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Matrigel influences morphology and cathepsin B distribution of prostate cancer PC3 cells

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Abstract. Increases in expression and activity of matrix-degrading enzymes such as the cysteine proteinases cathepsins B and L, and abnormal levels of their inhibitors, the cystatins, are associated with tumor cell invasion and metastasis. Environmental conditions have been shown to be causative factors in the development of a metastatic/invasive phenotype. We hypothesized that cell-matrix interactions affect the expression and activity of cathepsins B and L and their inhibitors in the prostate cancer cell lines, PC3 and DU145. To test this possibility, PC3 and DU145 were plated on uncoated surfaces or on surfaces coated with the reconstituted basement membrane, Matrigel. The cells were analyzed for cathepsins B and L immunolocalization, protein expression and activity 48 h after plating. Our data demonstrated that cathepsins B and L displayed a distinct punctate distribution with little co-localization; individual cells displayed a predominant staining for one or the other enzyme. Cathepsin B had a perinuclear distribution in PC3 grown on uncoated surfaces but a more peripheral staining in PC3 plated on Matrigel. Localization of cathepsin L remained predominantly perinuclear regardless of the plating surface. In addition to the translocation of cathepsin B from a perinuclear distribution to the cell periphery, growth of PC3 on Matrigel shifted cathepsin B activity from the cell extract to the media. There were no significant changes in cathepsins B and L immunolocalization or activity in DU145 with regard to plating surfaces. Likewise, the activity of endogenous cysteine proteinase inhibitors (CPIs) and protein expression of cystatin C remained unchanged in both cell lines. In conclusion, the interaction of PC3 prostate cancer cells with extracellular matrix components affects the distribution of cathepsin B protein and activity.

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Introduction

A primary cause of death from cancer is invasion and metastasis of cells from the primary tumor to distant sites. The ability of a cancer cell to detach from adjacent cells and its supporting extracellular matrix is partly dependent on the activities of a number of proteinases including matrix metalloproteinases, plasminogen activators, and the cathepsins (1-4). These proteinases facilitate the breakdown of cell adhesion molecules, basement membranes, vascular channels, and possibly other cell regulatory molecules; their activity may also be responsible for the release of regulatory factors from the surrounding matrix. It is no surprise that the activities and expressions of many proteinases are increased in a number of cancers and cancer cell lines. This study focuses on the cysteine proteinases, cathepsins B and L. Cathepsins B and L are lysosomal proteinases; they acquire mannose-6-phosphate (M6P) residues during their synthesis that target them to lysosomal vesicles via the M6P receptor. In many cancer cells, however, cathepsins B and L are either secreted or associate with the plasma membrane and with caveolae (5-8). Both proteinases can degrade components of the extracellular matrix *in vitro* (9-14) and can activate other matrix-degrading enzymes (15,16) suggesting that they contribute in a significant way to the metastatic ability of cancer cells. Lysosomal degradation of matrix components by the cathepsins has also been demonstrated in cancer cells (17,18).

The extracellular matrix is an important regulator of cellular differentiation and behavior; it also plays an active role in the development of the metastatic phenotype. A hallmark of metastasizing cells is their increased ability to migrate and invade adjacent tissue in most cases by altered expression of cell-cell and cell-matrix adhesion molecules. Adhesion molecules, in turn, participate in cell signaling cascades that relay information from the environment to the cell nucleus with resulting changes in gene expression patterns and cell behavior (19-21). Migration and invasion also involve cytoskeletal rearrangements necessary for cell locomotion; cytoskeletal components are responsible for the arrangement and location of cell organelles including lysosomes. We propose that the changes in cathepsin expression, activity and location result from the interaction of cancer cells with tumor-altered components of the extracellular matrix. Matrigel is a reconstituted basement membrane composed predominantly of laminin but also contains collagen IV, heparin sulfate proteoglycans,

entactin, and nidogen (BD Biosciences, SPC354234). Matrigel is used in many studies of cell-matrix interactions including prostate cell lines (22). Matrigel enhanced the growth of human prostatic carcinoma cell lines injected into nude mice (23) and increased their invasive and tumorigenic properties (24). Since cathepsins B and L are implicated in prostate tumor growth and invasion (3,4,25-29) we analyzed the effect of cell-Matrigel interactions on the expression and activity of cathepsins B and L in the prostate cancer cell lines, PC3 and DU145. This study demonstrates that the location of active cathepsin B is modulated by the interaction of PC3 cells with Matrigel.

Materials and methods

Materials. The prostate cancer cell lines, DU145 and PC3, were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Matrigel was obtained from Becton-Dickinson (Bedford, MA). All other culture reagents were obtained from Gibco/Invitrogen (Grand Island, NY). The cathepsin substrates, Z-phe-arg-aminomethylcoumarin (Z-phe-arg-NMec), Z-arg-arg-aminomethylcoumarin (Z-arg-arg-AMC), and the standard 7-amino-4-methylcoumarin (AMC) were purchased from Bachem (Torrance, CA). Centricon-YM10 columns were from Millipore (Bedford, MA). Reagent sources for the immunolocalization studies were as follows: 4',6-diamidino-2-phenylindole (DAPI) for DNA staining (Molecular Probes, Eugene, OR), polyclonal antibody to cathepsin L (Calbiochem, San Diego, CA), and polyclonal antibody to cathepsin B (R&D Systems, Minneapolis, MN). Secondary antibodies were donkey anti-rabbit conjugated to Rhodamine Red™-X for cathepsin L and donkey anti-goat conjugated to Cy™2 for cathepsin B (Jackson Immuno-Research, West Grove, PA). Antibodies used for Western blotting included polyclonal anti-cathepsin L (Calbiochem), polyclonal anti-cathepsin B, and polyclonal anti-cystatin C (Novus, Littleton, CO). Precast acrylamide gels were obtained from Invitrogen (Carlsbad, CA). The ECL kit was from Amersham (Piscataway, NJ). Protran® nitrocellulose transfer membrane was obtained from Schleicher & Schuell (Keene, NH). All other reagents were from Sigma (St. Louis, MO) or Fisher (Cincinnati, OH).

Cell culture. The human prostate carcinoma cell lines DU145, established from a metastatic lesion in the central nervous system of a 69-year-old man (30) and PC3, established from a lumbar vertebra metastasis of a 62-year-old man (31) were maintained according to ATCC specifications. The reconstituted basement membrane Matrigel (undiluted or diluted 1:3 with F-12K media) was spread on 60-mm plastic dishes (for enzyme analysis) or 22-mm round coverslips (for immunocytochemistry) and allowed to dry at room temperature for 1 h. Pre-coated 60-mm Matrigel dishes were also used for enzyme assays. The concentration of Matrigel per cm^2 of surface was calculated as ~70-80 $\mu g/cm^2$ for a thin layer of Matrigel or pre-coated dishes and 0.5-0.6 mg/cm^2 for a thin gel of Matrigel. PC3 and DU145 were plated at a concentration of 5.0×10^5 cells per 60 mm dish and at 2×10^4 cells per 22-mm coverslip. The cells were maintained in culture for a total of 48 h and fed with serum-free media 12-18 h prior to collection and analysis.

Immunocytochemistry. Cells were plated on a thin layer of Matrigel on 22-mm glass coverslips for immunolocalization of cathepsins B and L. Prior to fixation, cells were reacted with DAPI for 30 min at 37˚C for DNA staining. Cells were fixed in 3% paraformaldehyde and permeabilized with cold acetone. Non-specific binding sites were blocked with a 1:20 dilution of normal donkey serum in phosphate-buffered saline (PBS). Primary antibodies (polyclonal antibody to cathepsin L and polyclonal antibody to cathepsin B) were diluted 1:200 with PBS + 0.05% Tween, added sequentially to slides and allowed to react for 1 h and overnight, respectively. Secondary antibodies, diluted 1:50 with PBS + 0.05% Tween, were donkey anti-rabbit conjugated to Rhodamine Red-X for cathepsin L and donkey anti-goat conjugated to Cy2 for cathepsin B; slides were incubated with secondary antibodies for 1 h at room temperature following reaction with the primary antibody and extensive washing with PBS-Tween. Coverslips were mounted on glass slides with Mowiol (Calbiochem, La Jolla, CA) and examined with an Olympus confocal microscope.

Western blot analysis. Cells were collected by scraping in PBS and centrifuged at 5,000 g for 5 min. The cell pellet was resuspended in lysis buffer (1% nonidet P-40, 0.5% deoxycholate, 1% SDS, 1 mM sodium orthovanadate, 0.5 mM PMSF, 10 μg/ml aprotinin, 20 μg/ml leupeptin), lysed by sonication and placed on ice for 10 min. The homogenate was centrifuged at 13,100 rpm for 10 min at 4˚C and the supernatant saved. Protein (50 μg) from each sample was mixed (1:2, v/v) with sample buffer (125 mM Tris-Cl, pH 6.5, 20% (v/v) glycerol, 4% SDS, 0.025% bromophenol blue, 0.1 M DTT) and boiled for 5 min. Samples were loaded on 12% (for cathepsins B and L) or 16% (for cystatin C) precast SDS-PAGE gels and run at constant voltage in a Tris-glycine running buffer (25 mM Trizma base, 0.192 M glycine, 0.1% SDS).

Preparation of samples for Western blot analysis of cathepsins B and L and cystatin C in the media was as follows: 10 μl of the appropriate antibody was added to media (amount equivalent to 100 μg protein in media and previously concentrated 100-fold with a Centricon-YM10 column) and incubated overnight at 4˚C with gentle rocking. The immunocomplex was captured with 20 μl of protein G agarose beads with gentle rocking at 4˚C for 2 h. The agarose beads were collected by centrifugation (2 min at 14,000 g) and washed 3 times with lysis buffer. The beads were resuspended in 30 μl of sample buffer, boiled for 5 min, centrifuged and 40 μl supernatant loaded on SDS-PAGE gels as described above.

Following electrophoresis, proteins were transferred to Protran nitrocellulose membranes using the Invitrogen Trans-blot apparatus according to the manufacturer's specifications. The membrane was immersed in blocking buffer (5% NFDM in TBS-Tween) for 1 h at room temperature followed by incubation in primary antibody (diluted 1:1000 for cathepsins B and L, and cystatin C) in blocking buffer overnight at 4˚C. After extensive washing with 0.05% Tween-20 in TBS the membranes were incubated in secondary antibody (diluted 1:2000) for 1 h at room temperature. Cathepsins B and L and cystatin C protein bands were detected by chemiluminescence

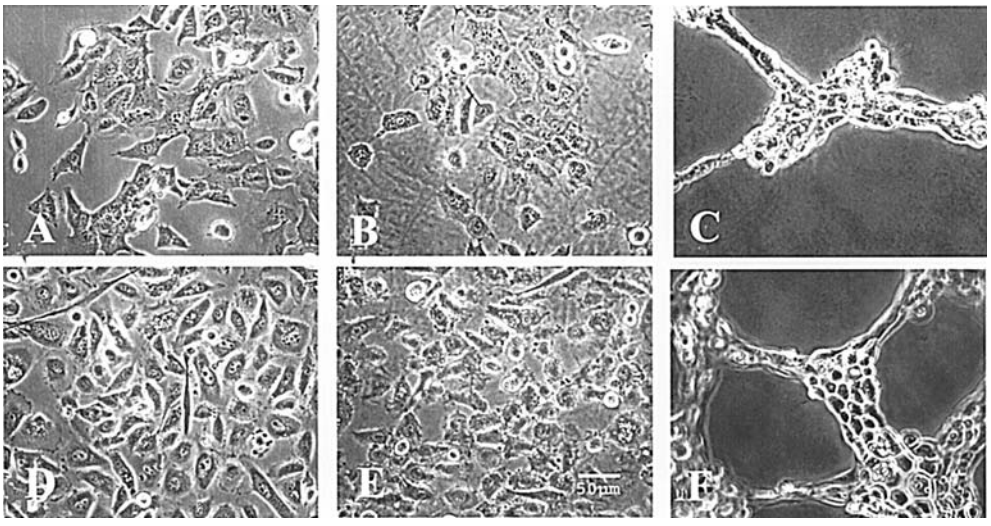

Figure 1. Phase contrast morphology of DU145 (A-C) and PC3 (D-F) grown on uncoated surfaces (A and D), or surfaces coated with either 70-80 μg Matrigel per cm^2 (B and E), or 0.5-0.6 mg Matrigel per cm^2 (C and F). Surfaces coated with 70-80 μg Matrigel per cm^2 form a thin protein layer for cell attachment in contrast to a gel as in surfaces coated with 0.5-0.6 mg Matrigel per cm^2. There are no apparent differences in morphology between cells growing on uncoated surfaces (A and D) or surfaces coated with a thin layer of Matrigel (B and E). Cells attached to a Matrigel gel clump together and form linear arrays (C and F). Size bar indicated in Fig. 1E.

using the ECL kit and procedure specified by Amersham Corp. (Piscataway, NJ).

Assays. Cells were fed with serum-free media 12-18 h prior to harvesting for enzyme and inhibitor analysis. On the day of harvesting, the serum-free medium was collected for proteinase and inhibitor assays. The cells were washed with PBS, pH 7.2, collected in PBS, centrifuged at 3,000 g for 5 min, and the pellet resuspended in PBS. The cells were lysed using a Tissue Tearor (Biospec Products Inc., Bartlesville, OK) at high speed for 5 sec, and immediately placed on ice. The cell pellet and homogenate were check microscopically at each step to ensure that the cells were completely lysed. The homogenate was centrifuged at 15,000 g for 15 min to remove cell residue, and the supernatant saved.

Cysteine cathepsin assays were done according to the procedure of Barrett and Kirschke (32) as described by Colella *et al* (25). Briefly, cathepsin L activity of the cell supernatant was measured using 12.5 μM Z-phe-arg-NMec as substrate in 250 mM acetate buffer, pH 5.4, containing 2.5 mM EDTA, and 1 μM dithiothreitol (DTT). The Z-phe-arg-NMec substrate is hydrolyzed by cathepsin L and to a small extent by cathepsin B; therefore, the activity is referred to as cathepsin L+B activity (32). Cathepsin B was measured with 12.5 μM Z-arg-arg-NMec in 250 mM phosphate buffer, pH 6.0, containing 2.5 mM EDTA and 1 μM DTT. The reactions were stopped by the addition of 100 mM monochloroacetic acid in 100 mM acetate buffer, pH 4.3. Fluorescence was measured in a Perker-Elmer spectrofluorometer at an excitation wavelength of 370 nm and an emission wavelength of 460 nm. Activity is expressed as μUnit/μg protein where 1 Unit is defined as 1 μmol substrate released per minute. Protein concentration was determined by the Lowry method using bovine serum albumin as standard (33).

The activities of secreted cathepsins B and L were determined as follows: Cell-conditioned media was concentrated with a Centricon-YM10 column. Cathepsin assays as described above for the cell extract were performed on concentrated media prior to and after incubation with 3M formate buffer, pH 3.0 for 10 min at 37˚C. Acid treatment ensures that cathepsin L-cystatin complexes are broken down allowing for more accurate measurement of cathepsin L in crude samples (34); incubation with formate buffer under acidic conditions also cleaves procathepsins B and L into their active forms (35,36).

Since there are no direct assays for measuring cystatin activity, total cysteine proteinase inhibitor (CPI) activity was indirectly measured in cell extract and conditioned media as described (25). Briefly, samples (cell extracts and conditioned media) were boiled for 5 min, centrifuged for 10 min at 15,000 g and the supernatant incubated with the cysteine proteinase, papain using Z-phe-arg-NMec as substrate as described above for cathepsin L+B. Inhibitory activity is expressed as μU inhibitory activity per μg protein where 1 μU of inhibitory activity represents the amount of papain activity inhibited per minute.

Statistics were calculated using the Student's t-test where $P \leq 0.05$ was considered significant. All values are the mean ± standard error of at least three separate experiments.

Results

Morphology of cells plated on Matrigel. The interactions between epithelial cells and components of their supporting matrix orchestrate the development of specific cell phenotypes and subsequently tissue and organ characteristics. Both cellular and stromal characteristics are altered during progression of cancer to the metastatic state; this includes the release of proteolytic enzymes that can also modify the external milieu (37). To determine if attachment to Matrigel affected cathepsins B and L expression in prostate cancer cell lines, PC3 and DU145 were initially plated on uncoated surfaces or surfaces coated with a thin gel of Matrigel (0.5-0.6 mg per cm^2). The Matrigel thin gel induced changes in cell shape and organization such that cells formed aggregates connected to other aggregates by linear arrays of elongated cells (Fig. 1C

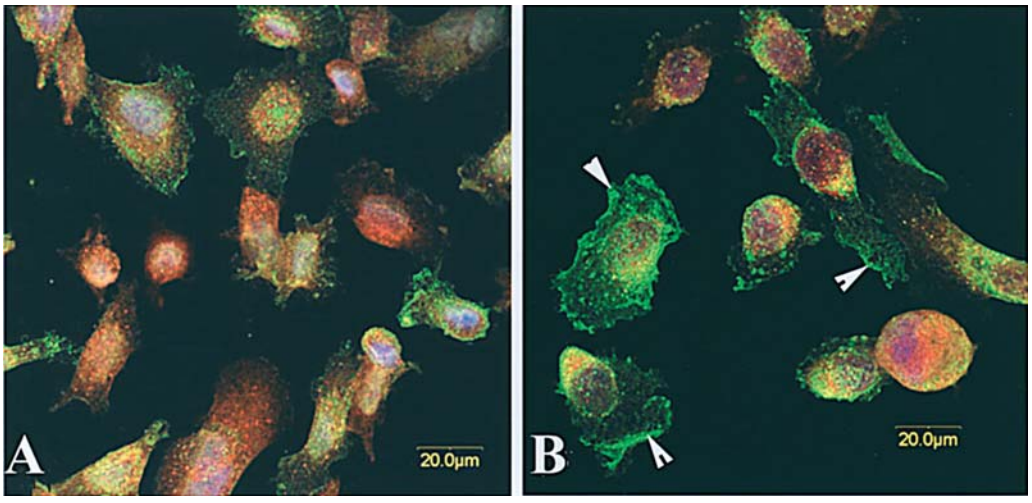

Figure 2. Immunolocalization of cathepsins B (green) and L (red) in PC3 cells plated on uncoated surfaces (A) or surfaces coated with 70-80 μg Matrigel per cm^2 (B). The cells were plated at 2x10^4 cells per 22-mm round coverslip and incubated at 37˚C for 48 h. Prior to fixation the nuclei were stained with DAPI (blue). Co-immunolocalization of cathepsins L and B was performed as described in Materials and methods. Cathepsins L and B appear as punctate material with individual cells showing a predominant staining for either cathepsin B (green) or L (red); there is little co-localization of cathepsins B and L (as indicated by yellow-orange staining). PC3 cells on Matrigel demonstrate more cathepsin B staining toward the cell periphery (arrowhead, B) when compared to cells plated on uncoated surfaces. The distribution of cathepsin L is similar in cells plated on coated or uncoated surfaces. DU145 cells demonstrated similar distribution of cathepsins B and L as PC3 plated on uncoated surfaces; however, B and L localization did not change when DU145 was plated on Matrigel (data not shown).

and F). The aggregates were seen as early as 4-5 h following plating. The goal of this study was to determine the effects of extracellular matrix components on cathepsins L and B distribution and activity. Therefore, to eliminate the possibility that changes in cell shape also affect cathepsins L and B expression, cells were plated on a thin layer of Matrigel (70-80 μg Matrigel per cm^2). The difference between a thin layer of Matrigel versus a thin gel is that the thin layer provides a complex protein layer for cell attachment without forming a 'basement membrane' type gel as the thin gel provides (Tech sheet, BD Bioscience SPC354234). There was no difference in the morphology of PC3 and DU145 plated on uncoated surfaces (Fig. 1A and D) compared to cells plated on a thin layer of Matrigel (Fig. 1B and E). We continued our studies using a thin layer of Matrigel to maintain consistency in cell shape between cells on uncoated and coated surfaces.

Immunolocalization of cathepsins B and L. Co-immunolocalization of cathepsins B and L was performed on prostate cancer cell lines plated on glass coverslips or coverslips coated with 70-80 μg Matrigel per cm^2. Cathepsins L and B appear as punctate material typical of a lysosomal/endosomal distribution in PC3 cells (Fig. 2). Individual cells show a predominant staining for either cathepsin B (green) or L (red). There is little co-localization of cathepsins B and L (as indicated by yellow-orange staining) and this is seen mostly around the nucleus. PC3 cells plated on Matrigel coverslips (Fig. 2B) demonstrated more cathepsin B staining toward the cell periphery when compared to cells plated on uncoated coverslips (Fig. 2A). Furthermore, there was more cathepsin B positive staining in cell ruffles (Fig. 2B, arrowheads). DU145 also displayed a typical lysosomal/endosomal distribution of cathepsins B and L; however, there were no apparent differences in the localization of cathepsins B in DU145 cells plated on glass compared to cells plated on a thin layer of Matrigel (data not shown).

Cathepsin L immunolocalization did not appear to be affected by the presence of Matrigel; cathepsin L displayed a perinuclear distribution in all groups of cells examined.

Cathepsin L+B, cathepsin B and cysteine proteinase inhibitor (CPI) activities. Cathepsin L+B, cathepsin B, and total CPI activities were measured in extracts and media of PC3 and DU145 cells grown on either uncoated surfaces or surfaces coated with a thin layer of Matrigel. DU145 exhibited higher cathepsin L+B (Fig. 3A and B) and cathepsin B (Fig. 3D and E) activity both in the cell extract and medium compared to PC3 consistent with our previous results (25) and with those of Freidrich *et al* (3). Cathepsin L+B activity was detected only in media that had been previously incubated with formic acid; the absence of proforms of cathepsin L on Western blots (Fig. 5) suggests that incubation of media at low pH destroys strong cathepsin L-cystatin complexes (34) rather than the presence of pro-forms of cathepsin L. Furthermore, incubation of media with formic acid results in the loss of measurable cathepsin B activity suggesting that cathepsin B is also secreted in its active form (Fig. 3E).

Matrigel did not significantly affect either cell-associated or secreted cathepsin L+B activity of PC3 and DU145 cells (Fig. 3); however, the percent of total cathepsin L+B activity secreted by PC3 was significantly higher when grown on Matrigel compared to plastic (Fig. 3C).

The distribution of cathepsin B activity between the cell extracts and media was affected by growth on Matrigel in the two cell lines (Fig. 3D-F). A decrease in intracellular activity of cathepsin B and an in increase in secreted cathepsin B was observed in PC3 cells plated on Matrigel compared to cells plated on non-coated controls. In contrast, DU145 cells on Matrigel exhibited increase intracellular B activity but no significant difference in secreted activity (Fig. 3D and E). The

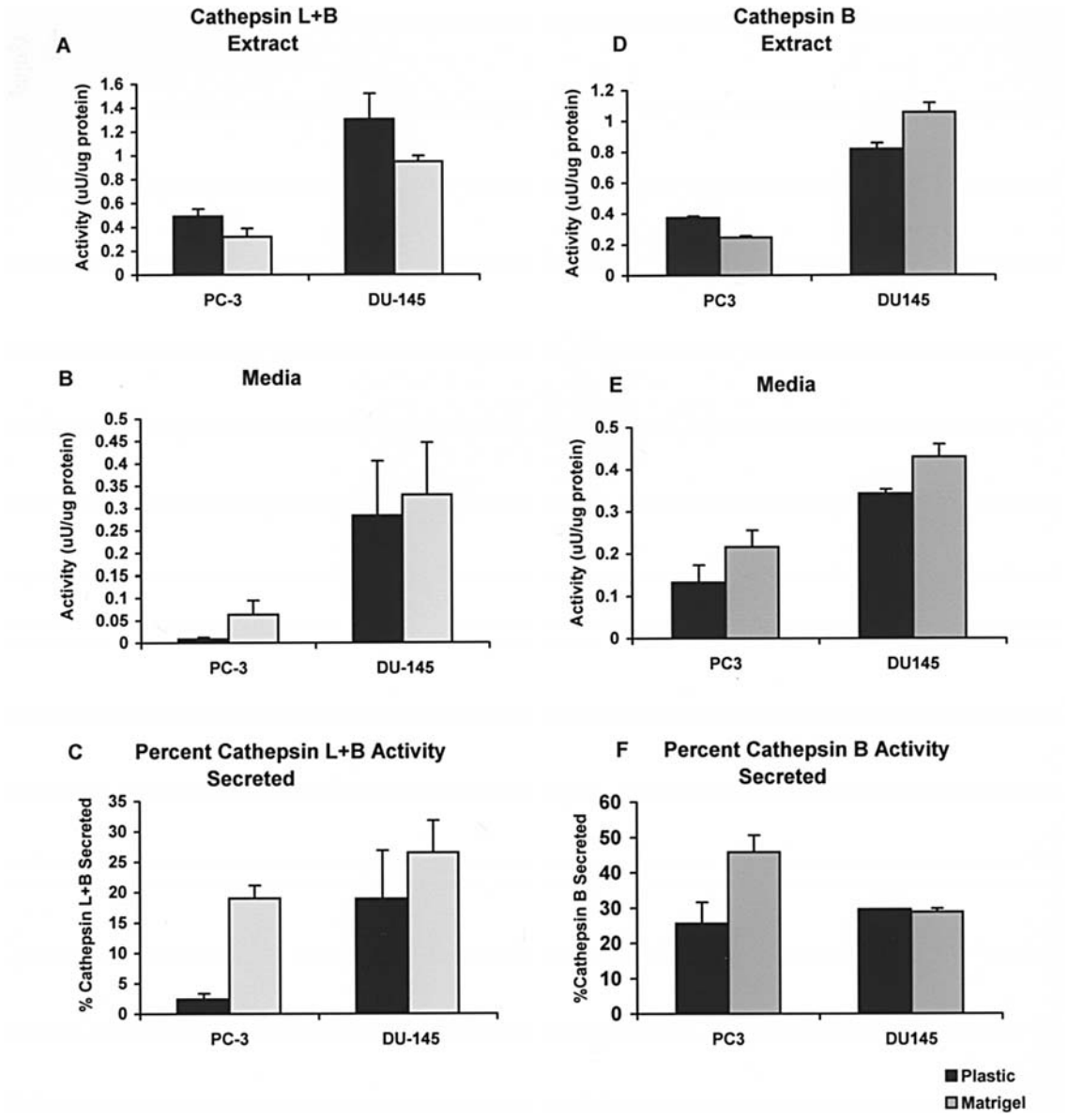

Figure 3. Cathepsin L+B (A-C) and cathepsin B (D-F) activities in cells grown on uncoated (black bars) or Matrigel-coated culture dishes (gray bars). PC3 and DU145 were plated at a concentration of 5.0×10^5 cells per 60-mm dish. The cells were maintained in culture for a total of 48 h and fed with serum-free media 12-18 h prior to collection. (A) Cell-associated and (B) secreted cathepsin L+B activities were not significantly different in either PC3 or DU145 cells plated on plastic compared to cells plated on Matrigel. (C) The percent of total cathepsin L+B activity found in media was significantly higher in PC3 plated on Matrigel (gray bars) compared to plastic (black bars). (D) Cell-associated and (E) secreted cathepsin B activity. PC3-Matrigel demonstrated a significant increase in secreted cathepsin B activity whereas DU145-Matrigel demonstrated a significant increase in cell-associated activity. (F) The percent of total cathepsin B activity secreted was significantly higher in PC3 when grown on Matrigel (gray bars) compared to plastic (black bars) whereas there was no difference in the percent of secreted cathepsin B activity of DU145 cells.

percent of total cathepsin B secreted confirms an increased secretion by PC3 but not DU145 (Fig. 3F).

Total cysteine proteinase inhibitory (CPI) activity was determined by the ability of cell extracts and media samples to inhibit the cysteine proteinase, papain. PC3 and DU145 had similar amounts of CPI activity in extracts (Fig. 4A) and media (Fig. 4B) as shown previously (25). Matrigel did not significantly affect cell-associated or secreted CPI activity in either cell line (Fig. 4).

Western blot analysis. Western analysis of cathepsins B and L and cystatin C was performed on cell extracts and media to determine if growth on Matrigel affected the protein levels of the cysteine proteinases and their endogenous CPI, cystatin C. Fig. 5 is a representative of three separate blots performed per protein. Cathepsins B and L appear as bands corresponding to the 31 kDa active forms of both proteinases. The inactive proforms of cathepsins B or L were not detected in the cell extracts or media. Western analysis indicates an increase in secreted cathepsin B protein in PC3 plated on Matrigel consistent with our activity data (Figs. 5 and 3B, respectively). An increase in the amount of secreted cathepsin B protein from DU145 grown on Matrigel is also evident in Western blots; however, the increase in secreted cathepsin B activity was not significantly different from cells grown on uncoated surfaces (Figs. 5 and 3E, respectively).

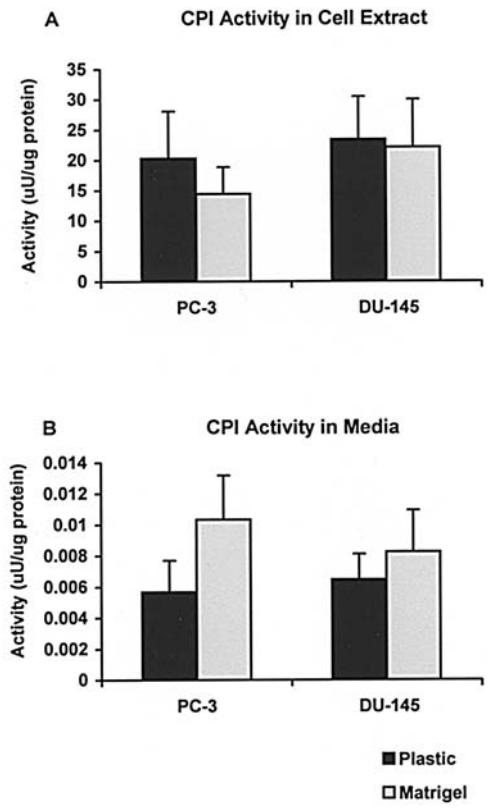

Figure 4. Cysteine proteinase inhibitor (CPI) activity in cells grown on uncoated (black bars) or Matrigel-coated (gray bars) surfaces. The cell extracts and media were boiled for 5 min, centrifuged to remove debris, and incubated with purified papain to measure total CPI activity. CPI activity in the cell extracts (A) or media (B) of both cell lines was not significantly different when comparing cells grown on Matrigel to those grown on plastic.

In agreement with cathepsin L activity data, Matrigel did not induce any changes in cell-associated or secreted cathepsin L protein in DU145 or PC3 cells.

A 14 kDa band corresponding to the cysteine proteinase inhibitor, cystatin C was present in the extract and media from both cell lines (Fig. 5); the amounts of cystatin C protein were equivalent in cells grown on plastic compared to those on Matrigel. An additional 25 kDA band was detected in the media of both cell lines; this band may represent cystatin C dimerization (38,39) or different glycoforms of the inhibitor. Various glycoforms of cystatin C similar in size to our 25 kDa band were also detected in media conditioned by hippocampal stem cells (40).

Discussion

This study demonstrates that the location of the lysosomal proteinase, cathepsin B, is affected by a cell's interaction with basement membrane components. This phenomenon is specific for cathepsin B as cathepsin L is not affected. Furthermore, we found that cathepsins B and L do not co-localize suggesting lysosomal sorting pathways specific for each proteinase. Others also have shown that cathepsins B and L do not co-localize to the same vesicle (16,41) suggesting differential localization of cathepsins B and L in some cells.

Cathepsins B, L and other lysosomal cathepsins can be sorted following synthesis of their pro-forms to either lysosomes, regulated secretory lysosomes/vesicles, or constitutory secretory vesicles; evidence for targeting to each of these vesicles has been reported (42,43). Pro-cathepsins B and L are processed to mature forms in the acidic milieu of the lysosome; the mature forms include a single chain of ~30-31 kDa that is further processed to the double chain species of ~25 and 5 kDa. Pro-cathepsin B, its mature forms or both are reportedly secreted from cancer cells depending on cell type and environmental factors. The pro-form of cathepsin B is secreted when normal and tumor breast fibroblasts are plated on collagen I gels (44) whereas collagen I induces the secretion of pro- and mature forms of cathepsin B from invasive melanoma cells (45). An active cathepsin B species of ~31 kDa is secreted pericellularly from a number of human cell lines (46). DU145 cells secrete mature, active forms of cathepsin B following their interaction with either type I collagen or bone fragments (47). In the present study, the secretion of active cathepsin B was up-regulated by Matrigel in PC3 but not in DU145 cells. This discrepancy may be due to the different types of matrices used in the two studies: Matrigel is composed predominantly of type IV collagen whereas bone is composed

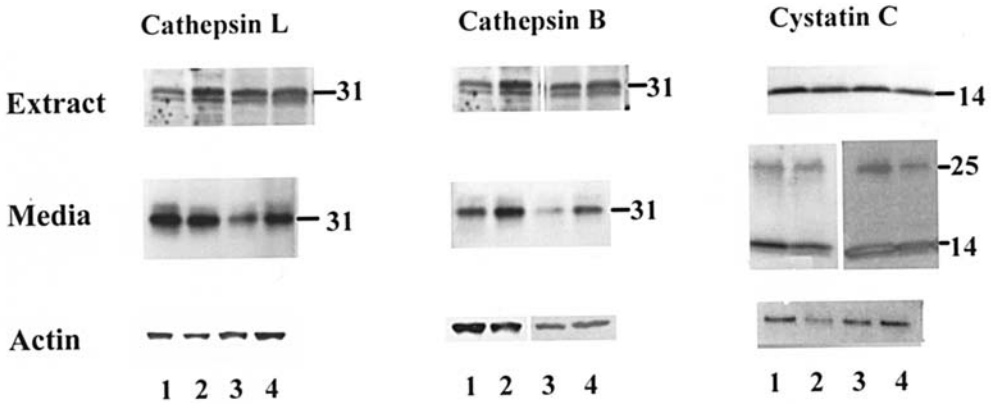

Figure 5. Western blot analysis of cathepsins L and B and cystatin C in cell extracts and conditioned media from PC3 (lanes 1 and 2) and DU145 (lanes 3 and 4) grown on uncoated (lanes 1 and 3) or surfaces coated with 70-80 μg Matrigel per cm^2 (lanes 2 and 4). Actin is included as the protein loading standard. Both cathepsins L and B appear as the mature single chain 31 kDa form in both extract and media; an increase in cathepsin B density is evident in conditioned media of both PC3 and DU145. The 14 kDa form of cystatin C is present in the extract and media; the media also demonstrates a 25 kDa form of cystatin C immunopositive band.

collagen. Cell binding to extracellular matrices occurs surface integrins and specific integrin subunits have been implicated to play a role in cathepsin B secretion (44,45). PC3 expresses different integrin subunits compared to DU145 and these integrin subunits correlate with an increased invasive ability of PC3 (48). The differences in integrin subunit expression between PC3 and DU145 could also be responsible for the significant increase in secreted cathepsin B activity by PC3 when grown on Matrigel.

Recent evidence demonstrated that a number of matrix-degrading proteolytic enzymes associate with caveolae (49), which are membrane lipid rafts that function in signal transduction, endocytosis, and lipid transport (50). Caveolae may function as organizing centers for proteolytic events leading to matrix degradation and invasion. Caveolin-1 is a major structural component of caveolae; caveolin-1 may play a crucial role in prostate cancer growth and is secreted by prostate cancer cells (51,52). Cathepsin B localizes to caveolae of colon cancer cells and caveolin-1 is implicated in the secretion of cathepsin B from colorectal carcinoma cells (8,53). It is possible that the selectivity of cathepsin B secretion from PC3 cells and not cathepsin L is due to the association of cathepsin B with caveolin-1. Although there is strong evidence that caveolin-1 functions as a tumor promoter in prostate cancer, its role in modulating cathepsin B secretion in prostate cancer cells is not known and needs to be assessed.

In this study, cells plated on uncoated surfaces or on a thin layer of Matrigel exhibited similar morphologies, but differed remarkably from cells plated on a gel-like matrix. PC3 and DU145 formed typical monolayers on uncoated surfaces and thin Matrigel layers; whereas on gels, the cells clumped together and formed web-like structures connecting aggregates of cells. Similar patterns of cell organization were seen in human airway smooth muscle (54), primary rat mammary (55), PC3 (22), and DU145 cells (56) when cultured on Matrigel gels. The difference between a thin layer of Matrigel versus a thin gel is that a thin layer of Matrigel is more rigid than a gel because of its closer association with the underlying glass/plastic surface. The physical properties of a matrix in addition to its constituents influence morphology; mechanical forces transmitted across the cell surface due to matrix flexibility modulate specific cell signaling pathways induced by the binding of matrix components to cell surface receptors such as the integrins (55,57). We conclude from our results that the web-like structures and cell aggregates formed on Matrigel gels are due to the more flexible properties of the gel surface compared to a Matrigel thin layer. We continued our studies on cathepsin activity using surfaces covered with a thin layer of Matrigel to minimize the influence of cell shape change on extracellular-matrix-mediated signaling. Changes in cell shape accompany changes in cytoskeleton organization; the latter promotes vesicular transport and fusion with the plasma membrane as has been shown (58-60). Although the effects of extracellular matrix components on lysosomal cathepsin activity and localization has been described in this study and by others, future studies that include the effects of morphology on lysosomal proteinase localization and secretion are needed. Understanding the dynamics of lysosomes and lysosomal cysteine proteinase secretion during cancer cell invasion is essential to the goal of eradicating cancer and its metastasis.

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