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Proteinases and their inhibitors in liver cancer

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Abstract

Proteinases are known to be involved in many cancer-related processes, particularly in the breakdown of extracellular matrix barriers in the course of tumor invasion and metastasis. In this review we summarize the current knowledge about the role of the most important matrix-degrading proteinases (cathepsins, matrix metalloproteinases, plasmin/plasminogen activators) and their respective inhibitors in liver cancer progression and metastasis.

Key words: Cathepsin; Cystatin; Hepatocellular carcinoma; Metalloproteinase; Plasminogen activator; Tumor invasion; Metastasis

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INTRODUCTION

Liver cancer represents the seventh most frequent malignancy, as manifested by more than 50.000 new cases per year. This corresponds to 6% of all cancers diagnosed in the year 2000[1]. The most common primary liver tumors are hepatocellular carcinomas (HCCs). Although many improvements have been made in terms of diagnosis and treatment, HCCs are usually associated with poor clinical prognosis, with a mean life expectancy of less than 6 mo. Surgical resection is only possible in 10%-20% of incidences and cures less than 5% of the patients. Tumor recurrence as well as intrahepatic and vascular metastasis severely affect the clinical outcome of this disease[2]. Interestingly, HCCs develop mainly in chronically injured tissue and are frequently associated with liver fibrosis. As a consequence of the development of fibrosis, HCC cells are often embedded in a stroma rich in extracellular matrix (ECM) proteins, which may culminate in the formation of a capsule surrounding the cancerous tissue[3]. However, aggressive HCCs have the capacity to penetrate such ECM barriers and spread into the surrounding parenchyma, leading to intrahepatic metastasis and portal venous invasion[4].

Various proteinases appear to be involved in the breakdown of ECM components during tumor invasion and metastasis, including plasmin and plasminogen activators, matrix metalloproteinases (MMPs), and cathepsins[5-7]. It has been shown that the synthesis of matrix-degrading proteinases is frequently upregulated in tumors. Cancer cells can also increase the proteolytic load in their environment by mobilization of proteinases from intracellular stores, and by acquisition and activation of proteinases released by stromal cells[8]. The degree of local ECM proteolysis is regulated by the concomitant secretion of endogenous proteinase inhibitors. The intricate balance between individual proteinases and their respective inhibitors implies that invasive tumor cells precisely coordinate ECM proteolysis with other cellular events required for effective invasion, such as cell-matrix attachment, detachment and migration[9].

Hepatocytes produce only a limited array of protein-

Table 1 Liver proteinases implicated in tumor progression and metastasis

Name	Type	Inhibitors	Localization	Physiological function
Cathepsin B	Cysteine	Cystatins (A-E)	Lysosomal	General protein turnover
Cathepsin D	Aspartic	-	Lysosomal	General protein turnover
Cathepsin L	Cysteine	Cystatins (A-F)	Lysosomal	General protein turnover
MMP-2	Metallo	TIMPs (1-4)	Extracellular	Matrix remodelling
MMP-3	Metallo	TIMPs (1-4)	Extracellular	Matrix remodelling
MMP-7	Metallo	TIMPs (1-4)	Extracellular	Matrix remodelling
MMP-9	Metallo	TIMPs (1-4)	Extracellular	Matrix remodelling
uPA	Serine	PAI-1, PAI-2	Extracellular	Fibrinolysis
tPA	Serine	PAI-1	Extracellular	Fibrinolysis

ases with matrix-degrading potential under normal, quiescent conditions. Besides plasminogen, the constitutively expressed enzymes most relevant to ECM degradation are the lysosomal proteinases cathepsin B, cathepsin D and cathepsin L[10-12] (Table 1). Other important matrix-degrading proteinases such as matrix metalloproteinases (MMPs) as well as plasminogen activators are usually undetectable. However, it has been reported that fetal rat hepatocytes can be stimulated to synthesize a selected range of MMPs and plasminogen activators[13]. Furthermore, expression of certain MMPs and plasminogen activators is enhanced during liver regeneration[14,15]. Nevertheless, even in the regenerating liver, ECM proteolysis is a tightly controlled process due to the concomitantly increased synthesis of proteinase inhibitors[14].

In the following sections, we review the current knowledge about the relevance of the balance between cathepsins, matrix metalloproteinases, plasminogen activators and their respective inhibitors for HCC progression and metastasis.

LYSOSOMAL PROTEINASES (CATHEPSINS)

In recent years, significant progress has been made in the biochemical and structural characterization of lysosomal proteinases. It has been shown that these enzymes participate in physiological processes other than bulk proteolysis in the lysosomes. Three proteinases appear to be present in all mammalian lysosomes: the aspartic proteinase cathepsin D, and the cysteine proteinases cathepsin B and cathepsin L[16]. Lysosomal cysteine cathepsins belong to the papain superfamily of cysteine proteinases, whereas cathepsin D is closely related to the major digestive enzyme pepsin[17,18].

Cathepsins are usually delivered in their zymogen forms to lysosomes. The acidic internal milieu of these compartments then triggers the largely autocatalytic proteolytic maturation of the latent proenzymes[19]. The rate-limiting factor in lysosomal targeting is the capacity of the endogenous sorting receptors, which results in the secretion of varying amounts of newly-synthesized proteinase precursors[20]. Under normal circumstances, these secreted forms exhibit only insignificant proteolytic activity. However, it was shown that at least secreted procathepsin B can be seen as a latent enzyme pool, which, upon (auto)activation in the acidic microenvironment around tumor cells, may cause local proteolysis[21].

Cathepsins can promote tumor invasion in different ways: (1) by direct cleavage of ECM/basement membrane components; (2) by activation of other proteinases[22-24] which in turn degrade ECM components; or (3) by cleavage of cell adhesion proteins on the cell surface, thus initiating the disruption of intercellular junctions[25].

CYSTEINE CATHEPSINS AND LIVER CANCER

The human genome encodes 11 cysteine cathepsins (B, C, F, H, L, K, O, S, V, X and W), all structurally closely related to the prototypic plant cysteine proteinase papain[26]. Cysteine cathepsins are often upregulated in various human cancers, and have been implicated in distinct tumorigenic processes such as angiogenesis, proliferation, apoptosis and invasion[7,25]. Using cathepsin knock-out mice, various groups have recently provided strong evidence for distinct functions of individual cathepsins in tumor progression and metastasis[27-29]. To date, the lysosomal cysteine proteinase most thoroughly studied in the context of cancer is cathepsin B, which has been reported to promote tumorigenesis in multiple ways[27,29,30].

So far, very little is known about cysteine cathepsins in liver cancer. However, there is some evidence that cathepsin B (CB) contributes to the invasive potential of hepatoma cells. Early studies reported differences between the subcellular distributions of CB in highly invasive murine Hepa cl9 hepatoma cells and normal hepatocytes, with significantly more CB associated with non-lysosomal membranes/vesicles in the tumor cells. This was attributed to transformation-induced changes to intracellular CB trafficking[31], a hypothesis further substantiated by subsequent morphological studies. While the enzyme was found to be restricted to perinuclear (presumably lysosomal) vesicles in an embryonic liver cell line, it was detected in vesicles adjacent to the cell membrane and in localized regions (possibly caveolae) of the surface of Hepa cl9 cells[32]. Evidence for the association of CB with caveolae in

tumor cells has been provided[33]. Moreover, it was found that CB synthesis and activity is significantly higher in Hepa cl9 cells than in normal liver cells[34]. Hence, these findings support the notion that alterations in the expression and subcellular distribution of CB contribute to the invasiveness and the metastatic potential of HCCs.

A detailed analysis of the biosynthesis and intracellular transport of another cysteine cathepsin, cathepsin C, in rat Morris hepatoma 7777 cells also revealed unusual features[35]. This can be at least partially explained by the deficiency of these cells in the main lysosomal sorting receptor, the mannose 6-phosphate/insulin-like growth factor Ⅱ receptor (M6P/IGF2R), a protein frequently absent and/or mutated in HCCs[36-38]. Intracellular sorting of cathepsin C in Morris hepatoma 7777 cells appears to involve MPR46, the second mammalian M6P receptor[39]. However, there is also evidence for M6P-independent membrane association and lysosomal delivery of cathepsin C in these cells[40].

In healthy tissue, the endogenous activities of cysteine cathepsins are tightly regulated by specific protein inhibitors, the cystatins. Type I cystatins (stefins) are located in the cytosol, whereas type Ⅱ cystatins are secretory proteins[41]. Alterations to the balance between cysteine cathepsins and cystatins have been postulated to contribute to tumor growth and malignant progression in various cancers[42]. Indeed, ectopic expression of cystatin C has been shown to reduce the tumorigenic and invasive potential of cancer cells[43,44]. Conversely, genetic ablation of this cystatin accelerated angiogenesis and tumor proliferation in a pancreatic cancer model[28]. Only very few reports have dealt so far with the role of cystatins in liver cancer. In one study, no obvious differences were found between the subcellular localizations of stefin A, stefin B and cystatin C in murine Hep cl9 hepatoma and embryonic liver cells[32]. However, a unique membrane-associated form of stefin A has been isolated from Hep cl9 tumors[45]. An intriguing novel cystatin, cystatin F, was identified in a screen for genes associated with liver metastasis[46]. The subcellular localization of cystatin F is highly unusual since this proteinase inhibitor is delivered to endosomal and lysosomal compartments[47,48]. It remains to be established whether the presence of cystatin F in lysosomes relates rather to the pro- than anti-invasive activity of this cystatin in malignant tumor cells.

CATHEPSIN D AND LIVER CANCER

The aspartic proteinase most extensively investigated in the context of cancer is cathepsin D (CD), with a particular emphasis on its role in breast cancer[49]. Comparatively little information is available on the relevance of this proteinase for liver cancer. CD was found to display a higher activity in hepatoma tissue than in normal human liver tissue. Interestingly, this coincided with an elevated M6P content of hepatoma cathepsin D[50]. Furthermore, the secretion of CD was markedly elevated in M6P/IGF2R-deficient rat Morris hepatoma 7777 cells when compared with normal hepatocytes. These cell types also differed in their ability to process CD into its mature forms. Remarkably, intracellular retention of CD in Morris hepatoma 7777 cells was largely insensitive to treatment with lysosomotropic bases, which are known to perturb M6P-dependent transport to lysosomes[51]. A similar observation was made for M6/IGF2R-positive human HepG2 hepatoma cells[52], thus ruling out that this phenomenon is linked to the M6P/IGF2R status of the cells. For HepG2 cells, evidence has been provided that biosynthetic transport of CD to lysosomes can occur in a M6P-independent manner[53]. This could be at least partially due to the transient association of procathepsin D with prosaposin[54]. It has been shown that prosaposin can undergo lysosomal delivery in the absence of a functional M6P receptor system, possibly via interaction with sortilin[55,56].

MMPS, TIMPS AND LIVER CANCER

More than 25 human proteins and plenty of homologues from other species are known to make up the MMP (matrix metalloproteinase) family. MMPs are classified into five subgroups regarding their preferential degradation of different matrix substrates: interstitial collagenases, type Ⅳ collagenases/gelatinases, matrilysins, stromelysins and membrane-type MMPs (MT-MMPs). Most MMPs contain several conserved functional domains, including a catalytic domain containing a highly conserved zinc-binding site and a hemopexin-like domain involved in substrate recognition[57-59]. All MMPs are initially synthesized as latent precursors. Conversion into the respective active species requires proteolytic removal of the inhibitory prodomain by other MMPs, serine proteinases or cathepsins[24,60-62].

MMPs are suggested as key regulators of tumor growth and metastasis. Based on their enzymatic properties, the MMPs most relevant to tumor invasion and metastasis are the type Ⅳ collagenases/gelatinases. The most prominent gelatinases, MMP-2 (gelatinase A) and MMP-9 (gelatinase B), are able to degrade type Ⅳ collagen and other components of the basement membrane, which is the first barrier tumor cells have to break through during metastatic dissemination[63]. Studies in transgenic mice have highlighted the importance of MMP-2 and MMP-9 for cancer progression and tumor invasion[64-66]. However, it should be pointed out that certain MMPs such as MMP-8 can also exhibit anti-invasive properties[67].

The biological activities of MMPs are controlled by TIMPs (tissue inhibitors of metalloproteinases), which act through the formation of a tight, noncovalent complex with their cognate enzymes. TIMP-1, TIMP-2 and TIMP-4 are soluble proteins, whereas TIMP-3 is membrane-bound[60,63].

Several MMPs have been implicated in liver cancer.

The induction/upregulation of various MMPs (e.g. MMP-2, MMP-3, MMP-7 and/or MMP-9) has been detected in tumorous liver tissue obtained from HCC patients[4,68,69]. Furthermore, synthesis of MMP-2 was observed in several malignant HCC cell lines, whereas their benign counterparts appear to lack this proteinase[70]. Moreover, the production of MMP-9 in transformed murine hepatocytes can be triggered by induction of epithelial-to-mesenchymal transition, concomitant with the acquisition of invasive properties[71]. Interestingly, hepatocyte growth factor (HGF) has been found to induce the synthesis of several MMPs in hepatoma cells. In particular, stromelysin-1 (MMP-3) became clearly detectable upon HGF stimulation of human HepG2 hepatoma cells. Intriguingly, invasion of HGF-treated HepG2 cells could be blocked by a synthetic MMP inhibitor as well as by antibodies to MMP-3. These results suggest that transformation-associated changes in MMP expression contribute to the invasive activity of malignant HCC cells[69].

Metastatic dissemination of tumor cells is also facilitated by reduced endogenous TIMP levels. It has been observed that the serum and tissue levels of hepatic TIMP-2 are significantly higher in HCC patients without metastasis than in those with metastatic disease. In the latter cases, both primary HCC tissues and intrahepatic metastases displayed low TIMP-2 levels. This qualifies TIMP-2 as an important prognostic factor in HCC patients[2].

It has been reported that antisense-mediated reduction of TIMP-1 accelerates tumor formation and disease progression in a mouse model of HCC. Conversely, ectopic overexpression of hepatic TIMP-1 interferes with oncogene-induced tumorigenesis. High TIMP-1 levels were found to inhibit tumor initiation as well as the progression to later stages in HCC development[72]. Using the same transgenic mouse strains, further studies revealed that TIMP-1 overexpression inhibits oncogene-induced hepatocarcinogenesis largely by reducing hepatocellular proliferation and tumor vascularization[73]. This was found to be due to the reduced levels of bioactive insulin-like growth factor Ⅱ (IGF-Ⅱ) in TIMP-1 overexpressing animals. It was postulated that the presence of ectopic TIMP-1 leads to reduced proteolysis of IGF-binding protein-3 (IGFBP-3) and thus elevated IGFBP-3 levels, which in turn lower the bioavailability of IGF-Ⅱ[74].

Collectively, these findings suggest that imbalances between MMPs and TIMPs may enhance the proteolytic load in HCC tissues and thus promote HCC progression and metastasis.

THE PLASMINOGEN ACTIVATING SYSTEM (UPA, UPAR, PAI-1) AND LIVER CANCER

Plasminogen activation plays an important role in tumor invasion and metastasis. This proteinase precursor circulates in the pericellular environment, waiting to be activated by proteolytic maturation. Plasminogen can be activated by either of two types of plasminogen activators: tissue-type plasminogen activator (tPA), or urokinase-type plasminogen activator (uPA). The precursor forms of tPA and uPA display significant enzymatic activity, but the catalytic efficiency of uPA is strongly increased by plasmin-mediated proteolytic processing[75,76]. The biological activities of uPA and tPA are controlled by two plasminogen activator inhibitors, PAI-1 and PAI-2. uPA is the enzyme of higher relevance for tumor biology, which is at least partially due to the occurrence of a cellular uPA receptor (uPAR). uPAR is a glycosylphosphatidylinositol (GPI)-anchored protein located at the cell surface where it binds uPA, which in turn interacts with plasminogen and activates the latter. In tumors, uPA is concentrated at focal adhesion points through association with uPAR, which is enriched in these regions. Thus, the highly specific ternary interaction of plasminogen, uPA and uPAR permits strictly regulated local proteolysis of ECM components at the contact sites between tumor cells and the basement membrane[77]. Studies in uPA-deficient mice have provided evidence for a decisive role of this proteinase in tumor progression and metastasis[78].

The expression of tPA in normal, quiescent liver is low or undetectable. However, stimulation of tPA synthesis is observed during hepatocyte proliferation[79]. Within normal quiescent liver tissue, uPA synthesis appears to be mainly due to the presence of non-parenchymal cells such as hepatic stellate cells and Kupffer cells. However, hepatocytes have the capacity to produce uPA when induced to proliferate[80]. In this context, it should be noted that uPA, via its capacity to trigger HGF activation[81], appears to play a crucial role in liver regeneration[82].

Interestingly, both plasminogen activators and uPAR are readily detectable in HCC tissues[83,84]. Among the components of the plasminogen activation system, uPA appears to be the most useful diagnostic indicator for intra-hepatic metastasis and a reliable prognostic factor for HCC recurrence[85]. Furthermore, the cumulative presence of uPA, uPAR and PAI-1 is a good predictor of HCC invasion and metastasis[84]. The cellular source of uPA and uPAR in HCC is still unresolved, since the expression of uPA and uPAR in HCC tissues appears to be largely confined to stromal and inflammatory cells[86]. However, human hepatoma cells produce uPA upon stimulation with HGF[87]. Furthermore, the invasiveness of HGF-treated HepG2 cells could be reduced by pharmacological uPA inhibition[88]. This suggests that uPA is a promising target for HCC therapy.

CONCLUSION

HCC is a severe and common disease all over the world. Novel drugs for HCC treatment are urgently needed. In the last decades, considerable information has been

gained about the role of matrix-degrading proteinases and their inhibitors in this disease. A number of proteinases and proteinase inhibitors have been identified as new markers for the prediction of HCC outcome. The available data suggest that synthetic proteinase inhibitors could be used to prevent HCC progression and metastasis. Given this knowledge, it appears possible that both HCC diagnosis and, hopefully, also its therapy, can be improved in the near future.

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