Chapter 7
Proteases and Cancer Development

Shudong Zhu and Zhoufang Li

Abstract Dysfunction of proteases is observed in many cancers. Signaling and functional roles of both intracellular proteases and extracellular proteases in the development of cancer are discussed in this chapter. As mitochondrial proteases, HtrA2/Omi regulates inhibitors of apoptosis proteins, while Lon protease degrades misfolded proteins and maintains the stability of the mitochondrial genome. Caspases are closely interconnected with mitochondria in apoptosis and serve as the major executors of the apoptosis machinery. Cathepsin proteases have multiple substrates including growth factors and extracellular matrix proteins. Matrix metalloproteinases trigger the release of growth and angiogenic factors and modulate extracellular matrix molecules. Changes of these proteases affect various aspects of cancer development, including transformation, apoptosis, invasion, and metastasis of cancer cells. Targeting these proteases is becoming an important approach to cancer treatment.

Keywords Protease • Cancer • Mitochondria • Caspase • Cathepsin • Matrix metalloproteinase • Apoptosis • Invasion • Metastasis

1 Introduction

Proteases are responsible for proteolysis, one of the most fundamental posttranslational regulatory systems. They are involved in various physiological processes. Dysfunction of proteases is observed in many cancers. Here we will discuss both

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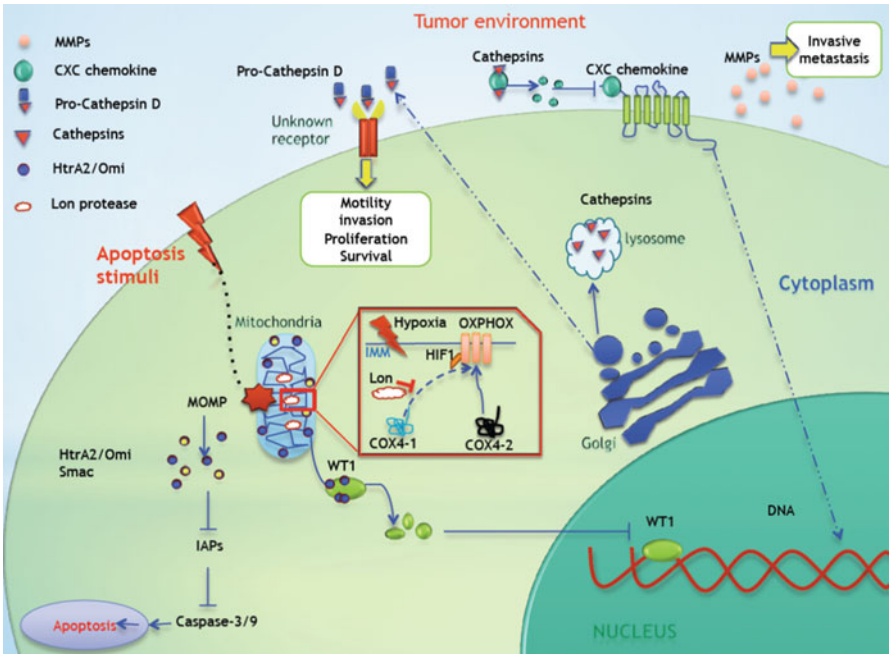

Fig. 7.1 Network of intracellular and extracellular proteases in cancer development. *MMP* matrix metalloproteinases, *HtrA2/Omi* high temperature requirement protein A2, *HIF* hypoxia-inducible factor, *COX* cytochrome C oxidase, *MOMP* major outer membrane protein, *IAP* inhibitors of apoptosis proteins, *WT1* Wilms' tumor suppressor protein

intracellular proteases and extracellular proteases in cancer development, including mitochondrial proteases, lysosome proteases, cytosolic proteases, and matrix metalloproteinases (MMPs). Figure 7.1 shows the network of the proteases in cancer development (Fig. 7.1).

2 Mitochondrial Proteases in Cancers

Mitochondria are essential for the survival and proliferation of normal and cancer cells. Besides their role as an energy factory for maintaining metabolism and proliferation of cells, they are also part of the apoptosis machinery. Dysfunction of mitochondria may lead to the development of various cancers.

Mitochondrial proteases play crucial roles in cancer development directly or indirectly. Some of them strictly control the quality of proteins in organelles by degrading misfolded and non-assembled polypeptides. Others are key factors in the apoptosis pathway. The roles of two mitochondrial proteases, including high temperature requirement protein A2 (HtrA2/Omi) and Lon protease, in cancers are summarized below.

2.1 HtrA2/Omi

HtrA2/Omi is a serine protease in the mitochondrial intermembrane space. It is a crucial regulator to maintain mitochondrial homeostasis, facilitating cell survival. However, HtrA2 acts as a proapoptotic factor under stress. HtrA2/Omi is widely expressed in a variety of cancer cell lines, including gastric cancer [1], ovarian cancer [2], prostate cancer [3, 4], and lymphoma [5].

HtrA2/Omi is associated with cancer development mainly because of its role in apoptosis. It promotes apoptosis in human cells in a caspase-dependent manner, as well as in a caspase-independent manner, via its proteolytic activity. In response to apoptotic signals activated by cisplatin [6] and staurosporine [7], HtrA2/Omi undergoes proteolytic processing, releasing the mature form of the protease from the mitochondria into the cytoplasm, where its exposed N-terminal Ala–Val–Pro–Ser motif mediates interaction with the BIR domains of its substrates, inhibitors of apoptosis proteins (IAPs), including the X-linked inhibitors of apoptosis (XIAP), cellular inhibitor of apoptosis protein-1 (cIAP1) [8], and cIAP2. Binding of HtrA2 with IAPs leads to the degradation of IAPs and activates caspase-3-dependent apoptosis pathway [7, 9, 10]. Hence, upregulation of Omi/HtrA2 sensitizes cells to apoptosis. On the contrary, decreased expression of HtrA2 increases the resistance of multiple cell lines against apoptotic stimuli, reduces cell death, and was observed in various human cancer cells [2, 9].

Besides the interaction of HtrA2 with IAPs and being involved in caspase pathways, other mechanisms of HtrA2 action have also been reported. For example, HtrA2 controls cell proliferation through WARTS kinase [11]. HtrA2 can also elicit its protease activity by cleaving the oncogenic Wilms' tumor suppressor protein WT1 at multiple sites under apoptotic stimuli. This leads to WT1's removal from gene promoter regions and enhances apoptosis [12]. Knockdown of HtrA2 prevents its proteolysis activity on WT1 [13]. HtrA2 also binds to receptors, such as integrin alpha 7 (ITGA7), and triggers cell death in cancers [4]. The activity of HtrA2 is directly regulated by Akt, providing a mechanism by which Akt induces cell survival at the post-mitochondrial level [14].

Abundant work has been done to examine the precise mode of action and the importance of HtrA2 in apoptosis in mammalian cells through biochemical, structural, and genetic studies. While the N-terminal Ala–Val–Pro–Se motif is crucial for binding with the IAPs, both the N-terminal alanine and the catalytic serine residue control the proapoptotic activity of mature HtrA2 protein. Ablation in either of the sites reduces the ability, whereas inhibition of both sites completely blocks the proapoptotic activity of HtrA2/Omi [10].

2.2 Lon Protease

Lon protease is an ATP-dependent serine peptidase. It plays vital roles in the degradation of misfolded and damaged proteins and maintains the stability of the mitochondrial genome. Overexpression of Lon is associated with tumor transformation

[15, 16]. Downregulation of Lon leads to massive apoptosis, disrupts mitochondrial structure, and causes cell death [17].

Lon protease maintains the respiratory system in tumors through one of its substrate, COX4-1 [18]. Cytochrome C oxidase (COX) is a terminal enzyme in the respiratory electron transport chain of mitochondria. There are two isoforms of COX4. COX4-1 is expressed under aerobic condition, whereas COX4-2 plays its major role under hypoxia. Rapid proliferation of cells may lead to hypoxia, which is lethal in normal cells. However, in malignant cells, the hypoxia induces Lon protease and COX4-2 expression through hypoxia-inducible factor-1 (HIF-1). COX4-1 is degraded by the Lon protease, whereas the level of COX4-2 is elevated to maintain the energy supply under hypoxia to promote tumor cell survival.

Lon protease also maintains the stability of the mitochondrial genome through its chaperon activity. Alteration of mitochondrial DNA is reported in cancer cells [19]. Mitochondrial DNA is in close proximity to the respiratory chain and is susceptible to high levels of reactive oxygen species (ROS) [20]. The D loop region controls replication and transcription. This three-stand segment in the mitochondrial genome where Lon binds contains many hot spots of mutation in cancer cells. However, despite our current level of knowledge, we still don't fully understand the precise mechanism by which Lon protease contributes to cancer development yet.

Biochemical and structural studies have revealed that Lon consists of three major functional segments. The N-terminal domain (N domain) is responsible for interaction with protein substrates. Blockage or mutation in N domain decreases its expression, reducing its proteolytic activity and even life cycle in *Podospora anserina* [21]. The second part is related to ATP binding and hydrolysis. ATP stimulates the proteolysis activity of Lon, whereas ADP inhibits this process [22]. The P domain carries the catalytic binding sites [23]. However, the P domain itself doesn't show proteolytic activity on protein substrates, but only on small peptides [24].

Recently, several drugs targeting Lon protease such as the obtusilactone A and sesamin have been shown to induce apoptosis in human lung cancer cells [25]. Synthetic triterpenoid CDDO and its derivatives also selectively inhibit Lon and cause the cell death in lymphoma [26].

3 Caspases

Evasion of apoptosis is one of the hallmarks of tumor development. Caspases are closely interconnected with mitochondria regarding their roles in apoptosis and serve as the major executors of the apoptosis machinery. Of the 12 caspases so far identified, 7 are involved in apoptotic pathways. CASP9, CASP2, CASP8, and CASP10 are categorized as initiator caspases which are recruited to the apoptosome when death stimulus occurs. The initiator caspases contain death-fold motifs, such as caspase recruitment domain (CARD, as in caspase 2 and caspase 9) or death effector domain (DED, as in caspase 8 and 10) at the prodomains. These initiator

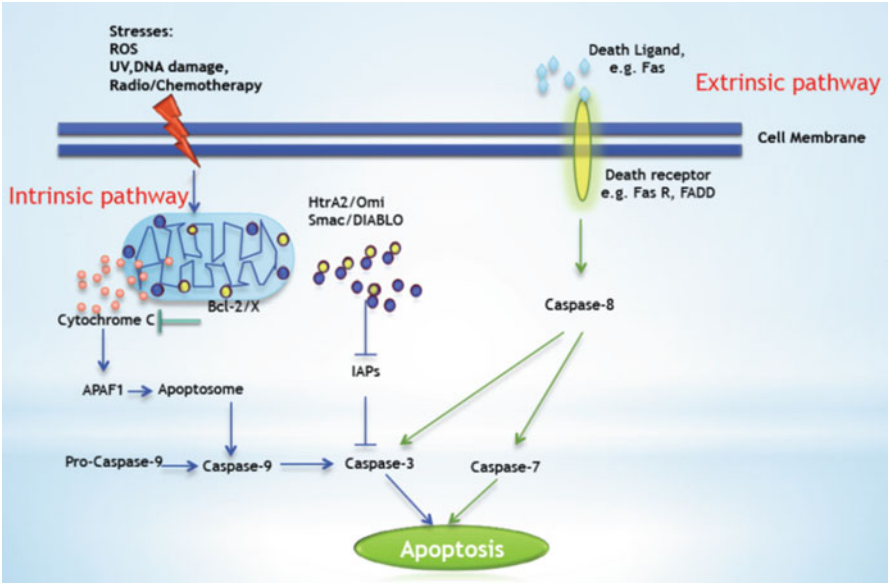

Fig. 7.2 Intrinsic and extrinsic apoptosis pathways. *HtrA2/Omi* high temperature requirement protein A2, *IAP* inhibitors of apoptosis proteins, *ROS* reactive oxygen species, *COX* cytochrome C oxidase, *Apaf-1* apoptosis-activating factor 1, *SMAC/Diablo* second mitochondrial activator of caspases/direct IAP-binding protein with low p*I*, *FADD* Fas-associated protein with death domain, *UV* ultraviolet

caspases further cleave the inactive pro-forms of members of another caspases family, the effector caspases, including CASP3, CASP6, and CASP7, which cleave protein substrates within cells and trigger the apoptotic process. Both groups of caspases play vital roles in apoptosis.

There are two main apoptosis pathways. One is the extrinsic receptor mediated and the other is intrinsic mitochondrial pathways. The extrinsic pathway is initiated by extracellular ligands through oligomerization of cell surface receptor and assembly of death-inducing signaling complex (DISC), whereas the intrinsic involves the participation of mitochondrial proteins such as Bcl-2 family and assembly of apoptosome (Fig. 7.2). Cells utilize an advanced machinery to get rid of genetic or biochemically abnormal cells through apoptosis. Repression of apoptosis, by either antiapoptotic proteins or mutation of the apoptotic executors (the caspases), can lead to cancers (Table 7.1) [27–31, 36, 44]. In particular, caspase 8 is associated with advanced and invasive cancers. In addition, recent evidence suggests that caspases per se could act as tumor suppressors. For instance, knockdown of caspase 2 not only promotes growth of mouse embryonic fibroblasts (MEFs) but also aggressively accelerates tumor formation [32]. Here, we will take initiator caspase 8 and effector caspase 3 as examples to illustrate the mechanisms by which caspases are involved in cancers.

Table 7.1 Alteration of caspase genes in various cancers

Cancers	Caspases	References
Breast	Caspase 3, 8, 10	[44]
Colorectal	Caspase 7, 8, 9	[28]
Gastric	Caspase 2, 6, 8	[30]
Head and neck	Caspase 8	[28]
Hepatocellular	Caspase 8	[29]
Lung	Caspase 8, 10	[31]
Neuroblastoma	Caspase 8	[36]

3.1 Caspase 8

Caspase 8 belongs to the family of cysteine proteases and is one of the initiator caspases in the extrinsic apoptosis pathway [33]. The activation of caspase 8 depends on the DISC in mammalian cells [34].

Several mechanisms are involved in caspase 8-induced cancer development. Caspase 8 plays a central role in the ligand-induced apoptosis of tumor cells. For example, the extracellular ligands of cytotoxic T lymphocytes such as TNF family death ligands (TRAIL, Fas/CD95 ligand, and TNF-a) interact with the corresponding death receptors (TRAIL receptors, Fas/CD95, and TNFR1), which then recruit adaptor proteins such as FADD in the cytosol, followed by recruiting pro-caspases 8. Caspase 8 is generated, and the active caspase 8 is capable of cleaving and activating downstream caspase 3 and other proteases. The cell death machinery is triggered and the tumor cells die [35].

In addition, genetic mutation or alteration in the caspase 8 gene or its regulatory sequence is associated with tumor formation. Silencing of caspase 8 caused by gene deletion or promoter hypermethylation has been identified in pediatric tumors and corresponding cell lines [36]. A six-nucleotide deletion in the promoter region of caspases 8 reduced the expression level of caspase 8 protease and was associated with squamous cell carcinoma of the head and neck cancer [37]. Other variants of caspase 8 such as caspase 8 long (caspase-8L) have also been identified in tumors. Caspase 8L is a splice variant produced by the insertion of a 136bp sequence between exon 8 and exon 9. This insertion leads to a premature stop codon, generating a truncated caspase 8 with only the DED domains but lacking the C-terminal proteolytic domain [38, 39]. This truncated, inactive caspase 8 can still be recruited to the DISC complex due to its retention of DED domains [40, 41]. As a result, caspase 8L acts as a dominant negative to the wild-type caspase 8 and therefore prevents cell death [42, 43].

Moreover, caspase 8 may also have multiple effects on tumor development. Caspase 8 can suppress oncogenic transformation, independent of its role in apoptosis. Loss of caspase 8 expression is often associated with amplification of the MYCN oncogene and increased expression of the corresponding protein. However, it is not clear if these two genetic alterations are functionally linked or if they just fit into a two-hit model providing a permissive environment for tumor growth.

3.2 Caspase 3 (Effector Caspase)

Caspase 3 belongs to the effector caspase family. It is both required in extrinsic and intrinsic apoptotic pathways. Signals from caspase 8 (extrinsic) or from the complex of caspase 9, apoptosis-activating factor 1 (Apaf-1), ATP, and cytochrome c (intrinsic) cleave the procaspase 3 and activate caspase 3 and then further cleave and activate caspases 6 and 7. Effector caspases target a broad spectrum of cellular proteins, ultimately leading to cell death. The proteolytic activity of mature caspases 9 and 3 is inhibited by inhibitor of apoptosis proteins (IAPs). In turn, IAPs are inactivated and caspase activity restored by regulatory proteins such as SMAC/Diablo (second mitochondri-derived activator of caspases/direct IAP-binding protein with low p*I*) or HtrA2/Omi, which are released from the mitochondria (Fig. 7.2).

Disturbances of caspase 3 have been reported in several types of cancers. In a screen of primary breast tumor samples for caspase 3 levels using PCR, northern blot, and western blot, approximately 75 % of the tumors as well as morphologically normal peritumoral tissue samples were found to lack caspase 3 transcripts and caspase 3 protein expression [44]. Interestingly, upregulation of caspase 3 has also been reported in clinical breast tumor samples, using immunohistochemistry [45, 46]. This discrepancy is likely due to the difference in the methods of analysis.

Structural and biomedical studies reveal that several mechanisms are involved in cancer development in association with caspase 3. For example, the different observations in the previous reports may be due to the different forms of caspase 3 being detected in cancer. CASP-3 gene can undergo alternative splicing, giving rise to two forms, the wild-type caspase 3 and a short-form caspase 3s which has antiapoptotic function [47]. Co-expression of two caspase 3 isoforms has been detected in diverse tumor cell lines, as well as in breast carcinomas, where the ratio of expression levels has been used as a prognostic marker to guide the use of cyclophosphamide-containing chemotherapy in patients [48]. At present, the exact role of caspase 3 in tumor formation/progression and tumor sensitivity to treatment is still unclear.

Inhibiting apoptosis is one of the important aspects of cancer; therefore finding ways to reverse this inhibition, and thus activate caspases, is important in cancer therapies and in the developing novel strategies in treating cancers. For instance, inactivation of caspase 8 has been shown to cause resistance to current treatment approaches; thus restoration of caspase 8 represents a promising therapeutic way to treat human cancers. Regulators of the caspase family are also promising pharmacological tools for treating of cancers.

Recently, a report from *oncogene* shows that caspase 3 activation in dying tumors cells in patients undergoing chemo- and radiotherapeutic regimens can not only induce cell death but also proteolytically activates a cytosolic Ca^{2+}-independent phospholipase A2 (iPLA2) (Fig. 7.3). iPLA2 then adopts the plasma membrane lipid as a substrate and produces arachidonic acid (AA) and releases soluble lipid messengers, notably prostaglandin E2 (PGE2), in a cascade of enzymatic reactions. The PGE2 stabilizes EP2 protein (a G-protein-coupled receptor expressed in the surface of tumor cells), which promotes tumor cell proliferation possibly through

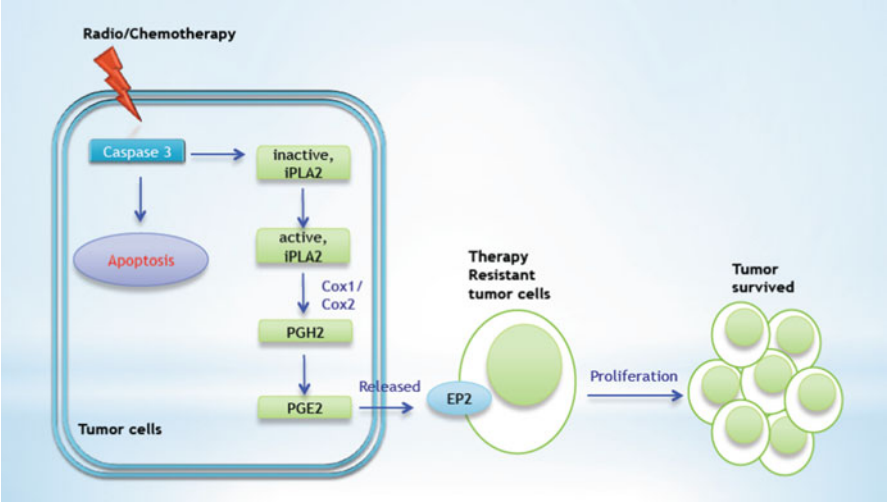

Fig. 7.3 Caspase 3-mediated stimulation of tumor cell repopulation during cancer therapy. *COX* cytochrome C oxidase, *iPLA2* Ca^{2+}-independent phospholipase A2, *PGH2* prostaglandin H2, *PGE2* prostaglandin E2, *EP2* E-prostanoid receptor 2

the WNT-β-catenin signaling pathway [49]. The report suggests that therapies that block the activation of caspase 3 alone may result in significant clinical and therapeutic failure.

4 Cathepsin Proteases

Cathepsin proteases are lysosomal peptidases ubiquitously expressed in animals. Most of them are activated and take effect in lysosomes with only few exceptions such as Cathepsin K, which takes effect in the extracellular matrix. Elevated Cathepsin proteases have been reported in several types of cancers including breast cancer [50–52], prostate cancer [53], colon cancer [54], and lung cancer [55]. Two representative Cathepsin proteases, the aspartyl Cathepsin D and the cysteine Cathepsin B, and their roles in cancers are summarized below.

4.1 Cathepsin D

Cathepsin D is a lysosomal aspartyl protease, a dimer of disulfide-linked heavy and light chains. Mutations in the Cathepsin D gene are involved in the pathogenesis of breast cancer and other diseases. Overexpression of Cathepsin D has been observed in breast cancer and prostate cancer and is associated with poor prognosis [56–59].

Cathepsin D has been implicated in activating growth factors, such as bFGF, which is known to be able to promote cancer cell growth and angiogenesis [60]. More direct evidence supports such roles of Cathepsin D: While overexpression of Cathepsin D was found to increase proliferation by reducing the cellular production of unknown secreted growth inhibitors [61], Cathepsin D also promotes cancer cell invasion and metastasis [62]. Interestingly, mutated Cathepsin D devoid of catalytic activity did not lose its mitogenic activity in cancer and endothelial and fibroblastic cells. Studies in estrogen receptor-positive breast cancer cell lines revealed that this housekeeping enzyme is highly regulated by estrogens and certain growth factors (i.e., IGF1, EGF). The regulation of Cathepsin D mRNA levels by estrogen is mainly due to increased initiation of transcription [63]. In addition, cancer cells and stromal cells overexpress pro-Cathepsin D [64]. The elevated pro-Cathepsin D in the extracellular matrix (ECM) of tumors also suggests an extracellular mode of action of Cathepsin D. The acidic environment of tumors facilitates the maturation of the pro-Cathepsin D and triggers downstream signaling pathways, either through some unknown cell surface receptor or to promote fibroblast outgrowth via a paracrine loop. This overexpressed and hypersecreted pro-Cathepsin D also stimulates motility and invasion of fibroblasts, or cancer cell angiogenesis, and results in enhanced tumor–host homeostasis. However, the mechanism is not yet clear.

Cathepsin D can also attenuate the immune response in mouse tumor models through its proteolytic activity, by degrading a series of chemokines including MIP-1a, MIP-1b, and SLC [65]. MIP-1a and MIP-1b recruit immature dendritic cells (DCs) to the tumor tissues, and then these immature DCs mature and express SLC receptor CCR7, which initiates an antitumor immune response at sites of the primary tumor [66, 67]. The cleavage of SLC by Cathepsin D interferes with the migration of antigen-loaded mature DCs to secondary lymphoid organs and thus attenuates the antitumor effect of the chemokines [68].

Researchers have tested major types of tumors for a correlation between Cathepsin D expression and cancer progression stages and the clinical outcome. Meta-analysis has established a correlation between high levels of Cathepsin D and poor disease-free survival in node-negative breast cancer patients [69]. Cathepsin D could also serve as a potential prognostic marker for lung cancer [55].

4.2 Cathepsin B

Cathepsin B belongs to the large family of cysteine Cathepsin proteases. Cysteine cathepsin proteases play important roles in cancer development. The Cathepsin B, as a major member in this family, is involved in several cancers, including breast cancer and melanoma [70, 71]. Cathepsin B is upregulated at the levels of mRNA, protein, and activity in these human cancers. For example, in inflammatory breast cancer (IBC), high levels of Cathepsin B are detected in the caveolar membrane microdomains. There is also a significant positive correlation between the expression of Cathepsin B and the number of positive metastatic lymph nodes in IBC.

Table 7.2 Cathepsins and inhibitors

Cathepsins	Cancers	Inhibitors
Cathepsin B	Melanoma	CA-074
	Breast cancer	
Cathepsin D		Pepstatin A
Cathepsin K	Breast cancer, bone metastasis	MK-0882
	Bone metastasis	SB-553484
Cathepsin L		SC-364671
Cathepsin S	Rheumatoid arthritis	RWJ-445380

Cathepsin B is proposed to be a prognostic marker for lymph node metastasis, and elevated Cathepsin B activity correlates to poor therapy outcome [71].

Cathepsin B expression is increased in tumor cells, particularly at the invasive edges with abundant stromal fibroblasts and inflammatory cells. Cathepsin B enhances the tumor proteolysis. It degrades extracellular matrix proteins, such as laminin and collagen IV, and activates the precursor form of urokinase plasminogen activator (uPA). Alternatively, it binds to the annexin II heterotetramer (AIIt) at the caveolar region on the tumor surface, with the AIIt binding site for Cathepsin B differing from that for plasminogen/plasmin or tissue plasminogen activator (tPA). Activation of Cathepsin B on the cell surface by AIIt leads to the regulation of downstream proteolytic cascades. In addition, AIIt also interacts with extracellular matrix proteins, such as collagen I and tenascin-C, forming a structural link between the tumor cell surface and the extracellular matrix. One assumption is that the complexes formed by Cathepsin B, AIIt, tPA, and tenascin-C facilitate the activation of precursors of proteases and initiation of proteolytic cascades, hence promoting the tumor cell detachment, invasion, and motility [72]. Increased secretion of pro-Cathepsin B has also been observed in tumors. The intracellular trafficking and location of Cathepsin B are also modulated in cancer cells [73].

Since Cathepsin B protease has recently emerged as an important member of proteolytic enzymes in cancer progression and invasion, it is not surprising that Cathepsin B inhibitors have been proposed as anticancer agents. Up to now, numbers of inhibitors of Cathepsin B have been identified either as endogenously expressed molecules, such as cystatins, or as chemically synthesized agents, such as CA-074. CA-074 significantly reduces the percentage of invading cells in melanoma [70] and limits bone metastasis in breast cancer [74]. Inhibitors for other cysteine Cathepsins such as K, L, and S (the CLIK series) have also been developed and tested in vivo [75–78]. In summary, Cathepsins in general have emerged as promising pharmacological targets for cancer therapy. A lot of synthetic Cathepsin inhibitors are under different stage of clinical trials (Table 7.2).

5 MMPs

MMPs are a family of zinc-dependent endopeptidases that facilitate cancer development by triggering the release of growth and angiogenic factors and by modulating extracellular matrix molecules. MMPs are present in the extracellular matrix in an

inactive form, and once activated, MMPs are further regulated by three major types of endogenous inhibitors, α2-macroglobulin, reversion-inducing cysteine-rich protein with Kazal motifs (RECK) [79], and tissue inhibitors of metalloproteinases (TIMPs) (reviewed in [80]). The bioactivity of TIMPs is further regulated posttranslationally by processes such as inactivation by serine protease cleavage [81]. So far, 23 MMPs are identified in vertebrates and categorized into several classes. Among them, MMP9 is one of the most studied protease that is actively involved in cancer development. Several other MMPs are also discussed briefly.

5.1 MMP9

The evidence for MMP9 contributing to neoplastic progression was from mouse model studies, where eliminating MMP9 significantly reduced the incidence of pancreatic islet carcinomas [82] and cervical carcinogenesis [83], while reconstitution of MMP9 restored cellular programs for the neoplastic progression and tumor development [84, 85]. The infiltrated leukocytes in these cancers are the major source of MMP9.

Several mechanisms are involved in MMP9-associated tumor progression. MMP9 alters the stromal microenvironment by mediating liberation of ECM-sequestered growth-promoting factors, such as basic fibroblast growth factor (FGF-2), or proteolytic cleavage of growth factor latent precursors, such as transforming growth factor (TGF-a) [86]. MMP9 is also found to be an active regulator of tumor angiogenesis: Vascular endothelial growth factor (VEGF) is a key factor in neovessel formation. However, VEGF requires both high MMP9 and the VEGF receptor (VEGFR1) to activate the angiogenic program. Low expression levels of VEGFR1 fail to induce subsequent metastatic cell growth in the presence of MMP9, while increases of VEGFR1 combined with a population of endothelial cells capable of inducing expression of MMP9 induce significant metastasis. It is now believed that activated MMP9 releases matrix-sequestered VEGFa to interact with its receptors, to be able to stimulate efficient vascular remodeling and angiogenesis necessary for metastatic cell growth and survival [82, 87]. Taken together, MMP9 not only induces a microenvironment favorable for primary tumor growth but is also crucial for the metastasis and survival of these metastatic cells [88, 89].

5.2 Other MMPs

Several other MMPs have been shown to enhance tumor progression. MMP1 increases susceptibility of chemical skin carcinogenesis [90]; MMP14, MMP2, or MMP3/stromelysin-1 promotes mammary carcinogenesis [91], while reduction of MMP11 attenuates chemically induced skin cancer [92]. Among them, MMP14 and MMP2 could release cryptic fragments of laminin-5 gamma 2 chain domain, which binds to the EGF receptor on tumor cells, thus activating downstream signaling

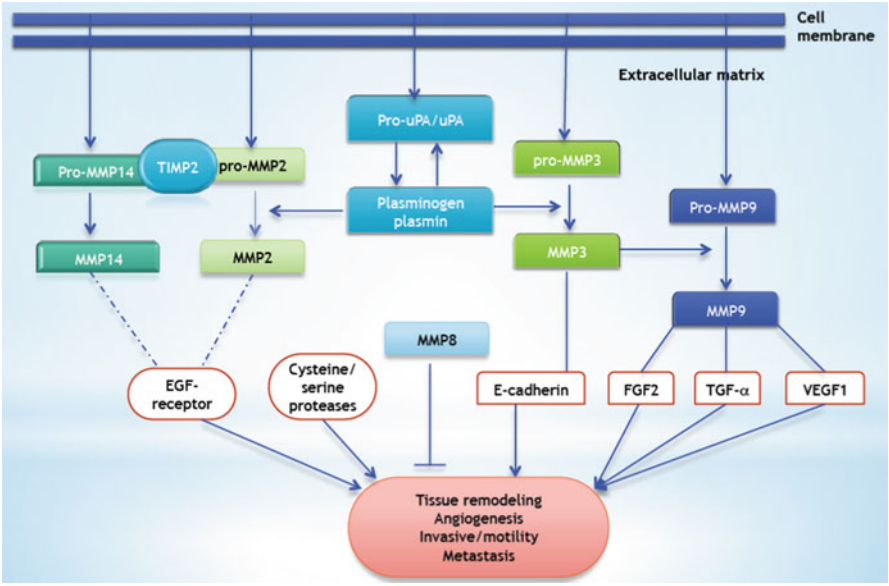

Fig. 7.4 MMPs and cancer progression. *MMP* matrix metalloproteinases, *uPA* urokinase plasminogen activator, *EGF* endothelial growth factor, *FGF-2* basic fibroblast growth factor, *TGF-α* transforming growth factor alpha, *VEGF* vascular endothelial growth factor

events that lead to tumor cell motility [93, 94]. Others such as MMP3 target the adhesion molecule E-cadherin which will trigger progressive phenotypic conversion of normal epithelial cells into the invasive mesenchymal phenotype, as observed in mammary epithelial cells [95]. Most of these MMPs are capable of altering cell–cell and cell–matrix interactions. While most MMPs promote tumor progression, however, some MMPs also exhibit antitumor functions. For instance, MMP8 (collagenase 2) reduces skin tumor susceptibility [96].

The mechanisms by which MMPs contribute to the malignant transformation and cancer metastasis are illustrated in Fig. 7.4. Due to the active involvement of MMPs in tumor progression, they have been selected as potential targets for anticancer therapy. Quite a lot of MMP inhibitors have been identified; however, the results of clinical evaluation in cancer patients of these MMP inhibitors have not been as promising as initially expected. The reason is not clear. In the case of MMP9, although the MMP9 inhibitor attenuated or blocked its activity, as a feedback control, several other proteases in parallel have been upregulated in compensation for the loss of the MMP9 proteolytic activity. Therefore, therapies that target a series of proteases or a protease pathway rather than an individual protease may have better outcomes.

6 Conclusions

Proteases including mitochondrial proteases, lysosome proteases, cytosolic proteases, and matrix metalloproteinases play important roles in the development of various cancers. Changes of these proteases affect various aspects of cancer development, including transformation, apoptosis, invasion, and metastasis of cancer cells. More details of the signaling pathways of the proteases are starting to be revealed. Targeting these proteases shows promise in cancer treatment.

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