases over basal levels of type IV collagenase secretion by the invasive and metastatic sublines (Fig. 7B). The parent cells showed only a modest 10-fold increase in type IV collagenase, and the 3 x N.I. cells secreted little or no type IV collagenase (i.e., 2 x 10^4 cpm) in the presence of PC-3 ML CM.

In Fig. 7C, the invasive activity of the same batch of cells was assessed, based on the percentage of 35S-radiolabeled cells which migrated across a Matrigel barrier in response to the different CM. The 3 x N.I. cells showed zero detectable invasive activity in response to CM from either the 3 x N.I. cells, the parent cells, or the PC-3 ML CM subline. The counts were near background levels and averaged 300, 400, and 600 cpm in response to the three different CM tested. Microscopic studies of the filters confirmed that few or no cells migrated across the barrier and that the cells appeared to stay on the Matrigel surface.

A small percentage of the parent PC-3 cells were invasive in response to CM from either the 3 x N.I. (0.1%), the parent PC-3 (0.2%), and the PC-3 ML subline (0.5%). Likewise, the 3 x I. cells and the 4 metastatic sublines tested did not exhibit a significantly enhanced response to the 3 x N.I. CM (0.6 to 1.0% invasion) and the parent PC-3 CM (0.5 to 1.2% invasion). We believe that an autocrine factor(s) produced by these latter sublines accounts for the low level of invasion observed.

The PC-3 ML CM had a strong stimulatory effect on the degree of tumor cell invasion exhibited by both the 3 x I. cells and the 4 metastatic sublines tested. The PC-3 ML CM (10 mg/ml) stimulated 6 to 7% of the total cells plated to migrate across the Matrigel barrier after a 6-h incubation. Microscopy confirmed that following attachment these cells did not remain on the Matrigel surface, but rapidly embedded themselves in the matrix.

In the above experiments, it was possible that either the dosage of the 3 x N.I. CM was insufficient or that the CM contained an inhibitory factor. We tested both possibilities, utilizing the experimental conditions described in Fig. 7. We found that the CM of 3 x N.I. cells at 30 mg/ml had no measurable stimulatory effect on any of the three properties measured. Basal levels were recorded for kinesin (1 x 10^6 cpm), collagenase IV secretion (2 x 10^5 cpm), and the extent of invasion (0.3%) demonstrated by the PC-3 ML subline. Also, the addition of aliquots of the 3 x N.I. CM (10 mg/ml final concentration) to the PC-3 ML CM (at 10 mg/ml) failed to inhibit the stimulatory activity of the PC-3 ML CM. Following a 6-h exposure to the mixture, immunoprecipitation revealed relatively high levels of kinesin (46 x 10^4 cpm), type IV collagenase (70 x 10^5 cpm), and an enhanced degree of invasion by PC-3 ML cells (7%). This indicated that inhibitory factors were probably not secreted by the 3 x N.I. cells.

Control Studies. In control studies, we have tested the influence of CM (10 mg/ml) from 3 other cell lines, including NIH-3T3 cells, fibroblasts (MEFs), and DU 145 cells (Tables 3–5). Immunoprecipitation analysis showed that the CM of these 3
cell lines failed to stimulate significant increases in the levels of kinesin (Table 3) or type IV collagenase (Table 4) in the 3 x N.I. cells or the PC-3 ML cells. Likewise, the CM from NIH-3T3, MEFS, and DU 145 cells (or CM from the 3 x N.I. and PC-3 ML sublines), did not effectively enhance the levels of kinesin (Table 3) or type IV collagenase (Table 4) produced by NIH-3T3 cells or the MEFS. Like the parent PC-3 cells, the DU 145 cells exhibited a reduced response to the PC-3 ML CM and DU 145 CM. Similarly, the DU 145 CM had only a partial stimulatory effect on the parent PC-3 and PC-3 ML sublines.

In agreement with the above results, Boyden chamber invasion assays revealed that the 3 x N.I., NIH-3T3, and MEFS did not exhibit any significant invasive activity in the presence of CM from any of the cell lines tested (Fig. 5). Some invasive activity (<=1%) was exhibited by DU 145 cells in response to the DU 145 CM or PC-3 ML CM, and the DU 145 CM stimulated some invasion by the PC-3 ML cell line (<1%).

In the presence of 10 μM cycloheximide, a protein synthesis inhibitor, the PC-3 ML CM (10 mg/ml for 6 h) failed to induce any marked increase in kinesin or type IV collagenase secretion. Basal levels of 1.0 x 10^6 cpm and 0.5 x 10^6 cpm, respectively, were recorded for these two enzymes in the PC-3 ML subline. The invasive activity of the PC-3 ML subline was 0% in the presence of drug.

Other studies showed that trypsinization (0.1% for 30 min) or heat denaturation (100°C for 10 min) completely destroyed the stimulatory activity of the PC-3 ML CM. In these experiments, the kinesin and type IV collagenase levels in PC-3 ML cells remained at basal levels of about 1.1 x 10^4 cpm and 0.5 x 10^4 cpm, respectively. Likewise, trypsinization of the cells (0.1% for 30 min) prior to the experiment eliminated their ability to respond to PC-3 ML CM (10 mg/ml), and basal levels of kinesin (0.9 x 10^4 cpm) and type IV collagenase (0.6 x 10^4 cpm) were recorded. Excess soybean trypsin inhibitor (1%) was added to inhibit the protease after 10 min.

Inhibition Studies. Normally, the levels of kinesin, type IV collagenase, and invasion were increased by at least an order of magnitude in response to PC-3 ML CM (Tables 2–5; Fig. 7). Control studies showed that absolutely no increased kinesin expression was observed if 1% bovine serum albumin, 10% fetal calf serum (O); 10 μg/ml epidermal growth factor (1); 10 μg/ml platelet-derived growth factor (2); or 10 ng/ml insulin-like growth factor (3); 100 ng/ml 2',5'-dideoxyadenosine (4) for 6 h. In some studies, the PC-3 ML CM were exposed only to 1% bovine serum albumin (5); 10% fetal calf serum (O); 10 μg/ml epidermal growth factor (1); 10 μg/ml platelet-derived growth factor (2); or 10 ng/ml insulin-like growth factor (3); 100 ng/ml formylmethionineleucinephenylalanine (4) for 6 h. The data were averaged from 5 experiments; bars, SD.

**DISCUSSION**

Kinesin serves as a mechanochemical force transducing ATPase (19, 20) which can power the orthograde translocation of organelles toward the cell surface (19). Since kinesin has been found in all tissues and cells examined, including all tumor cell lines tested (19–21), the enzyme may be universally important for vesicle transport and secretion in eukaryotic cell systems. How kinesin is regulated in terms of its synthesis, activation, and recycling are poorly understood (19, 20).

We have postulated that enhanced kinesin levels may be a prerequisite for accelerated protease secretion, which in turn is a key requirement for cell invasion of the basement membrane and ultimately metastasis. We were able to test this hypothesis by utilizing select PC-3 sublines which were isolated and subcloned on the basis of a differential capacity to migrate across a Matrigel barrier and to metastasize selectively to specific organ tissues in scid mice. Based on the first selection criterion, noninvasive and invasive sublines were obtained which differed substantially in their capacity to secrete type IV collagenase. The noninvasive sublines (i.e., 3 x N.I.) uniformly failed to secrete detectable amounts of a M, 72,000 type IV collagenase, whereas the invasive sublines (3 x I.) secreted the collagenase in abundance.

Four metastatic PC-3 sublines (i.e., PC-3 ML, PC-3 MK, PC-3 MR, PC-3 MC) were isolated from the 3 x I. cells. Like the 3 x I. cells, these sublines also secreted large amounts of
collagenase and were highly invasive in Boyden chamber chemotactic assays.

In this paper we have shown by three different immunocassays (Western blots, slot blots, and immunoprecipitation) that the PC-3 sublines differed significantly in the amounts of kinesin produced. In untreated cells, kinesin was undetectable in two different batches of the 3 x N.I. cells and barely detectable in the parent PC-3 cells. In comparison, the basal levels of kinesin were much higher in the highly invasive and metastatic PC-3 sublines. Culturing the sublines on type IV collagen (as opposed to plastic) accentuated these differences somewhat but did not appear to stimulate kinesin synthesis to any significant degree in any of these sublines.

We discovered that concentrated preparations of conditioned medium from either the 3 x invasive or the metastatic PC-3 ML sublines stimulated dramatic increases (i.e., over basal levels) in kinesin synthesis in the PC-3 ML, PC-3 MK, PC-3 MR, and PC-3 MC sublines. Most of the studies here, were carried out utilizing the CM of the PC-3 ML subtype. The stimulatory effects of PC-3 ML conditioned medium on kinesin were accompanied by both elevated type IV collagenase secretion and an increased invasive activity in the metastatic and invasive sublines. Cycloheximide, heat, trypsinization, and pertussis toxin independently blocked the stimulatory influence of the PC-3 ML conditioned medium, indicating that a specific autocrine regulatory factor (protein) may be involved. We suggest, therefore, that an autocrine factor(s) might somehow coordinate kinesin and type IV collagenase synthesis, leading to collagenase secretion and tumor cell invasion of the basement membrane.

In our studies, a prominent M, 72,000 type IV collagenase was secreted in accelerated amounts by the conditioned medium-activated, metastatic PC-3 sublines. Western blots showed that antibodies raised against the M, 72,000 type IV collagenase (Ref. 29; courtesy of W. Stetler-Stevenson) specifically cross-reacted with this protease, indicating that it is an identical type IV collagenase. Preliminary gelatinase assays further indicated that in addition to a prominent M, 72,000 gelatinase, the sublines also secrete minor amounts of a M, 92,000 and 56,000 gelatinase. Unfortunately, the gelatinase assays were not quantitative and did not show if the amounts of these proteases or their collagenolytic activity remain constant during cell activation or invasion. In future studies it is worthwhile to determine if any of the PC-3 sublines also secrete (or fail to) other known type IV collagenases or other proteases. For example, a wide variety of proteases have been identified which metastatically agressive tumor cells secrete, including heparanases (30), serine (6, 31), thiol (32), and metal-controlled (2, 33–35) enzymes. A variety of malignant tumor cells examined secreted excess levels of various proteases, including soluble and membrane-bound proteases (7, 36–38). In fact, the secretion of soluble type IV collagenase (2, 6, 8) was directly associated with conditioned medium-stimulated invasive activities of the tumor cells, and several sized type IV collagenase molecules (i.e., M, 72,000–95,000) were identified and implicated in the invasive process (5, 29, 39–44).

Whether other cellular processes might also be activated which are essential for invasion by the metastatic PC-3 sublines is not clear. Since the PC-3 ML conditioned medium was dialyzed to remove growth factors, and since the exogenous growth factors tested and a leukocyte cytokine failed to activate kinesin expression in the PC-3 sublines, we presume that at least these agents were not involved. We cannot rule out the possibility that a variety of different heat-, trypsin-, and pertussis-sensitive autocrine factors might be present in the PC-3 ML CM, however. For example, several factors might coactivate kinesin and/or protease synthesis, resulting in the release of collagenase plus a wide variety of other proteases. Overall, we believe that the processes activated may be fairly limited. Protein synthesis, in general, was not stimulated and the β-tubulin levels remained constant in all the PC-3 sublines even in the presence of high dosages of PC-3 ML conditioned medium (30 mg/ml).

Properties of Noninvasive Sublines. The above properties of the metastatic PC-3 sublines were difficult to detect in the highly selected noninvasive sublines. Qualitative slot blots and quantitative immunoprecipitation data have clearly shown that the highly noninvasive PC-3 sublines (3 x N.I.) did not express significant amounts of kinesin in the presence or absence of conditioned medium from any of the PC-3 sublines. A wide range of dosages of conditioned medium from the PC-3 ML or the 3 x N.I. sublines (0 to 30 mg/ml) failed to stimulate any noticeable kinesin synthesis in the 3 x N.I. cells. One possible explanation is that the 3 x N.I. cells do not secrete an autocrine factor(s) or express cell surface receptors for the appropriate autocrine factor(s).

The 3 x N.I. cells also did not secrete significant amounts of type IV collagenase in response to conditioned medium from the metastatic sublines. Neither did they appear to secrete an autocrine factor(s) which could activate type IV collagenase secretion or tumor cell invasion. Low and high dosages of conditioned medium (10 to 30 mg/ml) from the 3 x N.I. cells had negligible stimulatory (or inhibitory) effects on either the 3 x N.I. cells or the PC-3 ML subline. In general, we found that there was little or no change in either the kinesin levels or collagenase secretion in a wide variety of cell lines tested of low invasive and metastatic capacity (i.e., including the PC-3 parent, DU 145, MEF, or NIH-3T3 cells). In response to the PC-3 ML conditioned medium (10 mg/ml) these latter cells demonstrated only a marginal increase in kinesin synthesis, collagenase secretion, and invasive activity. Neither did the cell lines respond to conditioned medium prepared from themselves or the other cell lines of low invasive potential.

We were concerned that the striking differences among the noninvasive and metastatic sublines might arise inadvertently

<table>
<thead>
<tr>
<th>Cell line tested</th>
<th>Kinesin (× 10⁹ cpm)*</th>
<th>Collagenase (× 10⁹ cpm)*</th>
<th>% of invasive activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3-ML</td>
<td>44.0 ± 1.0</td>
<td>4.7 ± 0.3</td>
<td>85.0 ± 5.0</td>
</tr>
<tr>
<td>PC-3-MR</td>
<td>46.0 ± 2.0</td>
<td>5.2 ± 0.1</td>
<td>68.0 ± 6.0</td>
</tr>
<tr>
<td>PC-3-MK</td>
<td>49.0 ± 3.0</td>
<td>5.0 ± 0.2</td>
<td>60.0 ± 4.0</td>
</tr>
<tr>
<td>PC-3-MC</td>
<td>45.0 ± 1.0</td>
<td>5.5 ± 0.2</td>
<td>61.0 ± 3.0</td>
</tr>
</tbody>
</table>

* Pertussis toxin (60 ng/ml) was added for 3 h prior to and during the time the sublines were exposed to PC-3 ML CM (10 mg/ml for 6 h). For invasive assays the drug was always added with the PC-3 ML CM. Values were averaged from 5 separate experiments ± SD. The experimental conditions were the same as in Fig. 7.

Unpublished data.
as a result of insufficient care in the execution of the experiments. The conditions favoring maximum collagenase secretion and tumor cell invasion were worked out in earlier studies on DU 145 cells (5). Utilizing these conditions we made every effort to minimize differences which might arise as a direct result of variations in cell attachment, or in the experimental method. The cell batch, the cell passage (5 passages), the cell number (2 x 10^6), the cell volumes, the duration of the experiment, and the quantities of protein in the crude cell extracts were measured, standardized for each experiment, and ostensibly the same for all the cell lines tested. Still, there was some slight variation in the basal levels of kinesin or type IV collagenase detected among the cell lines, but this arose largely from the amount of label used, or the specific activity of the label (compare Tables 1 to 6).

We believe that cellular interactions with specific extracellular matrix components and cell attachment probably constitute part of the mechanism for transducing the signal for collagenase induction. Several reports have shown that fibronectin (11, 12), type IV collagen (5, 13), and laminin (9-11) can independently induce protease secretion by tumor cells. We found that culturing the PC-3 sublines on mouse type IV collagen uniformly enhanced cell attachment by both the noninvasive and the metastatic PC-3 sublines. Moreover, the basal levels of type IV collagenase secretion were slightly elevated in a pertussis toxin-insensitive manner in the metastatic cells, but not in the noninvasive PC-3 sublines.

It is also possible that the degree of cell attachment could alter microtubule patterns and thereby affect secretion. However, immunofluorescence studies in our laboratory have revealed the PC-3 sublines (i.e. 3 x 10^6 , PC-3 ML) cultured on type IV collagen or plastic failed to exhibit demonstrable difference in the organization of their microtubules. Similar microtubule networks were commonly observed emanating from a centrally located MTOC in all the PC-3 sublines. In addition, the organization of the microtubules remained unchanged in the presence of PC-3 ML CM (10 mg/ml for 3 h). Thus, gross changes in the microtubule organization are probably not involved, although subtle compositional or structural differences in the microtubule populations might exist and influence the secretory activity of the metastatic PC-3 sublines.

Pertussis Toxin Studies. Liotta et al. (14) have identified a M, 55,000 autocrine motility factor in the conditioned medium of human melanoma A2058 cells which can stimulate cell motility via a pertussis toxin-sensitive G-protein-associated receptor (15, 16, 45). Our data suggested that a similar protein or family of proteins and their receptors may be produced by the metastatic PC-3 sublines. The trypsinization data in this paper clearly suggested that a factor(s) in the conditioned medium of PC-3 ML CM might bind the cell surface receptor(s) to regulate kinesin expression in invasive and metastatic PC-3 sublines. Likewise, the observed inhibitory activity of pertussis toxin further suggested that a G-protein-dependent receptor might be involved.

In support of this interpretation, we found that agents (cholera toxin, forskolin, 8-BrcAMP, dideoxyadenosine) which directly inhibit or stimulate the adenylate cyclase pathways (46–49) had little or no influence on any of the PC-3 sublines in the presence or absence of the PC-3 CM. Normally, cholera toxin stimulates adenylate cyclase through the G-protein; forskolin stimulates the enzyme directly; and 8-BrcAMP acts as a cyclic AMP analogue. Likewise, the adenosine analogue, dideoxyadenosine, normally blocks adenylate cyclase independent of the G-protein. It would, therefore, appear that a factor(s) present in the PC-3 ML conditioned medium works independent of a cyclic AMP metabolic pathway and the adenylate cyclase system. The interpretation is consistent with the idea that receptor-mediated binding of a factor(s) (i.e., a heat-sensitive protein) in the conditioned medium of the PC-3 ML cells serves to up-regulate kinesin expression. One possible mechanism through which the factor(s) and the G-proteins act (46) might involve a phospholipase C second messenger pathway.

ACKNOWLEDGMENTS

We thank Hank Lane of Costar Corporation for the generous gift of the Transwell chambers.

REFERENCES


Regulation of Kinesin Expression and Type IV Collagenase Secretion in Invasive Human Prostate PC-3 Tumor Sublines

Mark E. Stearns and Min Wang