Hepsin and prostate cancer

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1. ABSTRACT

Hepsin is a membrane serine protease expressed in several human tissues including the liver, kidney, prostate, and thyroid. The physiological function of hepsin remains unknown. In vitro studies have shown that hepsin activates blood clotting factors VII, XII, and IX, pro-urokinase (pro-uPA), and pro-hepatocyte growth factor (pro-HGF). Recently, hepsin has been identified as one of the most up-regulated genes in prostate cancer. The hepsin up-regulation appears to correlate with the disease progression. In a mouse model of prostate cancer, hepsin overexpression promotes cancer progression and metastasis. In culture, anti-hepsin antibodies inhibited the invasion of human prostate cancer cells. This review will outline the molecular biology and biochemistry of hepsin and highlight recent data of hepsin in prostate cancer.

2. HISTORICAL BACKGROUND

Human hepsin was first identified in the late 1980s. In a search for novel trypsin-like proteases, Leytus et al. cloned human hepsin cDNA from a HepG2 cell library (1). Hepsin amino acid sequence contains all signature features of the trypsin-like proteases including the conserved activation cleavage site and the catalytic domain with active His, Asp, and Ser residues. Surprisingly, however, hepsin did not have a typical signal peptide sequence, which was present in almost all trypsin-like serine proteases known at that time. Instead, hepsin had a hydrophobic segment near its N-terminus, which was predicted to serve as a single-span transmembrane domain anchoring the protease on the cell surface. Although for many decades enteropeptidase was known to be associated
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Figure 1. Hepsin protein domain structure. The transmembrane domain (TM), scavenger receptor-like cysteine-rich domain (SR) and protease catalytic domain (Protease) with active site residues histidine (H), aspartate (D) and serine (S) are indicated. An arrow indicates the activation cleavage site. A disulfide bond (S-S) that connects the propeptide and the protease domain is also shown.

with the intestinal brush border membrane (2-6), the structural basis for its membrane association remained unknown until the full-length cDNA was cloned in the early 1990s (7, 8). Thus, hepsin became the first human trypsin-like serine protease known having an integral transmembrane domain.

Hepsin is most abundantly expressed in hepatocytes. Lower levels of hepsin mRNA are also found in several other tissues including the kidney, pancreas, prostate, and thyroid (1, 9). Because of its unique structural feature and distinct tissue distribution pattern, hepsin became an interesting molecule to study. Studies suggested that hepsin may play a role in blood coagulation (10), hepatocyte growth (11), and embryonic development (12). However, hepsin-deficient mice generated by gene targeting techniques were viable and fertile, and grow normally, indicating that hepsin is not necessary for embryonic development and postnatal survival (13-15). Hepsin-deficient mice had no detectable defects in liver histology and biochemistry. Blood clotting and tail bleeding times were also normal in these mice, indicating that hepsin is dispensable for normal hemostasis and liver function. Serum concentrations of bone-derived alkaline phosphatase were ~2 fold higher in hepsin-deficient mice than that of control mice but bone-derived alkaline phosphatase were ~2 fold higher in hepsin-deficient mice than that of control mice but bone-derived alkaline phosphatase were ~2 fold higher in hepsin-deficient mice than that of control mice but bone-derived alkaline phosphatase were ~2 fold higher in hepsin-deficient mice than that of control mice but.

3. HEPSIN PROTEIN

The full-length human hepsin cDNA encodes a polypeptide of 417 amino acids (1). As illustrated in Figure 1, hepsin has an overall structure of the type II transmembrane serine protease (16-18). The transmembrane is located near the N-terminus whereas the extracellular protease domain is at the C-terminus. Between these two domains is a macrophage scavenger receptor-like cysteine-rich domain. Like many two-chain serine proteases of the chymotrypsin family, the cysteine residues at position 153 in the propeptide and position 277 in the protease domain are predicted to form a disulfide bond that tethers the protease domain on the cell surface after the enzyme is cleaved at the conserved activation site (Figure 1). A recent study suggests that a soluble form of human hepsin may also exist (19).

Human hepsin has been purified from liver cells (20). On SDS-PAGE and Western analysis, hepsin appeared as a single band of ~51 kDa. Similar molecular mass was also reported for rat hepsin purified from the liver and recombinant human hepsin (21, 22). The result is consistent with the calculated mass of ~45 kDa for human hepsin, which contains a predicted N-linked glycosylation site at position 112 in the scavenger receptor-like domain (1). Purified human and rat hepsin proteins have been characterized biochemically (20, 22, 23). In small peptide-based assays, hepsin exhibited trypsin-like catalytic activity that favored Arg at the P1, Thr/Leu/Asn at the P2, Gln/Lys at the P3, and Pro/Lys at the P4 positions (24). The hepsin activity was inhibited by non-specific trypsin inhibitors. Herter et al, have reported that hepatocyte growth factor (HGF) activator inhibitors 1 and 2 are also potent inhibitors for human hepsin (24).

Recently, the 3-dimensional structure of a soluble human hepsin containing the scavenger receptor-like cysteine-rich domain and the protease domain has been determined (24, 25). The hepsin protease domain exhibited an architecture of two six-stranded β barrels, typical for a chymotrypsin family serine protease. In this regard, hepsin is similar to enteropeptidase (26) and matriptase/MT-SP1 (27), the other two type II transmembrane serine proteases whose protease domain structures are also shown to be chymotrypsin-like. In fact, the hepsin protease domain structure can be superimposed on the corresponding domains from tryptase, chymotrypsin, urokinase, enteropeptidase, and matriptase/MT-SP1 (25). In these proteases, unique loop structures contribute significantly to the substrate specificity of individual enzymes. The hepsin crystal structure revealed several loop structures that are distinctive. In particular, hepsin has a large loop between residues 241 and 256, which may represent a major determinant for its substrate recognition (24, 25).

The hepsin scavenger receptor-like cysteine-rich domain exhibited a structural fold that is homologous to other scavenger receptor-like domains (24, 25). The architect of this hepsin domain is most close to the scavenger receptor-like cysteine-rich domain in the Mac-2 binding protein. In the hepsin crystal structure, the scavenger receptor-like and protease domains formed a elongated structure of ~70 Å in length, lying on the cell membrane surface (24, 25). There is an interface of ~1200 Å² between these two domains and substantial interactions were observed. In addition to the disulfide bond connecting Cys-153 and Cys-277, a number of hydrogen bonds and salt bridges between the two domains were detected. These interactions are likely to reduce the flexibility of the protease domain on the cell surface. At this time, it is not known if the scavenger receptor-like domain has other functions such as interactions with substrates or inhibitors.

4. HEPSIN IN PROSTATE CANCER

Cancer cells are invasive and migratory. Degradation of the extracellular matrix is an essential step for cancer cells to spread and travel to distant locations.
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Many proteolytic enzymes such as metalloproteinases, uPA, cathepsins, and matriptases are implicated in cancer invasion and metastasis (28-34). Most recently, hepsin also is found to play a major role in prostate cancer. Using microarray techniques, a number of laboratories have examined gene expression profiles in prostate cancer tissues and identified hepsin as one of the most consistently up-regulated genes in human prostate cancer (35-44) (Table 1). In other quantitative studies, hepsin mRNA levels were found elevated in ~90% of prostate tumors examined, and in majority of the cases, the increase was >10-fold compared with that in noncancerous controls (45, 46).

The correlation between hepsin expression and prostate cancer progression has been examined. An initial study suggested that hepsin expression was more frequently associated with early prostate cancers (36), but later data indicated a correlation between hepsin expression and disease progression. For example, Stephan et al. showed that hepsin mRNA levels were significantly higher in prostate cancer of grade 3 than that of grade 2 (46). Stamey et al. and Riddick et al. also reported the correlation of high hepsin mRNA expression in advanced prostate cancers (35, 43, 47). These results were confirmed by Xuan et al. using monoclonal antibodies against hepsin that were developed in hepsin knockout mice (14, 21). Immunohistochemical staining using these specific antibodies showed weak hepsin expression in tissues from normal prostate, benign prostatic hyperplasia or low grade (2/3) prostate cancer. In contrast, hepsin expression was highly elevated in advanced prostate tumors (grades 4/5) and bone metastasis (21) (Figure 2). In another recent study, hepsin mRNA expression appeared to be lower in hormone-refractory prostate cancer compared to clinically localized prostate cancer (48).

More recently, single nucleotide polymorphisms (SNPs) in the human hepsin gene are found to be associated with prostate cancer susceptibility (49, 50). Witte et al. have reported that on human chromosome 19q12-13 a locus near the hepsin gene is associated with prostate tumor aggressiveness (51, 52). In case-control studies, SNPs in the hepsin gene showed significant allelic frequency differences between prostate cancer patients and normal controls of European origin (49, 50). In particular, one SNP was found to be associated with patients with high-Gleason score (50). However, most of the identified SNPs are located in hepsin gene introns and, therefore, unlikely to affect hepsin protein function. It is not known if these SNPs might alter hepsin gene expression in the prostate.

The functional importance of hepsin in cancer has been examined in cell-based assays but the results were inconclusive. An early study showed that anti-hepsin antibodies or antisense oligonucleotides against hepsin mRNA inhibited the growth of cultured human hepatoma cells (11). In another study, however, prostate cancer cell lines stably transfected with a hepsin expressing plasmid grew slower and were less invasive in culture (53). Similarly, stable transfection of hepsin cDNA in ovarian cancer cell lines promoted apoptosis and inhibited tumor cell growth in soft agar and in nude mice (54). On the other hand, Xuan et al. have shown that antibodies neutralizing hepsin protease activity did not block prostate or ovarian cancer cell growth in culture but inhibited their invasion in Matrigel assays (21). The reason for the different results from these studies is not clear but may reflect the different cell types and culture systems. Stably transfected cells also may change their phenotypes during selection processes. It would be important, therefore, to examine the role of hepsin in animal models of prostate cancer.

There are a number of well characterized mouse prostate cancer models (55, 56). In most the mouse models, however, hepsin mRNA expression is very low or undetectable (57, 58), making these models unsuitable for studying the role of hepsin in cancer. To create a mouse model that mimics hepsin overexpression in patients, Klesiowitz et al. made a transgenic mouse with high levels of hepsin expression in prostate epithelium (59). Hepsin overexpression did not affect cell proliferation but altered basement membrane structure in prostate tissues in these mice. When the transgenic mice were crossed with a non-metastasizing prostate cancer model (LPB-Tag), primary cancer cells became more invasive and metastatic. The animals developed metastatic lesions in distant organs such as the liver, lung and bone, indicating that hepsin promotes prostate cancer progression and metastasis in vivo (59). Together with the data by Xuan et al. (21), it appears that the role of hepsin is more important in cancer cell migration/invasion than cell proliferation.

5. HEPSIN IN OTHER CANCERS

High levels of hepsin mRNA are reported in other human cancers. In a study of ovarian cancer,
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Figure 2. Immunohistochemical staining of hepsin in prostatic tissues. A monoclonal antibody was used to stain hepsin protein (brown color) in tissues from normal prostate (A-B), benign prostatic hyperplasia (C-D), prostate cancer grades II/III (E-F), grades IV/V (G-H), and bone metastasis (I-J). The negative data with a control antibody is not shown. Reproduced with permission from (21).

Tanimoto et al. examined 44 tumors and found elevated hepsin mRNA expression levels in ~58% of low grade tumors and ~84% of more advanced ovarian carcinomas (60). In a recent microarray analysis, Bignotti et al. have shown that hepsin mRNA expression was significantly higher in primary ovarian serous papillary carcinomas compared to that in omental metastasis (61). The up-regulation of hepsin mRNA also was found in estrogen receptor alpha (ERα)-positive breast tumors compared with ERα-negative breast tumors (62).

In renal cell carcinomas, Zacharski et al. reported strong hepsin staining on tumor cell membrane in all seven cases examined (63). More recently, however, two groups reported different results. In a study of 27 cases of renal cell carcinomas by Roemer et al., hepsin mRNA levels in early stage cancer were similar to that in normal controls but the mRNA levels decreased in more advanced cancers (64). In the same study, patients with lower hepsin mRNA levels had poorer survival rates. In contrast, Betsunoh et al. studied 66 cases of renal cell carcinomas and found lower hepsin mRNA expression in early stage cancers (65). In some cases (11/66) of advanced cancer, however, hepsin mRNA was overexpressed (>3 fold) and patients with high hepsin mRNA levels had poorer survival rates (65). The reason for the apparently opposing results is not known but may be due to different methods for sample collection and experimental techniques used for quantifying hepsin mRNA expression levels. Clearly, additional studies with more patient samples are needed to better understand hepsin expression in renal cell carcinomas and its relationship with disease prognosis.

6. POSSIBLE MECHANISMS OF HEPSIN IN CANCER

The evidence of hepsin up-regulation in prostate cancer is striking but the biological significance remains unclear. Intuitively, hepsin as a proteolytic enzyme could degrade extracellular matrix proteins, allowing cancer cells to spread. Studies with hepsin overexpression transgenic mice indeed show disorganized basement membrane in prostate cancer tissues (59). However, it is unlikely that hepsin proteolytic activity alone was accountable entirely for degrading the basement membrane because matrix proteins usually are poor substrates for trypsin-like proteases. Biochemical studies have shown that hepsin has a rather narrow substrate specificity (24). Alternatively, hepsin could activate the proteases of the plasminogen/plasmin pathway which in turn activate matrix degrading metalloproteinases. Recently, Moran et al. showed that hepsin converted pro-uPA to active uPA (66), suggesting a possible role of hepsin in promoting the plasminogen/plasmin pathway and hence matrix metalloproteinase activation.

Up-regulation of uPA activity is notorious in many types of cancer (28). A recent study has shown that in prostate cancer tissues uPA was expressed mainly in macrophages but not cancer cells (67). It is unlikely that uPA activation is the only mechanism by which hepsin promotes cancer progression. If so, what are other potential mechanisms? For many years, serine proteases are known to have growth factor-like activities. Thrombin, for example, is a potent mitogen for vascular fibroblasts and smooth muscle cells (68). Several growth factors such as HGF and Gas6 are similar to blood clotting proteins in their sequences and structures (69, 70). In principle, hepsin could also activate growth factors. Recently, several groups have shown that hepsin activated pro-HGF in vitro...
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(21, 24, 71). HGF is a potent stimulator for the receptor tyrosine kinase c-Met, which plays an important role in tumor progression (72, 73). In fact, the HGF/c-Met pathway is considered as an important drug target (74-76). Thus, activating pro-HGF could be one of the possible mechanisms by which hepsin promotes cancer development. To date, however, many proteases including HGF activator (77), matrkapase/MT-SP1 (78, 79), plasma kallikrein (80), and blood clotting factor XI (80) have been shown to activate pro-HGF in vitro. It remains to be determined if hepsin-mediated pro-HGF activation is critical in the pathogenesis of human prostate cancer.

In addition to pro-HGF, hepsin, when tested in vitro, cleaved other proteins including blood clotting factors VII, XII, and IX (10, 24). As discussed earlier, blood coagulation in hepsin-deficient mice appeared to be normal, suggesting that these clotting factors may not be the physiological hepsin substrates (13). The recent identification of corin as the pro-atrial natriuretic peptide convertase indicates that type II transmembrane serine proteases may have a role in processing peptide hormones (81-85). Prostate cancers are known to be sensitive to steroid and peptide hormone stimulation. It will be interesting to examine whether hepsin also acts as a hormone-processing enzyme in prostatic epithelium.

7. PERSPECTIVE

Prostate cancer is a deadly disease but the underlying mechanisms are poorly understood. The findings of hepsin up-regulation in cancers, especially prostate cancer, are intriguing and suggest that this protease may play an important role in cancer invasion and metastasis. At this time, the biological significance of hepsin expression in human prostate cancer is not completely understood. Many questions remain unanswered. For example, what is the physiological function of hepsin? What are hepsin substrates in prostate cancer tissues? What is the relationship of hepsin with other proteases that are abundant in prostate tissues? What stimulates hepsin gene expression in the prostate? Undoubtedly, answers to these questions shall help to better understand the pathogenesis of prostate cancer.

If hepsin is indeed critical for prostate cancer progression, it may represent a new biomarker with better diagnostic and prognostic values than currently used biomarkers such as prostate specific antigen (PSA). More importantly, hepsin may represent an excellent drug target for prostate cancer. It is well known that trypsin-like serine proteases are pharmaceutical drug targets. As a cell surface protein, hepsin should be readily accessible by therapeutic agents in the circulation. Inhibition of hepsin is expected to have minimal side effects as hepsin-deficient mice exhibited no apparent abnormal phenotype despite intense scrutiny. As the hepsin crystal structure is available, structure-based drug design approaches should facilitate the development of potent and orally available small molecule hepsin inhibitors. Such inhibitors may represent a new class of drugs to treat prostate and other cancers.

8. ACKNOWLEDGEMENTS

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9. REFERENCES

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