Matrix Metalloproteinase Matrilysin Is Constitutively Expressed in Adult Human Exocrine Epithelium

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The proteolytic activity of matrix metalloproteinases is involved in normal and disease-related remodeling processes. One member of this family, matrilysin, can degrade a wide spectrum of connective tissue proteins, suggesting that this enzyme is involved in numerous and diverse biologic processes. In fact, recent studies have shown that matrilysin is expressed in developing hair follicles and glands. Using in situ hybridization and immunohistochemistry, we examined the sites of matrilysin expression in normal and diseased adult skin. In normal mature skin, matrilysin mRNA and protein was strongly and consistently expressed in ductal cells and in some secretory cells of all eccrine and apocrine glands and was not found in any other cell type. A similar tissue distribution was also found in numerous benign inflammatory skin lesions, and prominent expression of matrilysin mRNA and protein was also found in glandular disorders such as axillary hidradenitis and sweat gland tumors. These findings indicate that matrilysin is a constitutive product of the epithelium of dermal glands and that its expression may not be related to a disease-specific or remodeling process. Because of its extensive expression in dermal glands, we assessed whether matrilysin might be produced by all exocrine glands. Indeed, we detected matrilysin mRNA and immunoreactive protein in the ductal and glandular epithelium of mammary and parotid glands, pancreas, liver, prostate, and the serous acini of peribronchial glands of the lung. Thus, our findings indicate that matrilysin is constitutively produced by exocrine epithelial cells throughout the body. Because of its broad catalytic activity, we speculate matrilysin may participate in the normal function of exocrine glands by preventing glandular obstruction. Key words: eccrine glands/exocrine glands.


Matrilysin (also called MMP-7 and previously called punctuated metalloproteinase, or PUMP) is the smallest of the matrix metalloproteinases. It lacks the C-terminal hemopexin-like domain contained by all other members of this gene family [8] and thought to define substrate specificity [3]. Still, matrilysin has many characteristics of metalloenzymes, such as secretion in a latent form, activation in vivo by organomercurials, and inhibition by tissue inhibitor of metalloproteinases (TIMP) [9]. In addition, this enzyme has a broad substrate specificity, being able to degrade proteoglycans, type IV collagen, fibronectin, entactin, denatured collagens, and insoluble elastin [10,11], and it can also activate procollagenase [12], as well as other zymogens [13,14], and inactivate c1-antitrypsin [15]. These diverse catalytic activities suggest that matrilysin may function in tissue processes beyond its role in matrix remodeling.

Matrilysin is expressed in many forms of malignant tumors [16–21], in inflamed mesangial cells [22], during certain phases of uterine cycling [23], and during skin gland development [24], and it has been suggested that proteolytic activity of this metalloproteinase aids tissue growth [24] and contributes to tumor progression [25,26]. While studying the expression of metalloproteinases in normal skin, healing wounds, and various inflammatory skin diseases, we found that matrilysin was constitutively expressed by the epithelium of all sweat glands, confirming the stated observations of Karelina et al [24]. We thought that matrilysin may be a common product of exocrine tissue and indeed we found prominent expression in the ductal epithelium of all exocrine glands examined. This widespread and constitutive expression suggests that matrilysin serves an important and common function in exocrine organs.
MATERIALS AND METHODS

Tissues Formalin-fixed, paraffin-embedded archival specimens were obtained from the Department of Pathology, Washington University School of Medicine. We examined normal skin (n = 10), axillary hidradenitis (n = 4), cylindroma (n = 4), syringoma (n = 6), plus 46 samples of various benign inflammatory skin diseases, such as pyogenic granuloma, lupus erythematosus, necrobiosis lipoidica, Kerley's disease, pseudepitheliomatous elastocytic, actinic keratosis, milia, transient acantholytic dermatosis, and various forms of chronic ulcers [27]. In addition, we examined normal (n = 2) and mildly inflamed (n = 2) pancreas, normal parotid gland (n = 3), normal liver (n = 3), prostate with areas of nodular hyperplasia (n = 8), normal lung (n = 6), fibrocystic (n = 4) and atrophic (n = 1) nonlactating mammary gland, and mammary fibroadenoma (n = 3). All samples were from adult patients. Although some of these archival specimens were of tissues with mild inflammation, fibrosis, or cancer, our studies concentrated on areas with normal tissue morphology.

In Situ Hybridization An 800-bp human matrilysin cDNA subcloned into pGEM-7Zf(+) was provided by Dr. Lynn M. Matrisian, Vanderbilt University. In vitro transcribed antisense and sense RNA probes were labeled with 35S-UTP as described [27]. The specificity of this probe for matrilysin mRNA has been characterized by Northern hybridization and in previous in situ hybridization studies [18,23,28]. Sections were hybridized with 35S-labeled RNA probes (4 × 10^6 cpm/µl of hybridization buffer) and were washed under stringent conditions, including treatment with R.Nase-A, as described [27,29]. After autoradiography for 14 to 28 d, the photographic emulsion was developed, and the slides were stained with hematoxylin and eosin.

Antibody Purification Antiserum was raised in rabbits against a synthetic peptide corresponding to amino acids 93–108, and, as shown previously, this antibody specifically recognizes both the 28-kDa zymogen and the 19-kDa activated form of matrilysin [28]. For immunohistochemistry, anti-matrilysin antibodies were affinity purified from serum. For this, bovine serum antibody-conjugated synthetic peptide, which was also used for immunization, was coupled to Affi-Gel 100 (BioRad, Richmond, CA), and anti-matrilysin antibodies were adsorbed to the column as described in detail [30]. Essentially all immunoreactivity, as assessed by direct enzyme-linked immunosorbent assay [28], was eluted with 0.2 M glycine, pH 2.3, and was adjusted to the original volume of serum added to the column.

Immunohistochemistry Deparaffinized 5-µm sections were processed for immunohistochemistry as described [27]. Endogenous peroxidase activity was blocked by incubation in 0.3% H2O2 for 30 min at room temperature. Affinity-purified anti-matrilysin antibody was diluted 1:1000. Bound antibody was detected using a Vectastain ABC Elite kit (Vector Laboratories, Inc., Burlingame, CA) following the manufacturer's instructions. Enzyme activity was detected using 3,3'-diaminobenzidine tetrahydrochloride as chromogenic substrate. Sections were counterstained with Harris hematoxylin. For negative controls, we processed sections with preimmune serum at a comparable dilution, or we co-incubated affinity-purified antibodies with excess peptide antigen (5 µg/ml) to inhibit specific interactions with matrilysin in the tissue.

RESULTS

Expression of Matrilysin in Cutaneous Glands Using our affinity-purified antiserum, Karelina et al [24] found signal for matrilysin protein in the secretory cells of adult eccrine glands, but it was not clear from their studies if this signal represented active production, storage, or endocytosis of the protein. In our studies, we used in situ hybridization and immunohistochemistry to localize the cellular sources of matrilysin expression and the distribution of the protein. In all samples of normal adult skin, matrilysin mRNA was detected in eccrine sweat glands, and strong signal was consistently seen in all ductal epithelial cells of all types of dermal appendages (Fig 1a) plus inset, small arrows). As assessed by immunostaining with affinity-purified antibody, all mRNA-positive cells in eccrine sweat glands contained immunoreactive protein, but the relative intensity of immunostaining did not correspond to the relative autoradiographic signal strength seen by in situ hybridization. For example, strong immunoreactivity was detected in both the clear and dark cells of the secretory coils (Fig 1b, large arrows), which were typically negative (Fig 1a, large arrow) or only weakly positive for matrilysin mRNA. In contrast, relatively faint immunostaining was seen on the luminal edge of ductal epithelial cells at the region of the glycoctax (Fig 1b, small arrows), even though these cells consistently had strong signal for the mRNA. No specific staining for matrilysin protein was seen on sections processed with pre-immune serum (Fig 1b, inset) or incubated with specific antibody in the presence of excess peptide antigen (data not shown), nor was any autoradiographic signal seen on sections hybridized with a sense probe (data not shown). In all skin samples, no signal for matrilysin mRNA or protein was detected in epidermal, follicular, or myoepithelial cells or in any other cell type.

As in normal skin, expression of matrilysin was seen in dermal appendages of numerous, benign inflammatory skin conditions of diverse etiologies (see Materials and Methods). As before, the predominant signal for matrilysin mRNA was seen in ductal epithelial cells and strong immunostaining was consistently observed in the secretory portion of all glands (data not shown). However, no selective pattern of matrilysin mRNA expression or immunoreactivity was observed among any of these pathologic conditions. In axillary hidradenitis, prominent immunostaining was seen in the epithelium of apocrine glands (Fig 2A) similar to that in normal apocrine glands (data not shown). In cylindromas, matrilysin mRNA and protein co-localized and were seen only in the

Figure 1. Localization of matrilysin mRNA and protein in dermal exocrine glands. a) Sections of normal human skin were hybridized with 35S-labeled antisense RNA for matrilysin mRNA. Under dark-field illumination, autoradiographic signal for matrilysin mRNA was seen in the eccrine sweat ducts (small arrow), whereas no signal was seen in sweat coils (large arrow). Inset: as seen under higher magnification, signal for matrilysin mRNA was confined to ductal epithelial cells, whereas the outer cells (small arrow) were negative. Bar, 25 µm. Autoradiographic exposure for all in situ hybridization sections was 24 d. No signal was detected on sections hybridized with 35S-labeled sense RNA probe (not shown). b) Immunostaining of sections parallel to that shown in A demonstrates strong signal for matrilysin protein in sweat coils (large arrows) and weak signal on the luminal surface of sweat ducts (small arrows). Inset: no immunoreactivity was seen on sections processed with preimmune rabbit serum. Bar, 50 µm for inset.
Figure 2. Expression of matrilysin in sweat gland tumors and axillary hidradenitis. A, C) In cylindrornas, matrilysin mRNA (A) and protein (C) were detected in the luminal, differentiated epithelium (arrows). Bar, 50 μm. B, D, F, G) In syringoma, prominent signal for matrilysin mRNA (B) and protein (D) were seen in the numerous small ducts (arrows) characteristic of this tumor. Bar, 200 μm. F, G) The area around the arrows marked 1 in B and D are presented under higher magnification to show that the signal for matrilysin protein (F) and mRNA (G) was confined to the epithelium of the ducts. Bar, 20 μm. E) In axillary hidradenitis, strong signal for matrilysin protein was detected in all duct epithelial cells. Bar, 50 μm.
Figure 3. Matrilysin is expressed in the ductal epithelium of various exocrine glands. A) In parotid gland, strong immunostaining signal was seen in all ductal epithelium (large arrows). No matrilysin was detected in serous cells (stained blue). B) No signal was detected in sections processed with pre-immune serum. C) In liver, positive immunostaining for matrilysin was seen in the epithelium of bile ducts (large arrow), and no staining was detected in normal-appearing hepatocytes (small arrows). D) In pancreas, matrilysin was seen in large interlobular (large arrow) and small intralobular (small arrow) pancreatic ducts but not in any acinar cells. E) In peribronchial glands of the lung (a portion of the airway cartilage [c] is seen to the left), positive immunostaining for matrilysin was seen in the serous and ductal epithelial cells (large arrows) and not in any mucous cells (small arrows). A,B,E, bar, 100 μm. C,D, bar, 50 μm.

differentiated ductal cells (Fig 2A,C), but no signal was detected by either assay in the less differentiated cells of these tumors. In syringomas, matrilysin was prominently expressed in the cells lining the numerous small ducts characteristic of this disease (Fig 2B,D,F,G).

Matrilysin in Other Exocrine Glands  Because matrilysin was consistently expressed in the epithelium of dermal appendages, we reasoned that this protein may be found in other exocrine glands. Indeed, matrilysin was detected in exocrine tissue in parotid and mammary glands, liver, pancreas, prostate, and lung and, as we saw in skin, this metalloproteinase was typically expressed by the ductal epithelium. In parotid gland (Fig 3A) and pancreas (Fig 3D), prominent signal for matrilysin protein was confined to the epithelium of intralobular and interlobular ducts; no signal was detected in the serous or acinar portions of these tissues. Similarly, in liver, immunostaining for matrilysin was restricted to the epithelial cells of bile ducts and was not seen in hepatocytes (Fig 3C). In morphologically normal lung specimens obtained from the tumor-free margins of lobectomy samples and from recipient lungs of transplant patients with primary pulmonary hypertension, the serous and ductal epithelium of peribronchial glands contained immunoreactive matrilysin (Fig 3E, large arrows), whereas the mucous cells were consistently negative (Fig 3E, small arrows). In non-malignant prostate with age-related, mild hyperplastic changes, the glandular and ductal epithelia were strongly positive for both matrilysin mRNA and protein (Fig 4A,4'). In mammary
Figure 4. Expression of Matrilysin mRNA and Protein in Prostate and Mammary Gland. In serial sections of a prostate specimen with age-related hyperplasia, strong signal for matrilysin protein (A) and mRNA (A') was seen in the ductal (arrows) epithelial cells. Autoradiography was for 17 d. B) In mammary gland, positive immunostaining for matrilysin protein was detected in the epithelium of large acini and ducts (large arrows) and small terminal ductules (small arrows). B') No staining was seen on a serial section processed with pre-immune serum. Bar, 50 μm for all panels.

gland, the secretory epithelium of ducts and ductules stained positively for matrilysin protein (Fig 4B) and also had strong signal for the mRNA (data not shown).

DISCUSSION

We report that matrilysin is constitutively produced by the epithelium of dermal appendages, in agreement with the finding of Karelin et al [24], and we have extended these findings by showing that this important metalloproteinase is also expressed by most if not all human glandular epithelia. In all samples, matrilysin was prominently expressed by ductal epithelial cells and in many sites, notably eccrine, prostate, and peribronchial glands, many secretory cells were stained as well. In eccrine sweat glands, the secretory coils were strongly stained by antibody but had weak signal for matrilysin mRNA, whereas ductal cells strongly expressed matrilysin mRNA but had only a weakly immunoreactive signal (Fig 1). A possible explanation for these findings is that matrilysin is expressed at relatively low levels and stored in secretory epithelial cells but is expressed at higher levels and is readily secreted by ductal cells. Consistent with this idea are ultrastructural observations showing that secretory coil cells contain protein-storing granules that are released together with sweat during cholinergic
stimulation [31]. Although cells of the secretory coil and sweat ducts arise from the basal layer of fetal epidermis [32], their secretory functions may be controlled differently.

To begin to understand the functional significance of the constitutive expression of matrilysin in dermal appendages, we assessed whether this metalloproteinase is a characteristic product of all exocrine tissue, and our findings indicate that it is. Because mammary glands are specialized cutaneous apocrine glands, we were not surprised to find matrilysin protein in the epithelial structures of this tissue as well. Consistent with our findings, matrilysin mRNA was detected by Northern hybridization in both normal and neoplastic breast epithelial cells [33]. In the prostate specimens, we found prominent expression of matrilysin in glandular secretory and epithelial cells by both immunostaining and in situ hybridization in all samples examined. Earlier reports showed by Northern hybridization that matrilysin mRNA is present in normal and malignant lung [17,19], and here we demonstrate that this expression likely originates from the serous and ductal cells of peribronchial glands. We have also shown that matrilysin is produced in the ductal and glandular epithelium of parotid gland, pancreas, and liver. Thus, in addition to the skin, matrilysin is apparently a characteristic product of exocrine glands.

A significant finding of our studies is that, unlike other metalloproteinases, the production of matrilysin is constitutive and is apparently restricted to a few cell types, namely, exocrine epithelial cells. Despite its broad substrate specificity, we did not detect expression of matrilysin in other tissue structures or cell types, even in the numerous and various dissected human skin samples we examined. Expression of certain metalloproteinases is induced during normal remodeling processes such as production of collagenase by keratinocytes and fibroblasts during wound healing [27] and metalloelastases during salivary gland development [34,35], embryonic implantation [36], and breast lactation cycles [37,38], but evidence of constitutive expression of any member of this enzyme family has been limited to circulating cells [28,39,40]. Although matrilysin is apparently expressed by exocrine tissues, it may not be continually produced in all glandular epithelia. For example, in the cycling uterus, matrilysin is found in the endometrial epithelium only during the proliferative stages [23].

The common expression of matrilysin among exocrine epithelium suggests that this enzyme serves a critical role in glandular function. Other proteolytic activities have been found in exocrine secretions, and the function of these enzymes has often been thought to contribute to maintaining flow. For example, gelatinolytic proteinases have been implicated in semen liquefaction and secretion [41] and in tissue remodeling of rat prostate [42,43]. Sweat contains several proteolytic enzymes, including the potential metalloproteinase activators kallikrein and urokinase-type plasminogen activator [31]. In addition, and consistent with our findings, a caseinolytic protease of the size and pH optimum of matrilysin is present in eccrine sweat [44]. Although the functional significance of proteinases in sweat is not known, these enzymes may digest sweat glycoproteins and membrane debris, thereby maintaining a patent lumen and easing ductal flow. The broad and potent substrate specificity of matrilysin provides a reasonable candidate protease for fulfilling this biologic function.

Alternatively, matrilysin may function in basement membrane and interstitial remodeling. During sweat gland and follicle development, matrilysin is expressed by the invading epithelial front [24] suggesting that this metalloproteinase aids cell migration by degrading a barrier of extracellular matrix. Similarly, matrilysin has been detected in numerous carcinomas, and it has been suggested that this proteinase promotes metastasis [45]. However, because matrilysin is expressed in colon adenomas [21] and because overexpression of matrilysin in colon carcinoma cells does not enhance their invasive potential but does increase their tumorigenicity, this metalloproteinase may thus serve some role in the early stages of oncogenesis [26]. Because we examined fully mature tissues, the prominent and extensive expression of matrilysin we detected is likely not involved in morphogenesis or cell migration. As in a mechanism proposed for regulating cell behavior during mammary gland cycling [37], matrilysin may alter cell-matrix contacts, thereby modulating gland-cell phenotype and epithelial differentiation. This idea implies that matrilysin somehow modulates cell turnover or proliferation, but our findings argue against this idea. We consistently saw matrilysin in all duct cells in all samples, and it is unlikely that all cells in the many specimens we examined would be caught at the stage of differentiation. Apparently, with the exception of the uterus [23], matrilysin is expressed continuously in glandular epithelial cells. Thus, as discussed above, we speculate that because of its constitutive expression and broad catalytic activity, matrilysin may be involved in keeping ducts patent and maintaining the flow of secretions. Future experiments, such as a careful assessment of glandular function in matrilysin-deficient mice, are needed to address this hypothesis.

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REFERENCES


Stähle-Bickelth M, Parks WC: 92 kDa gelatinase is actively expressed by eosinophils and secreted by neutrophils in invasive squamous cell carcinoma. Am J Pathol 142:995–1000, 1993


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