Chapter 11

MATRIX DEGRADATION IN PROSTATE CANCER

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Abstract: Metastasis is the critical factor in the lethality of prostate cancer. Alterations in expression of cellular adhesion, cytoskeletal and cell motility proteins, and constituents of the extracellular matrix (ECM), are intimately involved in tumor cell invasion and metastasis. Proteolysis of ECM is a highly regulated process that has traditionally been considered fundamental to tumor cell invasion and metastasis, permitting physical passage of malignant cells. But proteolytic functions are now recognized as instrumental in tumor growth through release of growth factor and chemoattractant molecules, modification of cell surface receptors, and molecular processing of cytokines, other proteases, and ECM proteins. This chapter focuses on the control of proteolytic systems that cleave ECM proteins in studies of human prostate tissues.

Key words: Basement membrane, extracellular matrix, stroma, matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, cathepsin B, cathepsin D, stefin A, serine proteases, prostate specific antigen, kallikreins, plasminogen activators, hepsin, serpins, plasminogen activator inhibitor type I, maspin

1. INTRODUCTION

Metastasis is a critical aspect in the lethality of prostatic adenocarcinoma. The movement of prostatic cancer cells from the acinar epithelium through the basement membrane (BM) and interstitial stroma into blood or lymph vessels, with subsequent malignant cell migration and colonization of distant tissue sites, is facilitated by altered expression and degradation of cell

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adhesion and extracellular matrix (ECM) proteins. Although alteration in expression of cellular adhesion molecules, such as the integrins, and changes in cytoskeleton and of cell motility proteins are intimately involved in tumor cell invasion and metastasis, the focus of this chapter is the control of proteolytic systems that cleave BM and stromal ECM proteins, promoting growth of the tumor and passage of malignant cells in the prostate from one biological compartment to another. In addition, this review also focuses on studies of human prostate tissues. Our current understanding of the highly regulated process of ECM protein proteolysis is that, in addition to degradation of ECM proteins permitting physical passage of malignant prostatic cells through the BM and supporting stroma, proteases also influence prostatic tumor growth through the release of growth factor and chemoattractant molecules from the ECM, and by the proteolytic processing of cytokines, other proteases, and existing or newly formed ECM proteins.

2. BASEMENT MEMBRANE

The literature describing the BM and interstitial stroma of normal and cancerous human and animal tissues is extensive (1–6). At the ultrastructrual level the BM is resolved into the lamina rara (or lucida), lamina densa, and subjacent reticular lamina (Figure 1a). The BM controls the passage of macromolecules including proteins between the epithelium and subadjacent stroma (3). BMs are present not only around acini and ducts in the prostate, but also smooth muscle, nerve fibers, and endothelia of blood vessels and capillaries. BMs are attenuated in lymphatic vessels (1). Type IV collagen, entactin, laminin, fibronectin, and heparan sulfate proteoglycans are prominent proteins in the acinar BM. Type IV collagen is a triple helical molecule 400 nm in length comprised of 6 possible chains; the isoform of two α1(IV) and one α2(IV) chains being present in all BMs (5). The BM of fetal and normal prostate and prostatic intraepithelial neoplasia (PIN) contain the classical α1(IV) and α2(IV) chains of type IV collagen (1, 6, 7) as well as the more novel α5(IV) and α6(IV) chains, but not α3(IV) chains (6). The prostatic acinar BM demonstrates entactin (8) and strong Type VI and XV and faint Type XIX collagen immunoreactivity, whereas all of these components are localized in vascular and smooth muscle BMs in the prostatic stroma (9, 10). Laminins are a heterotrimeric group of high molelcular weight glycoproteins comprised of α, β, and γ chains of which there are 11 genetic chain variant forms (11). Immunogold electron microscopy shows a distribution of laminin (gold particles) predominantly in the lamina rara, and some in the lamina densa layers of prostatic acinar

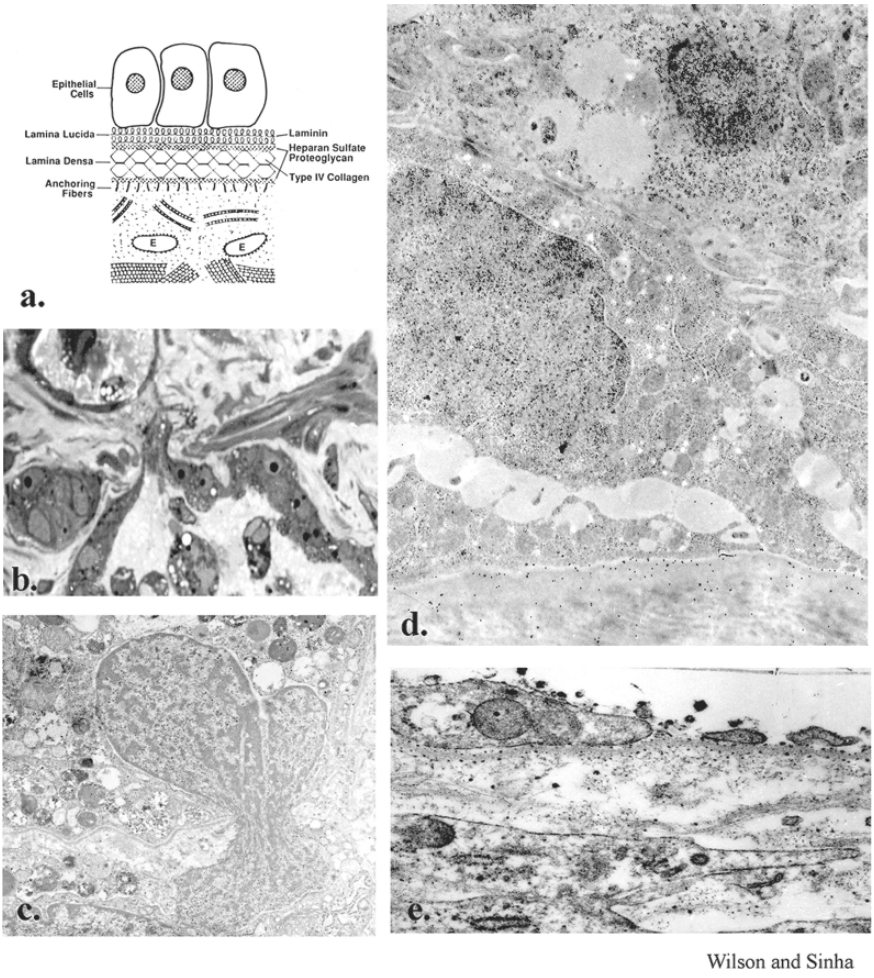


Figure 1. **A.** Diagrammatic representation of the relationship of epithelial cells with the underlying basement membrane and of its constituents in prostatic stroma. The basement membrane is distinguished cytolotically into the lamina lucida and densa layers. Laminin is subjacent to the epithelial cells whereas type IV collagen and heparan sulphate proteoglycan are intimately associated with the lamina densa. Anchoring fibers interface between the basement membrane and the subjacent connective tissue. The authors modified the figures of Martinez-Hernandez and Amenta (17) and Martin et al. (18). **B.** A micrograph of cancerous glands illustrates the migration of invasive cells from prostatic acini: some cells appear to be penetrating the basement membrane and invading the prostatic stroma, while other cells are still within acini. Also observe invasive cells and glands in the prostatic stroma. **C.** An electron micrograph of an invasive cell illustrates portions of the cell and nucleus within the acinus while the other portion protrudes into the prostatic stroma through a focally breached basement membrane. This invasive cell also illustrates well-developed Golgi complex, secretory and lysosomal granules (continued on page 224)

BMs (Figure 1d). Intense laminin localization is found in fetal prostate epithelial BMs, but BM areas locally thickened or non-reactive to laminin antibodies are also found (7). The α3, β3, and γ2 subchains of laminin 5, which is involved in hemidesmosome attachment of basal cells to the basal lamina, are present in the BM of normal prostate (11–13). Heparan sulfate proteglycans (7, 14), including perlecan, a multidomain heparan sulfate proteoglycan (15), are also components of BMs of prostatic acini and blood vessels, but not of smooth muscle (14). The distribution of heparan sulfate proteoglycans correspond to the distribution of heparan sulfate anionic charge sites in the acinar BM, which extend from the BM on bundles of collagen fibrils into the stromal interstitium and to fibroblasts. Anionic sites associated with the lamina rara and densa spanned over fenestrae of prostatic capillaries (Figure 1e), suggesting that these negatively charged sites may regulate passage of charged macromolecules through these fenestrae.

There are distinct disruptions to the BM of prostatic acini that permit physical passage of cancer cells into the stroma (Figures 1b, 1c). Breaching of the BM is accompanied by focal and wide spread diminished immunoreactivity for laminin, heparan sulfate proteoglycans and type IV collagen in prostatic acinar BMs in prostate cancers (1, 4, 14). However, distinct BM formations in contact with the stroma are still found in highly malignant prostatic lesions and metastases (4). Type IV collagen α5 and α6 chains are not detected in prostate cancers, whereas α1 and α2 type IV collagen chains continued to be expressed (6). Similarly, the message and not the protein of the β3 and γ2 chains of laminin 5 are detected in carcinoma cells (16). Changes in the production of BM constituent proteins, as well as degradation of BM proteins appears to be prerequisite for migration of prostate cancer cells through this structure (Figures 1b, 1c).

Figure 1. (continued) **D.** An electron micrograph illustrates localization of rabbit anti-laminin IgG in the basement membrane of a prostatic gland using goat anti-rabbit IgG complexed with 10–15 nm immunogold particles. Gold particles are distributed in both the lamina lucida and densa layers of the basement membrane. **E.** Micrograph illustrates localization of heparan sulphate-rich anionic sites using the cationic probe, polyethylenimine (PEI), in the basement membrane of prostatic endothelial cells. The presence of anionic sites over fenestrae indicates that negatively charged sites have the potential of regulating passage of charged macromolecules across the basement membrane of blood vessels, especially capillaries. Several studies have shown that heparan sulphate-rich anionic charge sites correspond with the distribution of HSPG in the BM (19–21).

3. INTERSTIAL STROMA

The ECM proteins of the prostatic interstitial stroma include collagens and non-collagenous proteins like fibronectin, elastin, and proteoglycans. Fibronectins are high molecular weight dimeric glycoproteins with binding sites for collagens, heparin/heparan glycoproteins, and cell surface receptors. Fibronectin is localized around glands and smooth muscle, whereas, collagen type III is distributed diffusely in the interstitial connective tissue of normal and benign prostatic hyperplasia (BPH) tissues (22, 23). Carcinoma is accompanied by an altered distribution (23) and increase in content (24) of fibronectin, due in part to a 3.5 fold increase in expression of the alternatively spliced fibronectin containing the ED-B segment. Expression of the ED-B domain is associated with fetal development, a variety of tumors, and wound healing (23). There is also an increased metabolism of collagen in prostate cancer, marked by an increase in collagen type I propeptides and collagen intermediate cross-linkage underscoring the increased synthesis of collagen, and increased matrix metalloproteinase (MMP)-2 activities correlated with collagen degradation (25). Galectin-1, a pleiotropic homodimer member of the β-galactoside-binding galectin family, and chondroitin sulfate containing proteoglycans, versican and decorin, are localized in the periglandular stroma of normal prostate and BPH, and are increased in prostatic carcinomas (26, 27). An increase in sialic acid (24), hyaluronan (28), and elastin (29) are also noted in prostate cancer.

In evaluating ECM components in prostatic carcinogenesis, it should be noted that changes in interstitial macromolecules also occur with formation of BPH. Gene expression by cDNA microarray analysis shows upregulation of ECM proteins laminin alpha 4 and beta 1, chondroitin sulfate proteoglycan 2, and lumican in BPH compared with normal prostate (30). There is a decreased amount of elastin message (31) and an increase in the ratio and size heterogeneity of the glycosaminoglycans chondroitin sulfate to dematan sulfate, with no quantitative change in hyaluronic acid and heparan sulfate content in BPH tissue (32).

Prostate adenocarcinoma, like several other solid organ cancers, demonstrates activation of the host stromal microenvironment or desmoplasia that features myofibroblasts and fibroblasts stimulated to express ECM components (33). Tenascin, a large ECM glycoprotein, is strongly expressed in the mesenchyme around developing prostatic glands, but is only weakly localized in periglandular matrix of normal or benign hyperplastic prostates, although it is consistently expressed in perivascular matrix of the same tissues (34, 35). In contrast to normal adult prostate and BPH, there is a broad and intense stromal distribution of tenascin in prostatic carcinomas (33–35).

In addition to tenascin, procollagen I and FAP (separase) are immunolocalized in stromal cells adjacent to PIN and carcinoma cells, indicating that these ECM changes occur early in carcinogenesis (33). Prostatic carcinogenesis is also accompanied by the down regulation of hevin, an acidic cysteine-rich ECM glycoprotein (36), and increased expression of osteopontin, an adhesive glycoprotein of the ECM containing a functional RGD cell binding domain (37).

4. PERICELLULAR PROTEOLYSIS

Proteolysis of ECM is fundamental to the invasion and metastasis of malignant cells in prostate cancer. MMPs, plasminogen activators, proteases of the coagulation system, and plasma membrane associated cathepsin B are representatives of diverse protease families that work in concert or in cascades to process ECM proteins (38). In general, regulation of expression of these protease groups occurs at the transcriptional, translational and post-translational levels. However, the specificity of control of extracellular proteolysis involves post-translation regulatory steps that include the production of these proteases in pro-enzyme inactive zymogens, activation of the zymogens commonly by proteolytic cleavage, specific subcellular localization through cell surface receptors or selective protein binding, and modulation of activity by endogenous inhibitor molecules.

4.1 Matrix Metalloproteinases

The MMP family is comprised of 25 or more structurally related enzymes that have been subclassified into 4 groups (collagenases, gelatinases, stromelysins, and matrilysins) based on ECM protein substrate specificity and into 8 groups based on their structure (5 secreted and 3 membrane-type sub-groups). Essentially any ECM protein can be cleaved by one or more of the MMP family (39–44). In many adenocarcinomas, matrilysin (MMP-7) is expressed in the epithelial compartment, whereas tumor cells induce (in part through EMMPRIN Extracellular matrix metalloproteinast inducer) other MMPs in adjacent host stromal cells. The stromal cell MMPs can subsequently be expressed in malignant epithelia of tumors that have undergone epithelial-to-mesenchymal transformation (42). The regulation of MMPs is complex and occurs at both transcriptional and post-translational levels. The MMPs are produced as latent zymogen molecules that must be proteolytically processed to become active. MMPs are localized to specific subcellular and extracellular sites since membrane-type MMPs (MT-MMPs)

are in the plasma membrane and secreted MMPs associate with select cell surface and ECM proteins (39, 41, 42, 44–46). Complex formation with tissue inhibitors of matrix metalloproteinases (TIMPs), a group of 4 glycoproteins with reported molecular sizes of 21–36 kDa, inhibits the activity of MMPs (40, 41, 44, 47, 48). In addition MMP activity can be thwarted by α2-macroglobulin, thrombospondin, TFPI-2 (tissue factor pathway inhibitor-2), NC1 domain of type IV collagen, CT-PCPE (carboxy-terminal fragment of pro-collagen C-terminal proteinase enhancer protein), and membrane-bound RECK (reversion-inducing cysteine-rich protein with Kazal motiffs) (42, 44, 46, 49). MMPs can also influence tumor growth and metastasis through cleavage of non matrix proteins that releases cell surface and matrix bound growth factors, exposes cryptic domains of ECM proteins that promote tumor cell migration, modifies growth factor receptors and adhesion molecules, regulates chemokine bioavailablity in chemokine directed cancer cell migration, and releases matrix-bound angiogenic factors and production of anti-angiogenic peptides from ECM proteins (42, 44–46).

Changes in expression of MMPs in the prostate is related to normal and pathological tissue organization changes (50). Morphogenesis in development and castration-induced regression of the prostate in the rat are marked by expression of the activated form of MMP-2 (51, 52). In the human prostate MMP-2 is upregulated in BPH compared with normal prostate (30), and MMP-2 has been localized by immunohistochemistry and *in situ* hybridization to basal cells, and to a lesser extent secretory epithelial cells, but not stromal cells of normal and BPH tissues (53–57). However, stromal cell MMP-2 immunoreactivity has also been reported (58). MMP-2 immunoreactivity is observed in PIN and is heterogeneous in intensity and location in prostatic adenocarinomas (Figure 2a) (54, 55, 57, 58), which express greater levels of MMP-2 protein and message (53, 59, 60). More intense MMP-2 staining in cribiform and solid/trabecular tumors is found in the cell layer adjacent to the stroma and in single or small clusters of tumor cells in the stroma (57, 61). MMP-2 mRNA transcripts, on the other hand, have been localized to stromal cells only (60, 62), or tumor but not stromal cells by others (54, 56). Increased expression of MMP-2, the ratio of MMP-2:TIMP-2 and particularly the active form of MMP-2, correlate with increasing Gleason score of prostatic cancers (56, 62, 63, 64). In addition, MT1-MMP, which has strong collagenolytic activity in its own right (46), and is involved in proMMP-2 activation (42, 44, 46), has a strong association of localizing with MMP-2 in prostatic tumors (58).

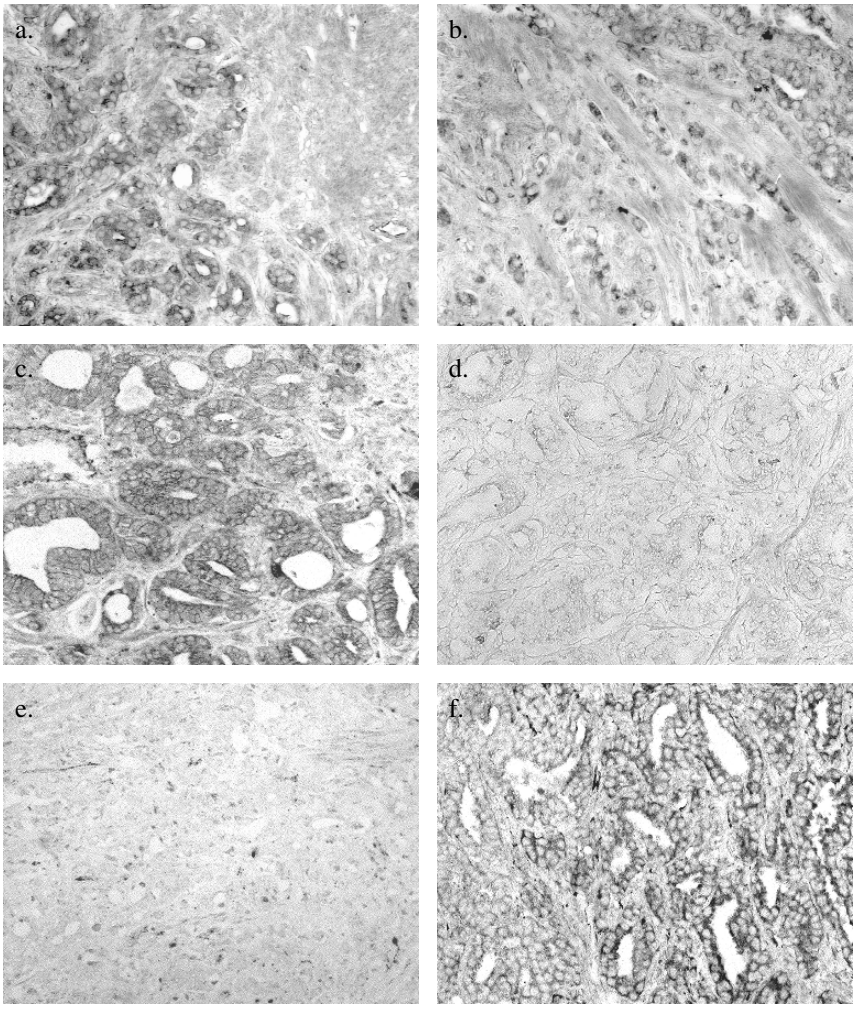


Figure 2. **A.** Immunohistochemical localization of MMP-2 in human prostate tumor cells (antibody from Dr. William Stetler-Stevenson). Tumor cells expressing MMP-2 are observed near the edge of this Gleason score 8 tumor, whereas, tumor cells more near the center of the did not show reaction products. **B.** Immunohistochemical localization of MMP-9 in human prostate tumor cells (antibody from William Stetler-Stevenson). MMP-9 expression is observed in prostate tumor cells (Gleason score 7), cells in the stroma, but only occasionally in acinar luminal cells. **C.** A micrograph illustrating strong tumor cell immunostaining for CB in a Gleason score 6 tumor. **D.** An adjacent section to that in Figure 2c illustrates markedly reduced immunostaining for stefin A. Comparison of Figures c and d shows a ratio of CB>stefin A in this Gleason score 6 tumor. **E.** This micrograph illustrates significantly reduced immunostaining for CB in a Gleason score 6 tumor. Compare with the CB immunostaining shown in Figure 2c. **F.** An adjacent section to that in Figure 2e illustrates strong immunostaining for stefin A. When compared to CB in Figure 2e, the ratio of CB<stefin A.

There are also discrepancies in reports of expression of MMP-9 in the prostate. MMP-9 immunolocalization has been reported as absent (57), or weak or absent in stroma but present in some prostatic cancers (Figure 2b), particularly those that are highly analplastic (65). MMP-9 is primarily in the pro-enzyme form (66). Likewise, expression of MMP-9 message was detected only in macrophages in areas of prostatic inflammation (60) and in the invasive edge of higher Gleason score tumors (64). The expression of MMP-9 in high grade prostate cancers may be indicative of mesenchymal-epithelial transformation that occurs with progression in tumors (67), particularly in view of the induction of MMP-2 and –9 in human epithelial cells in primary culture (68). Bombesin, a neuropeptide shown to stimulate MMP-9 secretion in human prostate tumor cell lines (69), is observed in the same cell populations expressing MMP-9 in higher grade prostatic tumors (70). A discrepancy between expression of bombesin and neuron-specific enolase indicates prostate cancer cells produce bombesin irrespective of neuroendocrine differentiation (70). The secretion of latent and active forms of MMP-2 and -9 has been observed in human prostatic secretions (71) and in experimental human prostatic organ and primary cell cultures (72, 73). However, Variani et al. (65) noted secretion of MMP-1, -2, and -9 in organ cultures of human prostate cancers in which the original tissues showed little or no immunohistochemical detection of these MMPs.

Epithelial cells in primary human prostate cancer also express greater levels of MMP-7 RNA and protein (59, 60). However the amount and proportion of the active and pro-enzyme forms of MMP-7 varied between cancers, and there was no correlation of extent of immunohistochemical MMP-7 expression with Gleason grade (60). Other studies show immunoreactivity of TIMP-2 (74) and MMP-3 and MMP-11 (57) in prostatic cancer stroma, the latter are particularly localized around blood vessels in the cancers. Messages for MT1 and MT3-MMP and TIMP-1 and –2 have been detected in both prostatic epithelial and stromal cells (75). There is also now data that show expression of members of the ADAMs (A Disintegrin And Metalloproteinase) and ADAMTS (ADAM with thrombospondin type I motifs) families in human prostate cancer cell lines (76). ADAMs are transmembrane proteins with disintegrin and metalloproteinase domains, however, only about half of the nearly 30 ADAMs have metalloproteinase activity and function in shedding of cell surface proteins, many of which function in growth regulation (42, 44).

Additional MMPs have been identified in the human prostate, but their role in prostate function or pathology have not been established. These

enzymes include MMP-26, which has amino acid sequence similarity with MMP-7 (77).

4.2 Cathepsins

The cathepsins are a large group of primarily lysosomal cellular proteinases that are classified functionally as to their pH optima and inhibitor specificity, and according to the amino acid structure of their active site. Cathepsins range is size from 14 to 650 kDa, can have exopeptidase and endopeptidase activities, and are routed to lysosomes by a receptor that recognizes mannose 6-phosphate on the enzyme molecule (78).

Cathepsin D is an aspartate endopeptidase that exists as a proenzyme of 48-52 kDa and a two-chain active form of 34 and 14 kDa (78). Cathepsin D is found in most cells but its activity is highest in phagocytic cells such as macrophages. Activation of procathepsin D removes a 44 amino acid peptide, which is able to stimulate proliferation of breast, colon, and prostatic cancer cells (79). Secretory and basal epithelial cells of normal and BPH are generally negative or weak in cathepsin D immunoreactivity, whereas normal transitional epithelium lining ducts, basal cell hyperplasia, and normal seminal vesicle are positive for cathepsin D expression (80, 81). Areas of PIN are positive for cathepsin D and carcinomas show heterogeneity in cathepsin D expression (80), with no particularly strong expression in tumor edges or tumor outside of the prostate (81, 82). The heterogeneity of lysosomes in the prostate is further supported by observations that cathepsin D positive ductal cells stained much more positively for the lysosome membrane-associated protein LAMP-2 than LAMP-1 (83). There does not appear to be a significant relationship between cathepsin D immunoreactivity and either Gleason grade (80, 82) or disease specific progression (84). However, quantification of cathepsin D levels in prostatic carcinoma biopsies showed no difference in tissue levels of cathepsin D over BPH in one study (85), increased levels in a second (86), and a correlation with tumor grade, but not postprostatectomy pathologic stage or disease recurrence in another (87). An examination of the molecular forms of cathepsin D show that prostatic carcinoma express active cathepsin D, whereas the proenyzyme form is predominant in normal and BPH tissues (88).

Cathepsin H is a cysteine type protease that can function as an aminopeptidase as well as an endopeptidase (78). The activities of cathepsin H do not differ between normal and cancerous prostate (89), but there is increased cathepsin H immunoreactivity in PIN and in prostatic cancers (90). An enzymatically active truncated form of cathepsin H with a 12 amino acid deletion in the signal peptide region has been detected in prostate cancer.

This truncated form of cathepsin H is localized in the perinuclear cytoplasm and less associated with lysosomes, but is secreted by prostate tumor cells (90). In contrast with cathepsin F, recently described in the prostate (91, 92), and some other cysteine cathepsins (C, O, K, W, Z), cathepsin B has been systematically studied in the prostate.

Cathepsin B is a cysteine endopeptidase with broad substrate specificity that includes ECM proteins such as laminin, fibronectin and proteoglycans. Cathepsin B and other cysteine-type cathepsins are inhibited by endogenous stefin (cystatin) protein inhibitors. It is usually found in perinuclear lysosomes of normal organs and nonmalignant tumors, but is found associated with the plasma membranes of many solid organ cancers (93–97). The exocytosis of the mature form of cathepsin B from cells (98) and localization of active cathepsin B at the tumor cell periphery, including cell surface and cell processes (99) support the concept that routing of cathepsin B to the cell surface facilitates degradation of ECM proteins and progression of malignant cells from one biological compartment to another (94, 99–103).

In the prostate, cathepsin B and stefin A protein and message are localized predominately in basal cells, and to a lesser extent in secretory cells, of normal prostate and BPH epithelia (104–108). There is heterogeneity in immunolocalization of cathepsin B and stefin A in prostatic cancers (Figures 2c–2f). Cathepsin B is localized by immunogold electron microscopy to cell processes, lysosomes, and vesicles in prostatic invasive cells (Figure 3a). The activities of cathepsins B are low in human prostate compared with other tissues (109), but its activity and that of cysteine protease inhibitors, have been found to be higher in normal vs cancer tissues (89), or that activities of cathepsin B did not differ in extracts of BPH and prostate cancer tissues, whereas, that of cyteine protease inhibitors was decreased in cancer (Figure 3b) (97). Cathepsin L activities have also been reported to be higher in normal vs cancer tissues (89), but its immunoreactivity absent in normal prostate (108). There is, however, strong localization of cathepsin B and L protein and message in neoplastic cells (105, 108, 110) in acini, isolated neoplastic cells and ragged glands in the stroma, and especially in the invasive edge of prostatic tumors (104, 105, 111). In addition, there is a shift of intracellular localization of lysosomes from the perinuclear cytoplasm to the cell periphery in prostatic carcinoma cells (97). In normal and neoplastic prostate there is co-localization of mature and pro-enzyme forms of cathepsin B in epithelial cells and a preponderance of pro-cathepsin B in stromal cells. However, mature but not pro-enzyme cathepsin B is localized in some neoplastic glands and subjacent stroma of prostatic cancers (112). Prostatic malignancy is also associated with

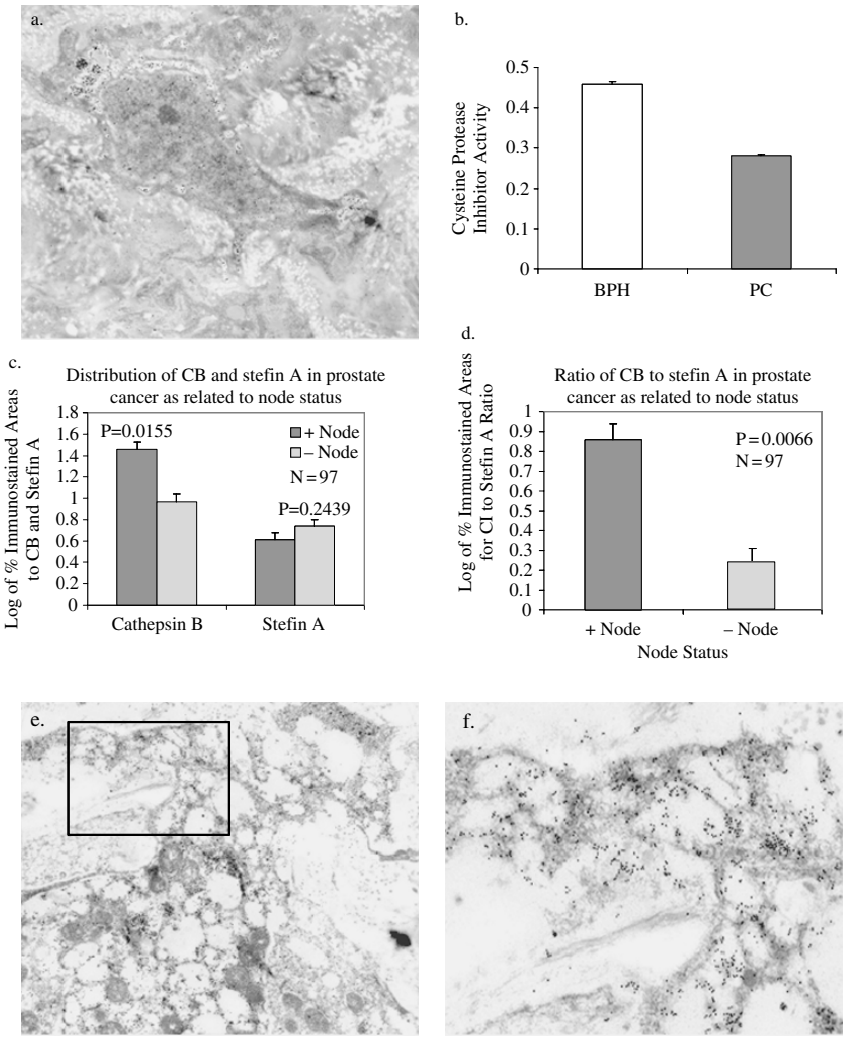


Figure 3. **A.** A micrograph illustrates an invasive prostate cancer cell localizing CB by immunogold electron microscopy. Invasive cell shows many processes, lysosome, and vesicles that have localized CB by immunogold techniques. **B.** Figure shows lower activity of cysteine protease inhibitors (CPI) in neoplastic prostate cancer (PC) when compared to benign prostatic hyperplasia (BPH). Activity is expressed in units/mg protein; columns, means; bars, SD (standard deviation) (97). An increase in CB activity in cancer may be due in part to lower levels of CPI, which may be involved in regulation of tumor aggressiveness. **C.** Figure illustrates the percent of immunostained areas for CB and stefin A in prostate tumors as related to positive and negative lymph nodes. Figure illustrates that CB was significantly higher ($p = 0.0155$) in tumor positive lymph nodes than in negative nodes. Differences in immunostaining for stefin A were not significant ($p = 0.2439$) in prostate tumors with positive and negative lymph nodes (114). Statistical analysis was conducted using Student's t-test.

increased cathepsin B and diminished cysteine protease inhibitor activites in the plasma membrane (113). Thus, there appears to be increased localization of the active form of cathepsin B at the cell surface of prostatic adenocarcinoma cells in an environment of low endogenous inhibitors, a situation that provides for localized, pericellular proteolysis of ECM proteins facilitating changes in tumor cell adhesion and mobility.

It has been recognized for some time that there is heterogeneity in clinical outcome for men with a given Gleason histologic score cancer; i.e., some men with Gleason 6 may succumb to this disease in a few years whereas others live 10 years or more. We found that within a given Gleason histologic score prostate cancer, tumors had varying levels of cathepsin B and stefin A expression, such that the ratio of the two could be $>$, $=$, or $<$ one (113). Our prediction was that tumors with a ratio of cathepsin B:stefin A $>$ one would be more biologically aggressive. Indeed, we have found a significant positive association of ratios of cathepsin B $>$ stefin A in primary prostatic cancers with the incidence of pelvic lymph node metastases (Figure 3c and 3d), and an increased rate of mortality of men whose prostatic cancers have a ratio of cathepsin B$>$stefin A (114). We are hopeful that the ratio of cathepsin B:stefin A will be useful in identfying the aggressive cancers within a given Gleason histologic score and that treatment regimens can subsequently be tailored accordingly.

4.3 Serine Proteases

There are two main groups of the serine class of protease that have been more highly studied in prostate pathobiology, the kallikreins, especially prostate specific antigen (PSA, hK3) and glandular kallikrein (hK2), and the

Figure 3. (continued) **D.** This figure shows the ratio of CB to stefin A immunostained areas in primary prostatic cancers as related to prostate cancer metastasis positive and negative lymph nodes. The figure illustrates a significantly higher ($p = 0.0066$) CB $>$stefin A ratio in primary prostate cancers when patients had tumor positive lymph nodes than with negative nodes. Sinha et al. (111) examined prostate cancer samples from 97 patients. Statistical analysis was conducted using Student's t-test. **E.** A portion of an invasive prostate cancer cell is present in the prostatic stroma, as evidenced by the adjacent collagen fibers, while the other portion is in the acinus. Micrograph shows secretory granules and vesicles containing PSA as evidenced by localization using anti-PSA-IgG and immunogold microscopic techniques (115). Portions of glandular epithelial cells show PSA-containing vesicles and some of them appear to be releasing PSA in prostatic stroma. PSA released in stroma may be one of the most important sources for serum PSA. Gleason score 8 tumor. **F.** Detail of an area of figure 3e illustrates distribution and leakage of PSA in prostatic stroma.

plasminogen activators. The plasminogen activator system has been most extensively investigated with respect to ECM protein modification, whereas PSA serum measurements have been utilized in detection and evaluation of prostate cancer. However, the recent identification of many new kallikreins and other serine proteases has generated new expectations for the role of these proteases in understanding prostate function and diseases.

The two plasminogen activators, tissue-type activator and urokinase are products of different genes, differ in their molecular weight, structure, and function, but cleave plasminogen through a common mechanism (38). Tissue-type activator is generally a fibrin activated, intravascular protease, but is implicated in some tissue remodeling events such as ovulation and bone remodeling. Urokinase on the other hand is a fibrin-independent protease mediating controlled extracellular proteolysis through its receptor (uPAR) localization to the cell surface where it generates cell surface bound plasmin. Plasmin hydrolyzes fibronectin, laminin, and other glycoproteins of the BM and stromal matrix, and it can activate other proteases such as proMMPs and activate and/or release growth factors bound in the ECM. Cells produce potent and specific inhibitors of plasminogen activators and plasmin, i.e., serine proteinase inhibitors (serpins), such as plasminogen activator inhibitor-type 1 (PAI-1) and – type 2 and protease nexin.

Localization of the plasminogen activator system components by immunohistochemistry in the human prostate have produced heterogeneous results. Tissue-type activator is localized in secretory cells of the central zone, but not peripheral zone in the prostate (116). Urokinase has been localized to tumor cell cytoplasm, stroma being negative, predominately in cancers with extracapsular extension (117). Similarly, uPAR is detected predominantly in cancers (118). Plasminogen activator activities are elevated in prostatic cancer compared with benign hyperplastic tissues (119, 120) and greater yet in prostatic cancer bone metastases (121). Both urokinase and tissue-type activator are present in human primary prostatic cancers (119, 121), but urokinase is the molecular form associated with prostatic tumor progression since it is proportionately greater in bone metastases over primary tumors (121) and it is the primary form of activator in established prostate tumor cell lines (reviewed in Wilson, 50). Immunologic measurements indicate that urokinase, uPAR and PAI-1 are increased in diploid prostate cancer tissues, whereas in aneuploid cancers urokinase levels remain elevated but uPAR and PAI-1 are decreased (122). There is no correlation of urokinase, tissue-type activator, uPAR, or PAI-1 tissue levels with cell cycle parameters in prostate cancer, indicating that urokinase is associated with non-cell cycle mechanisms in prostate cancer aggressive behavior (123). Increased expression of urokinase in hormone resistant prostate cancers may

be due in part to amplification of this gene (124). Serum levels of urokinase and uPAR have been reported in prostate cancer patients (125, 126) and their levels calculated as densities (dividing serum level by prostate volume) are significantly associated with patients with metastatic disease and diminished survival (127).

PSA (human glandular kallikrein 3, hK3) is a member of the human glandular kallikrein gene family, which originally was thought to have 3 members and now appears to contain at least 15 (128). PSA is proteolytically processed from a prepropeptide, sequentially to an active enzyme of 237 amino acids complexed with a single carbohydrate side chain. PSA has a chymotrypsin-like proteolytic function with no kininogenase activity (129, 130), and its main known biological function is cleavage of the gel forming proteins semenogelin 1 and 2 in the ejaculate. About 60-70 % of PSA in seminal plasma is in the catalytically active form , about 5 % bound to protein C inhibitor, and 30 % as 2 chain cleaved forms of the molecule (131). The clinical significance of PSA in the circulation of patients requires consideration of protease inhibitors, proteolytic processing, liver metabolism, and excretion in the urine (132). The majority of PSA in serum is bound to α1-antichymotrypsin or α2-macroglobulin, with the free uncomplexed PSA being enzymatically inactive. The α1-antichymotrypsin-PSA complex is immunoreactive, whereas PSA bound by α2-macroglobulin is enclosed in the structure of the inhibitor and not available for interaction with antibodies. Sera from patients with prostate cancer contain proportionately higher α1-antichymotrypsin complexed PSA and decreased free PSA, having a decreased ratio of free to total PSA as compared to men with BPH (133, 134). In patients with cancer associated elevated serum total PSA, pro-forms of PSA appear to be a considerable fraction of free PSA, whereas pro-forms of PSA were not detected in sera of those with BPH (135, 136).

PSA is able to proteolyze a number of peptides and proteins that implicate it in prostate cancer growth and invasion and metastasis. PSA may influence tumor growth since it can cleave insulin-like growth factor binding protein-3 (IGFBP-3), releasing IGF (137), activate latent tissue growth factor β (TGF-β) (138), and activate single-chain urokinase (139). A role for PSA in invasion and metastasis is indicated by its ability to degrade matrix proteins such as denatured collagen (gelatin) (140), and fibronectin and laminin (141). In contrast, PSA has antiangiogenic activity, which would impair tumor growth (142), and the levels of both PSA and α1-antichymotrypsin are considerably lower in cancer compared with normal prostate tissue (143). The demonstration of PSA localization by immunogold electron microscopy in the immediate extracellular environment (Figure 3e and 3f) is a manifestation of the PSA secreted by the invading tumor cell and supports a role

for PSA in the extracellular microenvironment. In this vein, other prostatic secretory proteases such as pepsinogen II (144, 145) and trypsinogen (146) may be expected to leak from acini with malignant cells or be released from invading tumor cells into the stroma. However, the activity of extracellular PSA, or other secretory proteases, must be tightly controlled by inhibitors or proteolytic processing, for with the levels of PSA produced and that reach the circulatory system, prostate cancer should be a much more aggressive cancer than it actually is.

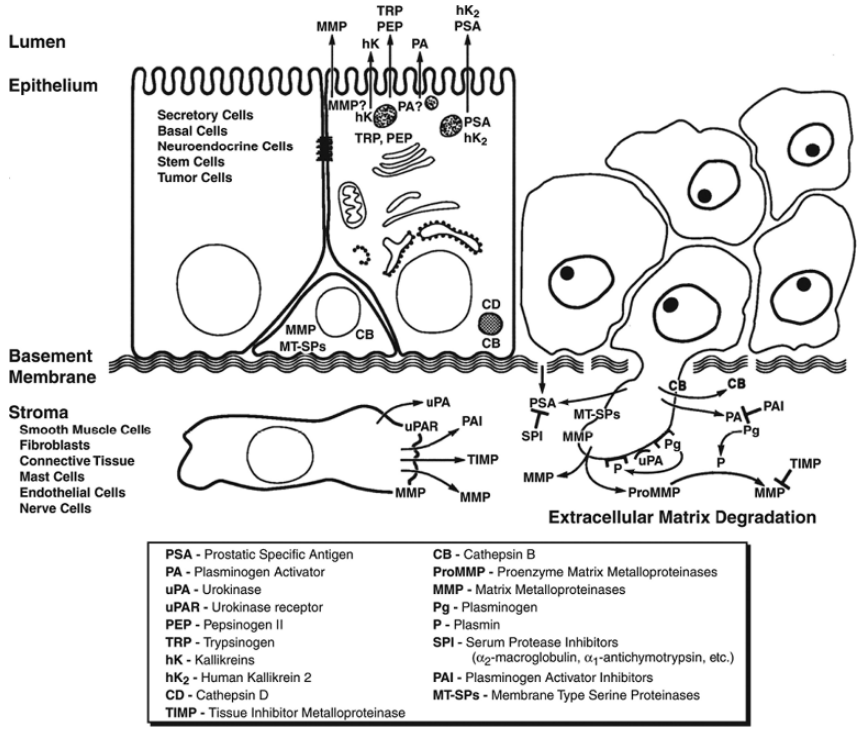
Another glandular kallikrein produced in a nearly prostate specific manner is hK2. hK2 has 78 % homology to PSA, but has a trypsin-like serine protease catalytic function (147, 148) and very low kininogenase activity (129, 130). The levels of hK2 protein and message increase in prostatic adenocarcinoma (149–151), which is in contrast to PSA which decreases in cancer cells (151). However, expression of pro-hK2 is greatest in primary cancers vs benign prostate or metastaic cancer (150). The increased levels of hK2 in prostate cancer cells may be due in part to amplification of the hK2 gene (151). The biologic function of hK2 may include activation of pro-PSA (152–154), and pro-hK2 (155), semen liquefaction (156), and processing of protein precursor molecules (157). hK2 may play a role in tumor cell invasion since it can directly hydrolyze ECM proteins like fibronectin (156) and may regulate the plasminogen activator system; i.e., hK2 can activate the single chain urokinase (154, 158) and inactivate PAI-1 (159). hK2 activity in turn may be regulated in part by serpins such as protease inhibitor 6 (PI6), with which hK2 complexes in prostatic tissue and which is increased in prostatic cancers (160, 161)

In addition to PSA (hK3) and hK2, hK4 (prostase), hK6 (neurosin), hK10 (NES1) and hK11 (hippostasin/PRSS20) proteins have been measured in human seminal plasma and in prostate tissue, indicating they are secretory enzymes, but there is little additional information available on their function in the prostate (162–170). hK10 immunostaining is detected in prostate secretory and neuroendocrine cells, but not in basal cells (171), and a general cytoplasmic distribution of hK6 and apical localization of hK4 are found in the prostatic acinar epithelium (162, 166). hK11 expression is found in normal and benign hyperplastic prostate (localized in prostatic secretory cells) and in prostate cancer cell lines. Interestingly, normal prostate and BPH express both prostate-type and brain-type alternatively spliced isoforms, but prostate cancer cell lines expressed only the brain-type hK11 (170). Higher levels of message but lower protein concentrations of hK4 are detected in prostatic cancers (162). The mRNA level for hK15 is upregulated in prostate cancer tissue, whereas that for hK5 is lower in prostate cancer with a negative correlation with Gleason score (172). Thus,

with divergent changes in kallikrein expression in prostate adenocarcinoma, their role as markers of prostate cancer is being pursued (128). The discovery of multiple alternatively spliced mRNA for kallikreins has opened another avenue of research on possible functionally diverse proteins in this group of serine proteinases. In the prostate this includes PSA (173–176), hK2 (176, 177), hK11 (170) and hK15 (178).

Several membrane-associated serine proteases have recently been cloned and identified in the prostate. Prostasin is a glycosylphosphatidylinositol-anchored membrane associated trypsin-like serine proteinase in prostatic secretory epithelial cells that is secreted into semen (179, 180). The expression of prostasin is down regulated in prostate cancer (181). TMPRSS2 is a protease with a transmembrane domain and trypsin-like protease activity and positively regulated by androgen in LNCaP cells (182, 183). Its mRNA in human prostate tissue is selectively expressed in basal cells of the prostate epithelium, but is also localized in prostate carcinoma (184). Membrane-type serine protease 1 (MT-SP1) or matriptase is a cell surface serine extracellular protease strongly expressed in the human prostate and in human prostate tumor PC-3 cells (183, 185). This protease with trypsin-like activity can cleave gelatin, protease-activated receptor 2, and single chain urokinase, implicating it in pericellular proteolysis that would facilitate prostate tumor growth and invasion of ECM (183, 186). Hepsin is a type II cell surface trypsin-like serine proteinase that is localized in basal cells of BPH epithelium (187, 188), but its expression is found in PIN (189) and greatly over expressed in cancer (188–193). The great extent to which hespin is expressed in prostate cancers and its cell surface localization make it a prime candidate for development of new diagnostic and therapy approaches for prostate cancer.

The control of serine protease activities in normal prostate function and in tumor invasion and metastasis is influenced by protease inhibitors. Maspin, a so-called ov-serpin due to its structural homology to chicken ovalbumin, can inhibit cell surface bound urokinase (194) and is strongly expressed in basal cells of normal prostate and BPH tissues (195). Maspin protein expression is dimininshed in prostate cancer, the loss of which is correlated with increased Gleason score and to higher tumor stages (195). In contrast to maspin, α1-antichymotrypsin and PI-6 levels increase in prostatic cancers (161, 196). The role of these and other serpins such as kallistatin (197), secretory leucocyte protease inhibitor (SLPI) (198), hepatocyte growth factor activator inhibitor (HAI) (199), monocyte/neutrophil elastase inhibitor (MNEI) (200), and huWAP2 (201) expressed in prostate remain to be established for normal organ function or pathology.



PSA - Prostatic Specific Antigen
PA - Plasminogen Activator
uPA - Urokinase
uPAR - Urokinase receptor
PEP - Pepsinogen II
TRP - Trypsinogen
hK - Kallikreins
hK_2 - Human Kallikrein 2
CD - Cathepsin D
TIMP - Tissue Inhibitor Metalloproteinase
CB - Cathepsin B
ProMMP - Proenzyme Matrix Metalloproteinases
MMP - Matrix Metalloproteinases
Pg - Plasminogen
P - Plasmin
SPI - Serum Protease Inhibitors (α_2-macroglobulin, α_1-antichymotrypsin, etc.)
PAI - Plasminogen Activator Inhibitors
MT-SPs - Membrane Type Serine Proteinases

Figure 4. A diagram depicting the distribution of proteases in the normal and neoplastic prostate, and the interaction of proteases effecting degradation of ECM proteins during tumor cell invasion of the stroma. Proteases are produced in the normal prostate for secretion and are depicted in the apical cytoplasm. A number of proteases including MMP-2, cathepsin B, and membrane-type serine protease are localized predominantly in basal cells. As neoplastic changes occur in the prostate, the basement membrane becomes compromised and PSA leaks into the stroma. As the tumor becomes invasive, tumor cells penetrate the basement membrane utilizing several proteases that can function at the cells surface. Urokinase is localized to the tumor cell surface through its selective receptor, facilitating the generation of plasmin. MT-MMPs and MMP-2 associated with MT1-MMP, cell surface cathepsin B, and membrane-type serine proteases also facilitate pericellular proteolysis of ECM proteins. The activities of these proteases is controlled through their generation as pro-enzyme forms which must be activated and by specific endogenous inhibitors. These proteases and inhibitors are produced by stromal host cells, as well as tumor cells, and their cooperative function controls the generation of growth factors, cytokines, and other regulatory molecules, as well as cleavage of ECM proteins permitting physical passage of malignant cells. Reproduced from Wilson (50) with permission of Microscopy Research and Technique.

CONCLUSION

ECM components modulate cellular behavior and function by poviding information in the cellular environment essential for development, morphogenesis, tissue remodeling, and maintenance of tissue specific functions. The progression of prostate cancer is associated with the synthesis of additional ECM components like tenascin and increased turnover of others. The processing and degradation of ECM proteins by cell surface associated proteinases are an important feature of controlling tumor cell invasion and metastasis, and tumor associated angiogenesis.

MMPs, proteinases of the coagulation and firbrinolysis sytstems, and cathepsins interact in this complex process of matrix turnover in orchestrating these malignant cell processes (Figure 4). It is becoming increasingly clear that the role of these proteinases in prostate cancer is much more broad than simply permitting passage of cancer cells through matrix barriers. Early stage cancers appear to be stroma-dependent tumors since many of these proteinases are produced by stromal cells upon molecular signaling influence of cancer and inflammatory cells. As cancers progress and undergo epithelial-meshencymal transformation, cancer cells express stromal cell proteinases. Cleavage of ECM proteins releases peptides that regulate tumor cell growth and migration. Likewise, endogenous inhibitors of proteinases can influence cellular behavior directly, in addition to their function in regulating proteinase activities. Most recently a number of kallikrein and other serine proteinases have been found in the prostate, for which a role in prostate cancer remains to be examined. The more extensively studied proteinases, and perhaps many of the newly discovered ones, work in concert in proteinase cascades, and function as membrane anchored or cell surface bound proteinases, or are shed into the immediate tumor cell environment. It is hoped we can take advantage of the properties of these proteinases in prostate cancer to devise better diagnostic methods and utilize them as targets for treatment interventions.

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