Localization of Collagenase at the Basal Plasma Membrane of a Human Pancreatic Carcinoma Cell Line

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ABSTRACT

We have recently presented biochemical evidence for collagen and gelatin degrading activities associated with plasma membranes of various human cancer cell lines. In this report we describe the localization of interstitial collagenase at the basal plasma membrane of the human pancreatic cancer cell line RWP-I, using immunofluorescence and ultrastructural immunogold labeling techniques. Collagenase was expressed on the extracellular face of the plasma membrane. Furthermore, the immunogold labeling was concentrated on the long, finger-like microvillous projections typically seen on the basal cell surface, while the short, brush-like projections characteristic of the apical cell surface were unlabeled. When the cytoplasmic face of the membrane was made accessible, the number of reactive sites increased markedly, indicating a high concentration of enzyme at the inner surface of the plasma membrane.

When plasma membrane fractions of RWP-I cells were prepared by differential centrifugation, high salt washes virtually failed to extract collagenase activity from the membrane, while detergent extraction with n-octyl glucoside, a detergent used in the purification of integral membrane proteins, yielded soluble collagenase activity. When detergent extracted membrane fractions were passed over an anticolagenase immunosorbent column, collagenase was specifically bound, as demonstrated by the TC50 and TC5 degradation of type I collagen by the bound material. Gelatinolytic activity did not bind to the column. Furthermore, immunoprecipitation of 125I-labeled detergent extracts of tumor membranes yielded a single Mr 55,000 band consistent with the zymogen form of the connective tissue collagenase. These morphological and biochemical findings suggest that collagenase is a tightly associated component of the basal plasma membrane, where it occupies a strategic location for directional proteolysis during cell migration and invasion.

INTRODUCTION

Dynamic interactions between cells and their extracellular milieu play a crucial role in neoplastic processes of tumor invasion and metastasis, as well as in normal phenomena such as tissue remodeling during embryogenesis and inflammatory responses. Basement membrane, extracellular matrix, and connective tissue fibrillary proteins present a natural barrier to the migration of cancer cells. Much of our insight into the proteolytic degradation of the extracellular components during this process comes from in vitro studies of malignant cells, which serve as a readily available model for cell migration in general. A spectrum of proteinases, such as interstitial collagenase (1, 2), type IV collagen degrading enzymes (3), gelatinase, serine proteinases (4), cathepsin B (5), and plasminogen activator (6) have been implicated in cancer invasion (7, 8).

Interstitial type collagen is the major structural protein of all tissues. Tumor cells can penetrate dense stroma only if a collagenase degrades the bundles of collagen (for review see Ref. 9) and tumor cell derived collagenases have been proposed as an important mechanism in their invasiveness (10, 11).

Collagenase, synthesized by normal connective tissue cells in culture, has been purified and its action on collagen types I, II, III (12, 13) and X (14) has been characterized. The enzyme has been shown to be a prototype of a secretory protein. It is synthesized as preprocollagenase, processed to a proenzyme within microsomal membranes and immediately secreted without intracellular storage (15).

In contrast, we described a metalloproteinase activity in the total cell extract of highly metastatic mouse melanoma cells that digests collagen type I and type IV and gelatin (16). Furthermore, the collagenolytic activity of human small cell carcinoma cells was highly enriched in the plasma membrane fraction (17). These observations suggest the existence of cell surface bound collagenase in human cancer cells and propose a proteolytic function associated with the plasma membrane. Their sanctuary status in the plasma membrane places them optimally for the effective destruction of substratum in a controlled, directional way. This also allows the local concentration of the enzyme to be maintained and may prevent it from binding to proteinase inhibitors present in the extracellular milieu (18).

In this study we have used an ultrastructural and immunochromatic approach to identify interstitial collagenase in RWP-I cells. Tumor collagenase was predominantly localized at basal type microvilli of plasma membrane where cells contact the substratum, suggesting that directional collagenolysis occurs at the cell-stroma interface.

MATERIALS AND METHODS

Cell Line

The human RWP-I pancreatic cancer cell line, kindly provided by Dr. D. L. Dexter (Department of Medicine, Roger Williams General Hospital, Providence, RI), has previously been characterized (19). For this study only cell cultures were used. Aliquots of these cells were injected into athymic mice and gave rise to large s.c. tumors. RPMI 1640 (GIBCO, Grand Island, NY) and 5–10% fetal calf serum (Flow Laboratories, Walkersville, MD) in 95% air and 5% CO2 at 37°C was used.

Antisera

Human procollagenase from the culture medium of rheumatoid synovial cells was affinity purified by exploiting cross-reactivity with anti-rabbit collagenase (20). The anti-rabbit collagenase antibody was directed against purified rabbit synovial fibroblast collagenase and has been described previously (20). Its monospecificity was established by double immunodiffusion, immunoprecipitation of 125I-labeled collagenase, and immunoblotting analysis. Cross-reactivity with human rheumatoid synovial procollagenase has been shown (20). The human antigen extracted on an anti-rabbit collagenase affinity column ran as a doublet with molecular weights of 52,000 and 55,000 on SDS-PAGE.

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The abbreviations used are SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; anti-pColl, sheep anti-human (rheumatoid synovial procollagenase) IgG; RAS, unconjugated rabbit anti-(sheep IgG) IgG; RWP-I, human pancreatic cancer cell line; MMP-3, matrix metalloproteinase-3, synonyms with stromelysin; APMA, p-aminophenylmercuric acetate; PBS, phosphate buffered saline.
The eluted antigen showed activity in a standard collagenase assay after activation by 4-aminophenyl mercuric acetate. Antiserum was produced by immunizing a sheep. The IgG fraction (referred to as anti-pColl) was isolated at a final concentration of 18 mg/ml. Anti-pColl recognizes interstitial type human collagenase and procollagenase, but not gelatinase and MMP-3/stromelysin as confirmed by immunoblot analysis. The working dilution was 1:60 (300 μg/ml).

**Immunofluorescence Microscopy**

Immuno localization of collagenase at the light microscopic level was carried out at room temperature. RWP-I cells were grown on coverslips to semisolid fermentation. The adherent cells were washed in PBS and fixed in 2% formaldehyde for 15 min. Others were washed in 150 mM NaCl, 50 mM Tris, 5 mM CaCl₂, pH 7.5. No difference in staining was noted between these two groups. Half of the coverslips were permeabilized in 0.25% Triton X-100/PBS for 5 min and the other half were kept in PBS only. The specimens were then incubated with primary antiserum (diluted 1:60 in PBS) for 60 min. After thorough washes in PBS, fluorescein isothiocyanate-conjugated rabbit anti-sheep IgG (H+L) was applied (diluted 1:150 in PBS) for 30 min. Preadsorbed anti-pColl as well as normal sheep serum were used as controls. The preparations were mounted with Aquamount, viewed with a Zeiss Axiomat microscope, and photographed with Kodak TMAX film.

**Immunogold Labeling of Collagenase Sites at Ultrastructural Level**

Colloidal gold particles (10 nm) coupled to rabbit anti-sheep IgG (H+L) IgG at 40 μg/ml protein concentration was purchased from EY Laboratories, Inc., San Mateo, CA. The specificity of the reagent was shown by incubating a nitrocellulose strip impregnated with 1 ng sheep IgG with 5 μl gold probe (20 μg/ml); bovine serum albumin (diluted 1:60 in PBS) for 30 min. Preadsorbed anti-pColl as well as normal sheep serum were used as controls. The preparations were mounted with Aquamount, viewed with a Zeiss Axiomat microscope, and photographed with Kodak TMAX film.

**Distribution Analysis of Gold Grains on Plasma Membranes**

The distribution of gold grains on the surface of 10 cells was assessed by image analysis in order to determine whether collagenase sites on the plasma membrane are concentrated on cell projections. The cells were selected at random and photographed at ×4000 which did not allow an evaluation of the presence or location of gold particles. All cells included a nuclear profile. All gold particles on the circumference of each cell were counted and grouped into sites on cell projections versus sites on the cell body. Small aggregates of gold grains were scored as one site and grains had to be directly attached to the membrane to be counted. Cell processes which were not in continuity with the cell body were considered part of the cell if the profiles were no further from the cell than the length of attached processes in the same plane of section. To normalize the number of grains in each categories, the perimeters were measured differentially with an electron image analysis device (Zeiss ZIDAC). Scanning electron micrographs of similar cells were taken to assess the 3-dimensional structure of the projections. For this, cells were grown on coverslips, fixed in glutaraldehyde, critically point dried, and examined with an AMC scanning electron microscope.

**Preparation of Plasma Membrane Associated Collagenase**

Postnuclear cell membranes were isolated from RWP-I cells using nitrogen cavitation followed by differential centrifugation as previously described in Ref. 16 and then subjected to sequential extraction. Extrinsic membrane proteins were extracted by 2 M KCl treatment (16). The remaining intrinsic membrane proteins were solubilized by treatment of tissue homogenates with 100,000 x g pellet with 1% n-octyl glucoside (20% in PBS on ice) followed by a second 100,000 x g centrifugation. The supernatant was divided into an upper half (lipid phase) and a lower half (protein phase). The pellet was treated with 1% n-octyl glucoside (Sigma Chemical Co., St. Louis, MO) overnight at 4°C and subjected to a last 100,000 x g centrifugation. The extracted proteins from each of the four supernatants were dialyzed against 25 mm cacodylate/5 mm CaCl₂/0.05% Brij 35 (Sigma), pH 7.2. Samples undergoing immunoprecipitation were concentrated by the addition of solid ammonium sulfate to 60% saturation and dialyzed against the above buffer. Aliquots of pellets and supernatants were activated with trypsin, then inactivated with soybean trypsin inhibitor and assayed for 3H-collagenolysis using 2 μg of labeled substrate at 27°C for 18 h as described in Ref. 17. RWP-1 conditioned medium was collected after 48 h from serum-free cell cultures containing 1 x 10⁷ cells/flask and spun at 770 x g for 10 min followed by 50,000 x g for 1 h. The proteins in the supernatant were precipitated with solid ammonium sulfate to 60% saturation, then resuspended, and dialyzed against the above buffer. Aliquots of pellets and supernatants were activated with trypsin, inactivated with soybean trypsin inhibitor, and assayed for collagen degradation, using 2 μg of 3H-labeled collagen at 27°C for 18 h as described in Ref. 17. The protein concentration of each extract and of conditioned medium was determined by the fluorosccaine method (21).

**Immunoblotting**

Samples (100 μl) of RWP-1 whole cell homogenate and sequential membrane extracts in various PBS dilutions were applied to nitrocellulose filters in a slot blot apparatus (Bio-Rad Bio-Dot SF) and the membrane was washed several times with PBS; 100 μl nonimmune rabbit IgG and PBS served as control. The nitrocellulose filters were soaked in reconstituted 5% (w/v) nonfat dried milk for 40 min. They were incubated with anti-pColl diluted 1:125 in PBS overnight at 4°C. After extensive washing, the filters were reacted with donkey anti-sheep IgG-IgG-alkaline phosphatase conjugate (Sigma) (1:1000 for 1 h at 37°C and the blot was developed in 0.1 M Tris-HCl, pH 9.5/0.1 M NaCl/5 mM MgCl₂ containing nitro blue tetrazolium (Sigma; 0.33 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (Sigma; 0.165 mg/ml). The reaction was terminated by rinsing it in water.

**Immunoadsorption of Collagenase**

RWP-1 membrane extracts (260 μl) containing 0.044 unit (1 unit degrades 1 μg of collagen/min) of total collagenase activity were applied.
to a column of Affi-Gel 10 (Bio-Rad, Richmond, VA) coupled with anti-pColl IgG (1.5 ml), which was equilibrated with 50 mM Tris-HCl/0.15 m NaCl/10 mM CaCl2/0.05% Brij 35/0.02% NaN3, pH 7.5. Specifically bound procollagenase was eluted with the above buffer containing 6 M urea and fractions of 200 µl were collected. The fractions were then applied to spin columns to remove urea as described in Ref. 22. Each fraction was assayed for the degradation of [14C]-acetylated type I collagen and [12C]-acetylated gelatin introducing 3200 cpm per tube (22) in the presence of 1 mM APMA and 0.04 ng of MMP-3/stronemel-lys in for 24 h at 24°C and 37°C, respectively. MMP-3 is required for the maximal activation of procollagenase (23, 24).

For the analysis of type I collagen degradation, 30 µl of the peak collagenase fraction were incubated with 15 µg type I collagen in the presence of 1 mM APMA/0.04 ng MMP-3 in a total volume of 50 µl at 28°C for 48 h as described above and then analyzed on SDS-PAGE (25).

Immunoprecipitation of Collagenase

One hundred µl of concentrated n-octyl glucoside membrane extracts (104 µg) were radioiodinated by using Iodo-Gen (1,3,4,6-tetrachloro-3a,6a-diphenylglycouril) (26) and free 125I ions were removed by spin columns (22). The sample was then subjected to immunoprecipitation by using 5 µl of anti-pColl IgG in a total volume of 400 µl according to the method of Nagase et al. (15). Antigen-antibody complexes were dissociated by boiling in SDS-PAGE sample buffer and the antigen was analyzed by SDS-PAGE and subsequent autoradiography.

RESULTS

Immunolocalization of Membrane Bound Tumor Collagenase.

To characterize the extent and overall distribution of collagenase, RWP-I monolayer cultures were studied with an immunofluorescence technique by using anti-pColl. A discrete punctate fluorescence was detected on approximately 50% of all cell cross-sections according to Nagase et al. (15). Antigen-antibody complexes were dissociated by boiling in SDS-PAGE sample buffer and the antigen was analyzed by SDS-PAGE and subsequent autoradiography.

Polarized Localization of Collagenase in RWP-I Cells. At the electron microscopic level, collagenase immunoreactivity was detected at the cell surface in a nonrandom distribution. Although there was considerable variation between individual cells, approximately 90% of all cell cross-sections were labeled with gold grains. The short plump microvilli on the apical surface were virtually devoid of gold labeling (Fig. 2b), while the long, thin finger-like processes, which are typical for the cell base, showed a moderate degree of collagenase reactivity (Figs. 2a and 3a). The number of reactive sites increased greatly when both the extracellular and the cytoplasmic face of the plasma membrane became accessible to the immunoprobe (Fig. 3). The detached fragments of plasma membrane were heavily labeled with gold grains in an irregular fashion. These fragments also showed a distinct morphology. They usually formed long convoluted strings with many small uniform ruffles decorating the main thread (Fig. 3b and control Fig. 4). Labeling was associated with these structures. To show that these peculiar string configurations were plasma membrane rather than rough endoplasmic reticulum or nuclear membrane, we have the following evidence. (a) Some cells showed very early stages of plasma membrane detachment, with loops peeling off (Fig. 4a). Their morphology was identical to the detached membrane fractions (Fig. 3b); (b) previously, we demonstrated that the 100,000 × g pellet of RWP-I cell homogenates contains a membrane band which is highly enriched in collagenase activity and exhibits the same ruffled profile as seen in this study (see Ref. 27, Fig. 2, lower right). Other cell organelles such as nuclei, mitochondria, and various vesicular structures showed only occasional sparse labeling. No dense core granules which might contain collagenase were noted.

Three types of controls demonstrated the specificity of the immunolabeling at the electron microscopic level. Preabsorption of anti-pColl with excess of purified human synovial procollagenase (Fig. 4a, b) and substitution of anti-pColl with normal sheep serum (data not shown) completely abolished immunogold labeling. Preincubation of the anti-pColl treated specimens with unconjugated secondary antibody (RAS) blocked the subsequent immunogold labeling virtually completely (Fig. 4c). Immunolabeling of intact cells was abolished as well (data not shown).

Quantitative Distribution Analysis of Collagenase Sites on Intact Plasma Membrane. A comparison of the number of particles per unit area of membrane on cell body versus projections gave a measure of the differential distribution on the...
COLLAGENASE IN HUMAN PANCREATIC CARCINOMA

Fig. 2. (a) Basal plasma membrane projections, which are long and finger-like, are decorated with anti-pColl immunogold complexes at the extracellular face. (b) Apical projections, which are short and brush-like, are unlabeled; x 24,000.

Fig. 3. (a) Membrane on cell projections (small arrow) and on the cell body (big arrow) are immunolabeled with anti-pColl. (b) Detached plasma membrane fragments are heavily labeled with anti-pColl immunogold complexes, indicating a much larger number of enzyme sites at the cytoplasmic face. Note the irregular distribution and the formation of ruffles (arrow) as shown in Fig. 4; x 24,000.

intact cell surface (see Fig. 3a). We assume that a single gold particle or an aggregate of gold particles attached to the membrane represents one collagenase site. Each pair of numbers in Table 1 was generated from the same cross-section of a cell. The total perimeter of cell body versus projecting membrane profiles on each cell circumference was measured and the data normalized. Table 1 shows that the plasma membrane of projections exhibited twice as many collagenase sites than smooth areas.

Extraction of Collagenolytic Activity from Detergent Treated Plasma Membranes. Trypsin activated RWP-I whole cell homogenate, postnuclear preparations, and plasma membrane
COLLAGENASE IN HUMAN PANCREATIC CARCINOMA

Fig. 4. RWP-I controls. (a) and (b) Preabsorption of the primary antibody with affinity purified immunogen. (a) Some cells show very early stages of plasma membrane detachment with formation of the typical ruffled structures. (b) Large convoluted strands of plasma membrane show complete blockage of immunolabeling. (c) Preabsorption of the antigen-antibody complex with unconjugated anti-(sheep IgG) prior to anti-(sheep IgG)-gold conjugate; × 24,000.

enriched fractions degraded 10, 40, and 1560 ng of type I [3H]collagen/mg protein/18-h incubation, respectively. Treatment with buffered 150 mM NaCl, pH 7.5, followed by 2 M KCl for 30 min resulted in no significant loss of membrane collagenolytic activity. In contrast, treatment with n-octyl glucoside resulted in extraction of 96% of the final collagenolytic activity (1115 ng substrate degraded/mg protein/18 h). RWP-I conditioned medium also contained secreted collagenolytic activity; 48-h conditioned medium degraded 41 ng of [3H]collagen/mg protein/18-h incubation following trypsin activation. Ammonium sulfate precipitated (0–60% saturated) conditioned medium digested 244 ng of [3H]collagen/mg protein/18-h following trypsin activation.

Immunoblotting of Detergent Extracted Plasma Membrane Fractions. The distribution of immunoreactive collagenase was followed through each step of the membrane extraction sequence. Aliquots of whole cell homogenate contained highly concentrated collagenase-like material which could only be extracted by the final n-octyl glucoside step (Fig. 5). However, high salt extraction alone, which removes loosely associated proteins, or butyl alcohol extraction, which removes certain intrinsic membrane proteins yielded very little immunoreactive material. These results suggest that collagenase is a tightly associated membrane protein.

Selective Binding of Membrane Collagenase to an Immunoaffinity Column. Detergent extracted RWP-I membrane fractions, which selectively bound to an anti-pColl affinity column, were examined for collagenolytic and gelatinolytic activity (Fig. 6). The degradation of [3H]-type I collagen and [3H]gelatin after APMA and stromelysin activation at 27°C and 37°C, respectively, across the column showed that collagenase activity was eluted from the affinity column with 6 M urea, while gelatinase...
did not bind to the column and was recovered in the flow through fraction. The elution profile of collagenase seems to be composed of two individual peaks, probably due to antibody populations of lower and higher affinity. When peak fractions of the eluate were incubated with type I collagen at 27°C and then analyzed by SDS-PAGE, the TC^ and the TC^ products were formed. No other degradation products were found (Fig. 6, inset). These products are considered specific for collagenase. Conversely, the peak of the gelatinase fraction produced multiple smaller fragments after incubation with gelatin at 37°C (data not shown).

Immunoprecipitation of Collagenase. To identify the species and determine the molecular weight of the membrane bound collagenase, the detergent extract was radioiodinated and precipitated with anti-pColl. As shown in Fig. 7, a single species with a molecular weight of about 55,000 is recognized (Fig. 7, Lane 2). The membrane bound collagenase required activation to produce collagenolysis, indicating that the enzyme is present in a latent form (data not shown). The M, 55,000 species is consistent in molecular weight with the procollagenase of connective tissue (15, 28). Other metalloproteinases, which have partial homology with collagenase, are not recognized by our antibody.

DISCUSSION

In the present study we have demonstrated tumor collagenase at the plasma membrane. The enzyme seems to be primarily

![Fig. 7. Identification of membrane collagenase by immunoprecipitation. ^125I-labeled detergent extract of plasma membrane was immunoprecipitated with anti-pColl. Lane 1, whole plasma membrane extract; Lane 2, a M, 55,000 collagenase is the only immunoreactive membrane component. Molecular markers are phosphorylase a (M, 94,000), transferrin (M, 77,000), bovine serum albumin (M, 68,000), heavy chain of lgG (M, 55,000), ovalbumin (M, 43,000), and carbonic anhydrase (M, 29,000). kDa, molecular weight in thousands.]

![Fig. 6. Purification of membrane collagenase by immunoaffinity chromatography. Detergent extracted RWP-I membrane preparations were applied to an anti-pColl immunoaffinity column and the bound collagenase was eluted as described in "Materials and Methods." Each fraction was assayed for collagenolytic activity in the presence of 1 mM APMA and 0.04 ng stromelysin at 27°C for 24 h. Gelatinolytic activity was measured in the presence of 1 mM APMA at 37°C for 18 h; 3200 cpm/tube were introduced. The activities obtained were within the linear range of the assays. Inset: type I collagen degradation analyzed by SDS-PAGE. Lane 1, 15 μg collagen only; Lane 2, 15 μg collagen incubated with 0.018 unit of purified human synovial fibroblast collagenase at 27°C for 48 h (23); Lane 3, 15 μg collagen incubated with 30 μl of fraction 48. TCA and TCB products are formed. Proteins were stained with Coumassie brilliant blue R-250.]

![Table 1 Differential distribution of gold grains on plasma membrane of cell projections versus cell body in intact RWP-I cells. A cluster of gold particles is counted as 1 grain. For definition see Fig. 3a.]

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* Projection index = Grains on projected membrane/relative length unit
* Relative length unit.

![Fig. 5. Immunoblot analysis of RWP-I membrane collagenase. Aliquots of whole cell homogenate and of each extraction step were analyzed with anti-pColl. Only detergent treatment was able to extract a significant amount of collagenase. Left row: wch, whole cell homogenate; 79 μg; kcl, KC1 extracts, 390 and 39 μg; but, butyl alcohol extracts, lipid phase, 18 and 3 μg; right row: but, butyl alcohol extracts, aqueous phase, 89 and 20 μg; glu, N-octyl glucoside extracts, 104, 21, and 5 μg.]

### Table 1 Differential distribution of gold grains on plasma membrane of cell projections versus cell body in intact RWP-I cells

A cluster of gold particles is counted as 1 grain. For definition see Fig. 3a.
present in the zymogen form and is activated with organomer-
curials and MMP-3 in vitro. In vivo, other matrix proteinases,
such as the plasminogen activator/plasmin system could func-
tion as activators as well as regulators of membrane associated
collagenase (29). Also, based on sequence homologies at the
site of activation between all members of the matrix metallo-
proteinase family known so far, autoactivation and mutal acti-
vation have recently been shown to be important mechanisms
for these enzymes (23, 24, 30). The collagenase molecules are
concentrated at the cytoplasmic face of the membrane with
some degree of expression at the extracellular surface. To
our knowledge, this is the first ultrastructural immunolocalization
of a metalloproteinase. Our studies were possible because tumor
collagenase cross-reacts with an immunoprobe raised against
rheumatoid synovial fibroblast collagenase. Immunoprecipita-
tion indicates that the tumor collagenase is a M, 55,000 mole-
cule consistent with the molecular weight of the secreted con-
nective tissue procollagenase. Furthermore, the immunological
cross-reactivity as well as the pattern of substrate degradation
suggest a high degree of conservation in the primary structures
between the two molecules, and even possible identity. The fact
that tumor collagenase was extractable from plasma membrane
with detergent, but not with high salt washes, is evidence that
the enzyme is tightly associated with the membrane and not
just loosely adsorbed. When the activity of membrane associ-
cated collagenase is compared with secreted collagenase in con-
ditioned medium, it appears that the cell membrane represents
a major portion of enzymatic activity in RWP-I cells. However,
differential half-life, activation status, and local inhibitor con-
centration ultimately will determine the activity in vivo.

Our morphological data can reflect a number of possible
associations of enzyme and plasma membrane; e.g., (a) the
enzyme may be directly anchored within the lipid bilayer of
the membrane. Phosphatidyl inositol has been described as a co-
valently linked lipid anchor for a number of membrane associ-
ated enzymes (31). (b) The enzyme may undergo transmem-
braneous secretion but be partially or completely captured by
a high affinity membrane bound collagenase receptor. This type
of pathway has been shown to apply to the urokinase-type
plasminogen activator in normal and malignant cells (see Ref.
32 for a brief review). The failure to extract collagenase activity
from plasma membrane preparations with high salt solutions
makes this possibility unlikely but does not entirely exclude it.
(c) Collagenase may be transiently accumulated at the inner
surface of the plasma membrane and subsequently be released
as soluble enzyme via an exocytosis-like secretory pathway. The
ultrastructural image we observe could then be due to enzyme
in transit through the membrane. (d) The expression of colla-
genase on the cell surface may be a transient phenomenon that
precedes the shedding of portions of the plasma membrane as
vesicles. Such a pathway has been described for cathepsin B-
like cysteine proteinase (33). Also, electron microscopic studies
on invasive carcinoma in vivo suggested destruction of connec-
tive tissue by membrane vesicles in the immediate vicinity of
the invading epithelial cells (34, 35). Although we favor the
possibility of a genuine collagenolytic activity of plasma mem-
brane at points of contact between cell and substratum, our
data do not definitively distinguish among the above alterna-
tives. A combination of mechanisms is also conceivable.

The pathway of collagenase in normal mesenchymal cells has
been well described and appears to be a simple secretory one,
but it is not known whether there is concentration at the cell
surface. In pulse chase experiments of human skin and rabbit
synovial fibroblast cultures, collagenase appears rapidly in the
culture medium within 35 min after synthesis (15, 28). Immu-
nofluorescent collagenase sites are found abundantly within the
connective tissue matrix, whereas the cytoplasm is relatively
free of enzyme (36–38). These findings are consistent with the
secretion and diffusion of soluble enzyme into the matrix.

The notion of proteolytic activity at points of contact has
recently become more important. Urokinase-type plasminogen
activator has been localized to points of local contact in normal
and sarcoma cells (39). Fibrinolysin/urokinase-type plasminogen
activator has been localized to points of local contact involving the activation of an integral membrane proteinase by pp60" which itself is
present at the inner surface of the plasma membrane (40). Our
findings of selective distribution of collagenase at basal type
microvillous projections supports directional proteolysis via
contact sites at the cell-stroma interface. Electron microscopic
studies of reconstituted basement membranes have demon-
strated that tumor cell invasion of extracellular matrix proceeds
by the formation of specialized pseudopodia that form adhesion
contacts and maximize local hydrolysis by membrane bound
proteinases at the interfacing surface area (39). Various human
breast cancer cell lines required direct contact for the degrada-
tion of endothelial basement membrane (41). Clearing of sub-
strate with release of degradation products of basement mem-
brane type IV collagen, laminin and fibronectin occurs only
along the path of tumor cells and beneath cellular processes,
suggesting that it is due to cell surface proteinases rather than
secreted enzymes (42–44).

Collagenase localization studies in tumor cells have been
limited until now. Only light microscopic studies using immu-
nofluorescence have been performed. In head and neck tumors
(45, 46) and in melanoma (38), the intercellular stroma exhibits
bright immunofluorescence when stained for collagenase. In
permeabilized cells, there is a sharp border between cytoplasm
and matrix, thus outlining the silhouette of the tumor cells,
while more than 90% of the tumor cells themselves are negative.
This is consistent with the existence of collagenase domains on
the cell surface, particularly in view of the limited resolution
of immunofluorescence. Our high resolution ultrastructural study
lends further support to this concept.

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COLLAGENASE IN HUMAN PANCREATIC CARCINOMA

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Ute M. Moll, Bernard Lane, Stanley Zucker, et al.


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