

Emerging roles of proteases in tumour suppression

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Abstract | Proteases have long been associated with cancer progression because of their ability to degrade extracellular matrices, which facilitates invasion and metastasis. However, recent studies have shown that these enzymes target a diversity of substrates and favour all steps of tumour evolution. Unexpectedly, the post-trial studies have also revealed proteases with tumour-suppressive effects. These effects are associated with more than 30 different enzymes that belong to three distinct protease classes. What are the clinical implications of these findings?

Proteases have fundamental roles in multiple biological processes and are associated with a wide variety of pathological conditions, including cancer¹. Our view of the proteolytic world has expanded considerably after the recognition that beyond their nonspecific functions in protein catabolism, proteases act as processing enzymes that carry out highly selective cleavage of specific substrates and influence cell behaviour, survival and death². The sequencing of the human genome and certain model organisms has revealed that the impressive diversity in protease functions derives from the evolutionary invention of a large number of structurally and catalytically diverse enzymes with the common ability to hydrolyse peptide bonds³. Thus, the human degradome — the complete set of proteases produced by human cells — consists of at least 569 proteases and homologues distributed into five classes: 194 metalloproteinases, and 176 serine, 150 cysteine, 28 threonine and 21 aspartic proteases (FIG. 1). Interestingly, the mouse and rat degradomes are even more complex, with at least 644 and 629 members, respectively⁴. On the basis of these recent data, it seems clear that any attempt to understand the biological and pathological relevance of proteases must take into account the large diversity of proteolytic systems operating in all cells, tissues and diseases, including cancer.

Functional diversity of proteases

The association of proteases with cancer can be traced back to 1946, when Fisher proposed that tumour-associated proteolytic activity could be responsible for the degradation of the cell matrix and the

subsequent invasion of the tumour into the surrounding normal tissue⁵. This concept focused on extracellular and pericellular proteolysis, the degradation of matrix components, and the facilitation of tumour invasion and metastasis. Individual proteases began to be identified in the 1970s, with pro-metastatic activities associated with secreted members of the serine and cysteine protease and metalloproteinase families. Molecular biology studies over the next two decades found many proteins with a role in cellular invasion, as indicated by gain-of-function and loss-of-function experiments in models of both experimental and spontaneous metastasis. We now recognize that proteolytic enzymes contribute to all stages of tumour progression, and not only to the later stages as was originally thought^{6–8}. Intracellular and extracellular proteases can function as signalling molecules in various cellular processes that are essential for cancer biology. These protease-regulated processes include proliferation, adhesion, migration, differentiation, angiogenesis, senescence, autophagy, apoptosis and evasion of the immune system.

The function of intracellular proteases in signalling cascades is generally associated with the removal of damaged or undesirable products. Lysosomal cysteine and aspartyl cathepsins mediate the degradation of endocytosed proteins⁷. The cysteine proteases of the caspase family function in a tightly regulated cascade of proteolytic activities that results in apoptosis⁹. The cysteine autophagins contribute to the ‘self-eating’ effect that is observed during starvation conditions¹⁰. The deubiquitylases (DUBs), which are also cysteine

proteases, are responsible for removing protein modifications such as ubiquitin and small ubiquitin-related modifier (SUMO) from proteins¹¹. The organization of these intracellular enzymes in proteolytic cascades serves as a protective mechanism. Indeed, there are several examples of loss-of-function mutations in these proteases in various human tumours^{9,11}, indicating that they can function as tumour suppressors in the classical sense (FIG. 2; TABLE 1).

Conversely, extracellular proteases are thought to be actively involved in facilitating tumorigenesis. These enzymes are frequently overexpressed in malignant tissues, often as a result of the activation of oncogenic transcriptional pathways⁶. The prevailing view that proteases promote tumour progression and metastasis led to the development of small-molecule inhibitors for the treatment of cancer, in particular of molecules targeting matrix metalloproteinases (MMPs) and plasminogen activators². However, clinical trials showed that these molecules had, at best, no effect and in some patients treated with broad-range metalloproteinase inhibitors, there was a suggestion of an acceleration of tumour growth. This finding suggested the possibility that some proteases might have anti-tumour roles^{12,13} and forced a re-evaluation of the prevailing concepts. Recent studies based on the generation of loss-of-function animal models have provided definitive evidence of the existence of extracellular proteases with anti-tumour properties^{14–16} (FIG. 2; TABLE 1). These results support an emerging and paradoxical role for proteases in tumour progression, which is the focus of this Perspective.

Intracellular proteases

Caspases. These enzymes constitute a family of cysteine proteases that have a fundamental role in apoptosis. Because deregulation of apoptosis is one of the hallmarks of cancer, there has been great interest in evaluating whether genetic alterations of caspases occur in malignant tumours. The first clear evidence in this regard derived from studies on the caspase 8 gene (*CASP8*) that was found to be deleted or silenced through DNA methylation in neuroblastomas with amplification of the oncogene *MYCN* (REF. 9). Further studies have shown that *CASP8* is inactivated by somatic mutations in other malignancies, including head and neck, lung, colorectal and gastric carcinomas as well as diverse paediatric tumours^{17–19}. Interestingly, loss of *CASP8* increases the

risk of metastasis in patients with neuroblastoma, which has led to the proposal that this protease is a metastasis suppressor that regulates the survival and invasive capacity of neuroblastoma cells²⁰. Different groups explored the possibility that other caspases might be inactivated in cancer and found that the caspase 10 gene (*CASP10*) — which is functionally related to *CASP8* and located in the same region on chromosome 2 — is also frequently inactivated by mutation in various human malignancies^{19,21,22}. Likewise, other family members such as caspase 3, caspase 5, caspase 6 and caspase 7 have occasionally been found to be mutated in human tumours^{23–26}. Altogether, these results indicate that the inactivation of caspases might represent an additional strategy for cancer progression (FIG. 3), although it is still unclear whether such mutations are driver mutations or passenger mutations that occur during cancer progression.

Deubiquitylases. It is now well established that deregulation of ubiquitin-dependent signalling pathways underlies many human diseases, including cancer¹¹. The attachment of ubiquitin and ubiquitin-like modifiers to their target proteins is a reversible process that is catalysed by members of this large family of DUBs. Some DUBs were originally identified as oncogenic proteins, but recent work has shown that other family members are *bona fide* tumour suppressors. This is the case for the protease encoded by *CYLD*, a tumour-suppressor gene that is mutated in patients with *familial cylindromatosis*, a disease characterized by the formation of multiple tumours of skin appendages²⁷. Reduced or absent expression of *CYLD* has also been detected in tumours from other origins, pointing to a more general tumour-suppressor function for this enzyme²⁸. *CYLD* limits inflammation and tumorigenesis through deubiquitylation of different components of the nuclear factor- κ B (NF κ B)-signalling pathway, including tumour necrosis factor (TNF) receptor-associated factor 2 (*TRAF2*) and *TRAF6*, inhibitor of NF κ B kinase γ (*IKBK γ*) and *BCL3* (REF. 29).

Similar to *CYLD*, herpes-virus associated ubiquitin-specific protease (*HAUSP*) could also indirectly act as an anti-tumour enzyme through its ability to catalyse the deubiquitylation and stabilization of the tumour suppressor p53 (REF. 30). Consistent with this, *HAUSP* overexpression is sufficient to induce p53-mediated anti-tumour effects such as apoptosis or cell-growth

arrest. Likewise, reduced *HAUSP* expression has been found in different human carcinomas³¹. In addition to *CYLD* and *HAUSP*, other DUBs might have roles as anti-tumour enzymes. For example, the desumoylating enzyme sentrin-specific protease 1 (*SENPI*) targets a reptin chromatin-remodelling complex that promotes the expression of the metastasis suppressor KAI1 (also known as *CD82*), which blocks the invasive activity of cancer cells³².

Autophagins. These cysteine proteases belong to a group of four related enzymes involved in proteolytic processing events that are associated with autophagy¹⁰. This process was originally described as a physiological response to starvation, but recent studies have also shown its relevance in cancer³³. The finding that the inactivation of autophagic genes such as beclin1 (*BECN1*) promotes tumorigenesis, whereas their overexpression blocks tumour development, has suggested that autophagy is a tumour-suppressive mechanism. Consistent with this, mutant mice deficient in autophagin 3 (also known as *Atg4c*) show a high incidence of carcinogen-induced fibrosarcomas, which has been correlated to the decrease in autophagy found in *Atg4c*^{-/-} fibroblasts³⁴. This observation adds autophagin 3 to the growing list of anti-tumour proteases and opens the possibility of evaluating similar protective functions in the remaining members of this family of cysteine proteases.

Extracellular proteases

Extracellular proteases of all major catalytic classes might act to suppress tumour progression. Most work in this regard has focused on members of different families of metalloproteinases, but proteases belonging to other catalytic classes, including cysteine proteases and serine proteases, might also function as anti-tumour enzymes^{7,8}. The growing category of extracellular and pericellular proteases with tumour-defying functions includes MMPs, ADAMTSs (disintegrin-metalloproteinases with thrombospondin domains), neprilysin (also known as *MME*), cathepsins, kallikreins, tectin (also known as *PRSS21*), prostaticin (also known as *PRSS8*) and dipeptidyl peptidase 4 (also known as *DPP4*) (FIG. 2; TABLE 1).

Matrix metalloproteinases

The MMP family comprises 23 human enzymes that have long been associated with cancer invasion and metastasis owing to their ability to degrade the extracellular matrix^{6,35}. However, recent studies have shown that

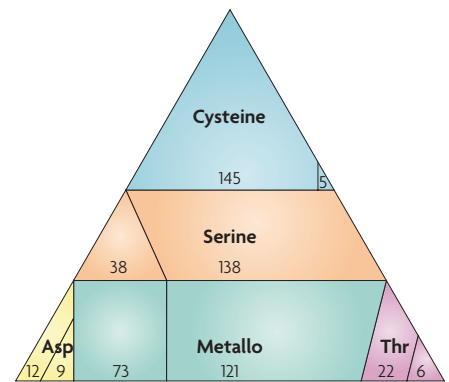


Figure 1 | **Classification of human proteases.** All identified human proteolytic enzymes are classified into five catalytic classes: metalloproteinases, serine, threonine, cysteine and aspartic proteases. Numbers at the left sections of each catalytic class correspond to intracellular or integral-membrane enzymes, whereas numbers at the right sections refer to extracellular or pericellular enzymes. The pyramidal structure of the figure does not imply a hierarchical organization of proteolytic systems.

several members of this family, including collagenase 2 (also known as *MMP8*), macrophage metalloelastase (also known as *MMP12*) and matrilysin 2 (also known as *MMP26*), provide a protective effect in different stages of cancer progression. Furthermore, other MMPs such as stromelysin 1 (also known as *MMP3*), gelatinase B (also known as *MMP9*), stromelysin 3 (also known as *MMP11*) and *MMP19*, which were originally recognized as pro-tumorigenic proteases, might also function as protective enzymes in some specific situations.

MMP8. The anti-tumour properties of *MMP8* were the first to be demonstrated *in vivo*¹⁴. *MMP8* is a protease that is mainly produced by neutrophils and is associated with inflammatory conditions. Studies with *MMP8*-deficient mice have shown that the absence of this protease strongly increases the incidence of skin tumours in mice. Conversely, bone-marrow transplantation experiments in these mice showed that neutrophil-derived *MMP8* is sufficient to restore the anti-tumour protection mediated by this metalloproteinase. Further studies aimed at elucidating the basis of the protective effect of *MMP8* showed that the loss of this enzyme causes profound abnormalities in the inflammatory response induced by carcinogens, finally leading to a sustained inflammation that generates a more favourable environment for tumour development¹⁴. The relevance of *MMP8* as a

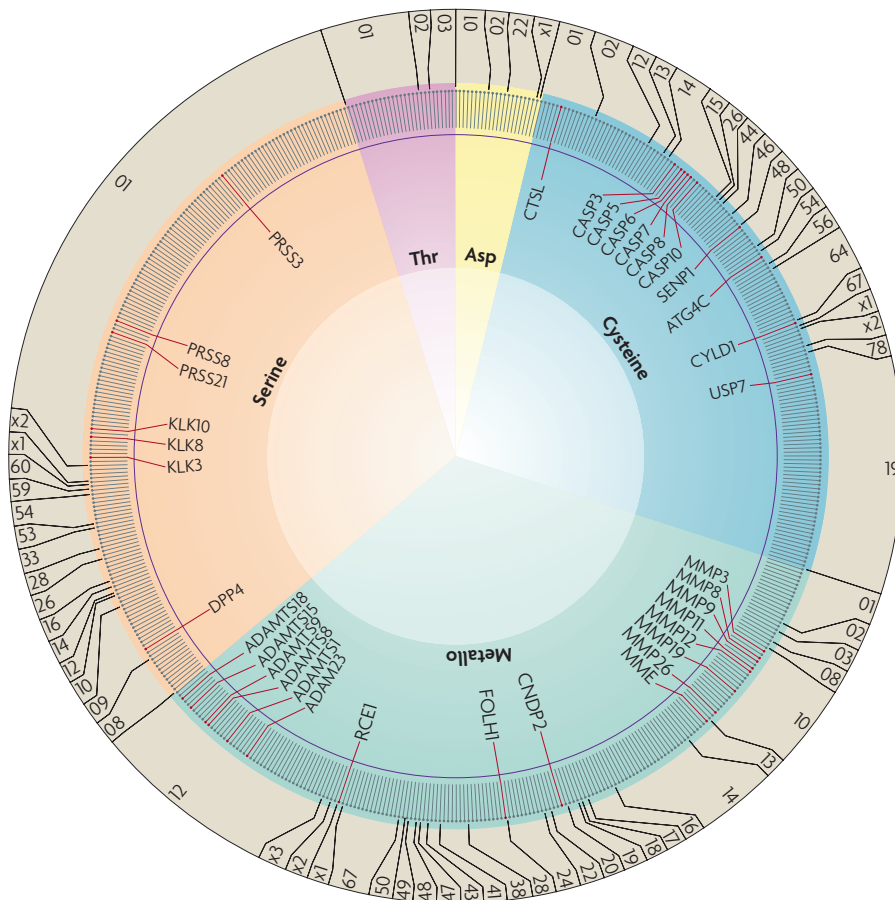


Figure 2 | **Distribution of tumour-protective proteases in the human degradome.** Human proteases are distributed in 5 classes and 69 different families. Each individual enzyme is indicated by a grey line, and those with tumour-protective properties are shown in red. Numbers at the edge represent the different protease families of each catalytic class, according to the MEROPS database numbering.

protective factor in cancer has been further shown by experiments carried out in human breast cancer cells. The downregulation of MMP8 in non-metastatic breast cancer cells increases their metastatic potential, whereas its overexpression in metastatic breast cancer cells reduces this potential³⁶. We have also found that MMP8 expression confers a good prognosis to breast cancer patients (C.L.O., unpublished observations). Altogether, these results indicate that MMP8 is an important tumour-protective factor with the ability to influence both the primary tumour growth and the metastatic potential of malignant cells.

MMP12. MMP12 is mainly produced by macrophages, and its expression has been linked to reduced tumour growth rates in mice³⁷. Likewise, studies using *Mmp12*^{-/-} mice have revealed a protective role for stromal MMP12 in lung tumour growth³⁸. Further studies have also shown that this protease

suppresses the growth of lung metastases both in spontaneous and experimental metastasis models³⁹. The precise role of MMP12 in human cancer is still unclear, because there are several reports that associate its expression in hepato-cellular and colon carcinoma with favourable outcomes, whereas in other tumours its expression correlates with a worse prognosis^{40–42}. It has been proposed that the cellular source of MMP12 can determine its effects; when expressed by tumour cells, the clinical outcome tends to be unfavourable, but when produced by host macrophages it confers a good prognosis⁴³. The anti-tumour effects of MMP12 could derive from its ability to cleave plasminogen and generate angiostatin, a potent angiogenesis inhibitor that blocks endothelial cell proliferation and migration⁴⁴. Consistent with this possibility, MMP12 expression in human hepatocellular carcinomas correlates with the formation of hypovascular tumours that produce angiostatin⁴⁰.

MMP26. MMP26 is a recently described member of the MMP family, which is absent in rodents and could have a specific function in human tissues⁴⁵. MMP26 expression levels are strongly induced in diverse hormone-regulated carcinomas but, contrary to most MMPs, its expression contributes to a favourable clinical outcome⁴⁶. The anti-tumour properties of this protease might derive from its ability to regulate the expression levels of oestrogen receptor β by cleaving it and so influencing oestrogen signalling in hormone-dependent malignancies⁴⁶. Moreover, the finding that MMP26 is produced at high levels by macrophages and polymorphonuclear leukocytes has suggested that, similar to MMP8, this protease could be part of an anti-tumour inflammatory response that contributes to a better clinical prognosis in certain patients with cancer⁴⁶.

MMP3, MMP9, MMP11 and MMP19. These enzymes represent examples of dual proteases in terms of cancer-modulating properties. MMP3 was first described as a potent pro-tumorigenic protease⁴⁷, but recent studies have shown its protective role in skin cancer¹⁵. For example, squamous cell carcinomas in *Mmp3*^{-/-} mice are less differentiated and grow faster than controls. Likewise, transgenic mice overexpressing MMP3 in their mammary glands develop fewer 7,12-dimethylbenz[a]ant hracene (DMBA)-induced tumours than control mice, further showing the protective role of this MMP in certain mouse models⁴⁸. The observation that MMP3 expression accelerates the rate of apoptosis in transformed cells might help to explain its anti-tumour effect⁴⁸.

Similar to MMP3, MMP9 has also been frequently associated with tumour progression⁶, but in some instances it might have an anti-tumour effect. For example, the human papilloma virus (HPV) 16-induced carcinomas that arise in a *Mmp9*-null background are more aggressive and of a higher grade than those in wild-type mice, indicating a suppressive effect for this protease during tumour progression⁴⁹. Clinical studies have shown that the overexpression of MMP9 is associated with a favourable prognosis in patients with node-negative breast cancer and inversely correlates with liver metastasis in patients with colorectal cancer, thereby validating the protective role of this enzyme that is observed in some experimental settings^{50,51}. As in the case of MMP12, the protective effects of MMP9 might derive from its capacity to generate

angiogenesis inhibitors such as angiostatin and tumstatin, a peptide fragment from the NC1 domain of the $\alpha 3$ chain of type IV collagen^{52,53}.

MMP11 also has dual roles during mouse mammary tumour progression, increasing the ability of cancer cells to give rise to primary tumours but repressing the development of metastases⁵⁴.

Finally, the decreased susceptibility of *Mmp19*^{-/-} mice to develop chemically induced skin tumours suggests that MMP19 may promote tumour growth⁵⁵. However, it also functions as a negative regulator of early steps of tumour angiogenesis and invasion⁵⁶, providing an additional example of a protease with dual roles in cancer.

ADAMTSs

These are members of a family that includes 19 human enzymes, which show some structural relationships with other metallo-proteinases such as MMPs and ADAMs (disintegrin and metalloproteinase domain proteases)⁵⁷.

ADAMTS1. This was the first identified member of the ADAMTS family. It has a complex domain organization and undergoes autoproteolytic cleavage to generate N-terminal and C-terminal fragments that contain at least one thrombospondin domain. Several reports have shown that *ADAMTS1* inhibits angiogenesis and reduces tumour growth and metastasis formation, although data are not univocal in this regard^{58–60}. Further studies have shown that the proteolytic status of *ADAMTS1* determines its anti-tumour effect as the cleavage fragments are responsible for the anti-metastatic properties of this enzyme⁶¹. The ability of *ADAMTS1* to inhibit endothelial cell proliferation by sequestering vascular endothelial growth factor (VEGF) and its capacity to release anti-angiogenic peptides from thrombospondins might also contribute to the protective properties of this protease in cancer^{62,63}. Consistent with this, *ADAMTS1* is downregulated in breast, colorectal and lung carcinomas through cancer-specific promoter hypermethylation^{64–66}.

ADAMTS8, ADAMTS9, ADAMTS15 and ADAMTS18. *ADAMTS8* — which is a secreted protease with anti-angiogenic properties — is dramatically downregulated in tumours from diverse origins, and this silencing is often associated with promoter hypermethylation^{64,67,68}. Likewise, because expression of *ADAMTS9* is downregulated or lost through epigenetic

Table 1 | **Proteases with anti-tumour properties**

Gene	Protease name	Anti-tumour mechanism	Refs
Intracellular proteases			
<i>ATG4C</i>	Autophagin 3	Activation of autophagy	34
<i>CASP3</i>	Caspase 3	Induction of apoptosis	23
<i>CASP5</i>	Caspase 5	Induction of apoptosis	24
<i>CASP6</i>	Caspase 6	Induction of apoptosis	25
<i>CASP7</i>	Caspase 7	Induction of apoptosis	26
<i>CASP8</i>	Caspase 8	Induction of apoptosis	9,17–20
<i>CASP10</i>	Caspase 10	Induction of apoptosis	19,21,22
<i>CYLD</i>	CYLD	Negative regulation of NF κ B pathway	27–29
<i>SEN1</i>	Sentrin protease 1	Induction of CD82 tumour suppressor	32
<i>USP7</i>	HAUSP	Stabilization of p53	30,31
<i>CNDP2</i>	Glu-carboxypeptidase like B	Inhibition of proliferation and invasion	78
<i>CTSL1</i>	Cathepsin L	Inhibition of proliferation	81
<i>RCE1</i>	Ras-converting enzyme 1	Inhibition of proliferation	80
Extracellular proteases			
<i>ADAM23</i>	ADAM 23	Not determined	79
<i>ADAMTS1</i>	ADAMTS 1	Angiogenesis inhibition	59,61–63
<i>ADAMTS8</i>	ADAMTS 8	Angiogenesis inhibition	64,67,68
<i>ADAMTS9</i>	ADAMTS 9	Angiogenesis inhibition?	69
<i>ADAMTS15</i>	ADAMTS 15	Angiogenesis inhibition?	70
<i>ADAMTS18</i>	ADAMTS 18	Angiogenesis inhibition?	70,71
<i>DPP4</i>	Dipeptidyl peptidase 4	Inhibition of invasion	95–97
<i>FOLH1</i>	Prostate membrane-specific antigen	Inhibition of invasion	77
<i>KLK3</i>	Kallikrein 3	Activation of TGF β	82
<i>KLK8</i>	Kallikrein 8	Inhibition of invasion	85
<i>KLK10</i>	Kallikrein 10	Inhibition of proliferation	86,87
<i>MME</i>	Neprilysin	Inhibition of proliferation and angiogenesis	72–76
<i>MMP3</i>	Stromelysin 1	Stimulation of apoptosis	15,48
<i>MMP8</i>	Collagenase 2	Regulation of inflammation	14,36
<i>MMP9</i>	Gelatinase B	Angiogenesis inhibition	49–53
<i>MMP11</i>	Stromelysin 3	Metastasis suppression	54
<i>MMP12</i>	Macrophage metalloelastase	Angiogenesis inhibition	37–40
<i>MMP19</i>	MMP19	Angiogenesis inhibition	56
<i>MMP26</i>	Matrilysin 2	Regulation of oestrogen-signalling	46
<i>PRSS3</i>	Trypsinogen IV	Not determined	98–100
<i>PRSS8</i>	Prostasin	Inhibition of proliferation and invasion	90,91,94
<i>PRSS21</i>	Testisin	Not determined	89,92,93

HAUSP, herpes-virus associated ubiquitin-specific protease; MMPs, matrix metalloproteinases; NF κ B, nuclear factor- κ B; TGF β , transforming growth factor β .

mechanisms in oesophageal carcinomas, it is a candidate tumour-suppressor gene⁶⁹. The relevance of ADAMTSs as tumour-suppressor enzymes has received unexpected support from a large-scale

genomic study showing that *ADAMTS15* and *ADAMTS18* are among the genes that are most frequently mutated in a panel of breast and colorectal cancers⁷⁰. Furthermore, *ADAMTS18* is frequently

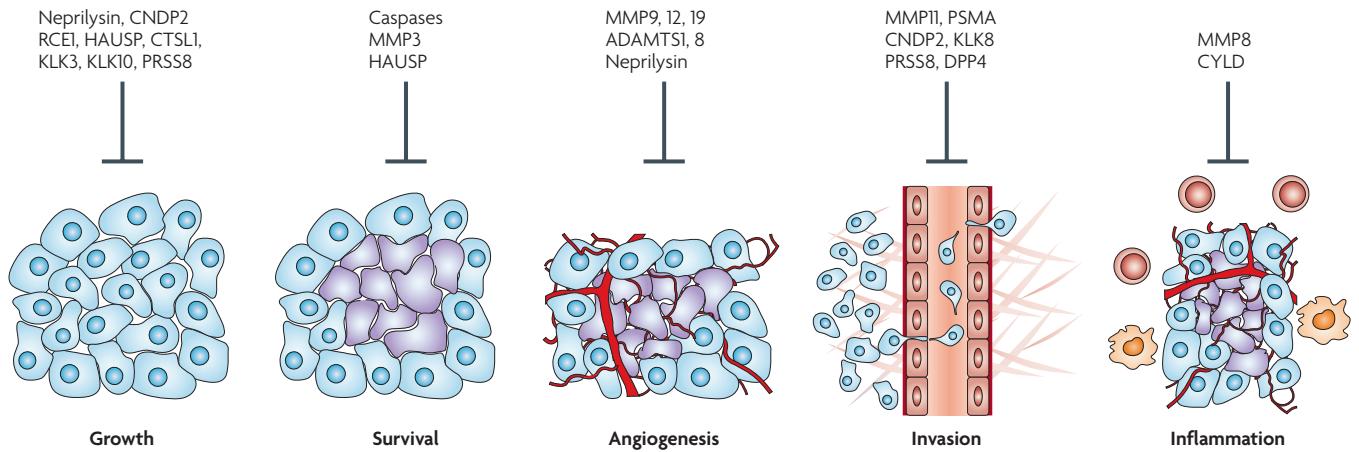


Figure 3 | **Functional roles of anti-tumour proteases at different stages of cancer progression.** Proteases belonging to different catalytic classes show tumour-defying functions by inhibiting stages of cancer progression, such as angiogenesis or invasion, or by their ability to modulate the inflammatory responses that are elicited by cancer cells.

epigenetically inactivated in various carcinomas⁷¹. Recent work from our laboratory has confirmed and extended these observations with the finding that some loss-of-function mutations within the genes that encode ADAMTSs occur in cancer, thus reinforcing their putative role as novel tumour suppressors.

Neprilysin

This protein, encoded by *MME*, is a cell surface metallopeptidase, the expression of which is lost in several human tumours, including prostate carcinomas⁷². Neprilysin is involved in the inactivation of neuropeptides that are implicated in the growth of androgen-independent prostate cancer. It also inhibits angiogenesis through the proteolysis of fibroblast growth factor 2 (*FGF2*) and potentiates the effects of the phosphatase and tensin homologue (*PTEN*) tumour suppressor through direct protein–protein interaction^{73,74}. Loss of neprilysin expression through the hypermethylation of the *MME* promoter has been shown to result in Akt kinase activation and contributes to the clinical progression of prostate cancer⁷⁵. Interestingly, lentiviral-mediated *MME* transfer inhibits prostate cancer growth, supporting the relevance of this protease in tumour suppression⁷⁶.

Other anti-tumour metalloproteinases

Recent studies have identified prostate-specific membrane antigen (*PSMA*; also known as *FOLH1*) — a type II transmembrane carboxypeptidase — as a tumour-suppressor gene in prostate cancer. Ectopic expression of *PSMA* in PC3 prostate cancer cells reduces their invasiveness, whereas knockdown of *PSMA* increases the invasive

properties of LnCaP prostate cancer cells⁷⁷. Carboxypeptidase of glutamate-like-B (*CPGL-B*; also known as *CNDP2*) has also been proposed as a candidate tumour suppressor in human hepatocarcinoma. Stable transfection of *CPGL-B* inhibits cell proliferation and invasion, and suppresses tumour formation in nude mice⁷⁸. Nevertheless, it seems that the proteolytic activity of *CPGL-B* is not directly involved in these tumour-suppressive functions⁷⁸. Similarly, *ADAM23* — which is a proteolytically inactive member of the ADAM family of metalloproteinases — has also been proposed as a candidate tumour suppressor gene that is silenced in breast and gastric cancers by homozygous deletion or aberrant promoter hypermethylation⁷⁹. However, its precise role in carcinogenesis remains largely unknown. Finally, deficiency in Ras-converting enzyme 1 (*RCE1*) — which is a metalloproteinase involved in the activation of oncoproteins of the Ras family — accelerates the development of *Kras*-induced myeloproliferative disease and increases the proliferation of *Rce1*-deficient haematopoietic cells. The molecular basis of this finding, which was unexpected for a protease presumed to have oncogenic properties, is unknown, but might derive from the lack of processing of an isoprenylated protein that suppresses cell proliferation⁸⁰.

Cysteine cathepsins

In humans, these enzymes form a family of 11 lysosomal proteases that are highly expressed in malignant tumours. They are secreted into the extracellular milieu and have multiple roles during cancer progression⁷. Causal relationships between deregulation of cysteine cathepsins and cancer

progression have been established through pharmacological inhibition or by studies with mutant mice that are deficient in specific family members. However, contrary to all previous studies on this protease family, genetic ablation of cathepsin L (*CTSL1*) in an HPV16-induced skin carcinogenesis mouse model leads to the formation of early-onset aggressive tumours. Further analysis has shown a marked hyperproliferation of *Cts11*^{-/-} keratinocytes, which can facilitate cancer development⁸¹. These findings represent the first description of a cysteine cathepsin with a protective role in cancer, although it may be circumscribed to squamous cell carcinomas of the skin.

Kallikreins

Kallikreins constitute a family of trypsin-like serine proteinases, the expression of which is frequently altered in hormonally regulated human carcinomas⁸. Many studies have linked the overexpression of human kallikreins (hKs) in cancer to a poor clinical prognosis, but the finding that increased levels of some hKs predict a favourable outcome suggested their relevance as anti-tumour proteases. Functional analyses have confirmed the tumour-suppressive actions of several hKs, including hK3, hK8 and hK10 (also known as *KLK3*, *KLK8* and *KLK10*, respectively). *KLK3* might suppress tumour growth by activating transforming growth factor β (*TGF β*), which is a central component of anti-mitotic pathways⁸². *KLK3* might also act as a negative regulator in hormone-dependent breast cancer owing to its ability to increase the conversion of oestradiol — which is a potent oestrogen — to the less potent oestrone⁸³. Furthermore, *KLK3* might inhibit cell migration and

adhesion through protease-activated receptor (PAR) signalling, and also possesses anti-metastatic properties that are probably derived from its anti-angiogenic activity⁸⁴.

KLK8 suppresses tumour cell invasiveness through its ability to modify the extracellular microenvironment by cleaving fibronectin. This cleavage suppresses integrin signalling and impairs cancer cell motility by inhibiting actin polymerization⁸⁵. Consistent with this, expression of KLK8 confers a favourable clinical prognosis to patients with non-small-cell lung cancer⁸⁵.

KLK10 is downregulated in several tumours, which first suggested its putative role as a tumour suppressor⁸⁶. Accordingly, *KLK10*-transfected breast cancer cells show reduced proliferative activity and diminished potential to generate tumours in nude mice⁸⁶. Similar to the case of other anti-tumour proteases, the loss of KLK10 expression in human malignancies is caused by hypermethylation of its promoter region⁸⁷.

Finally, there are other kallikreins, such as *KLK9*, *KLK13* and *KLK14*, which can also act as tumour suppressors as shown by the fact that their loss of expression in diverse tumours confers a good prognosis to cancer patients and by their ability to generate angiostatic factors^{8,88}. Nevertheless, evidence to support a role for these three proteases as tumour-defying enzymes is still insufficient.

Testisin and prostaticin

Testisin and prostaticin are glycosylphosphatidylinositol (GPI)-anchored serine proteases, the expression of which is lost in testicular and prostate cancers, respectively^{89,90}. Prostaticin can suppress tumour growth and invasion of prostate and breast cancer cells⁹¹, and the ectopic expression of testisin in testicular tumour cells suppresses their tumorigenicity⁹², although opposing results have been found in ovarian cancer cells⁹³. Testisin is silenced in testicular tumours by DNA hypermethylation⁹², whereas prostaticin is downregulated in prostate cancer by a combination of promoter methylation, decreased expression of transcriptional activators, such as *SREBP2*, and increased expression of transcriptional repressors, such as *SLUG*⁹⁴.

Dipeptidyl peptidase 4

This enzyme is a cell surface serine protease that was first reported to suppress the malignant phenotype of melanocytic cells⁹⁵, and it was subsequently associated with protective functions in different human malignancies^{96,97}. DPP4 overexpression upregulates E-cadherin and tissue inhibitors

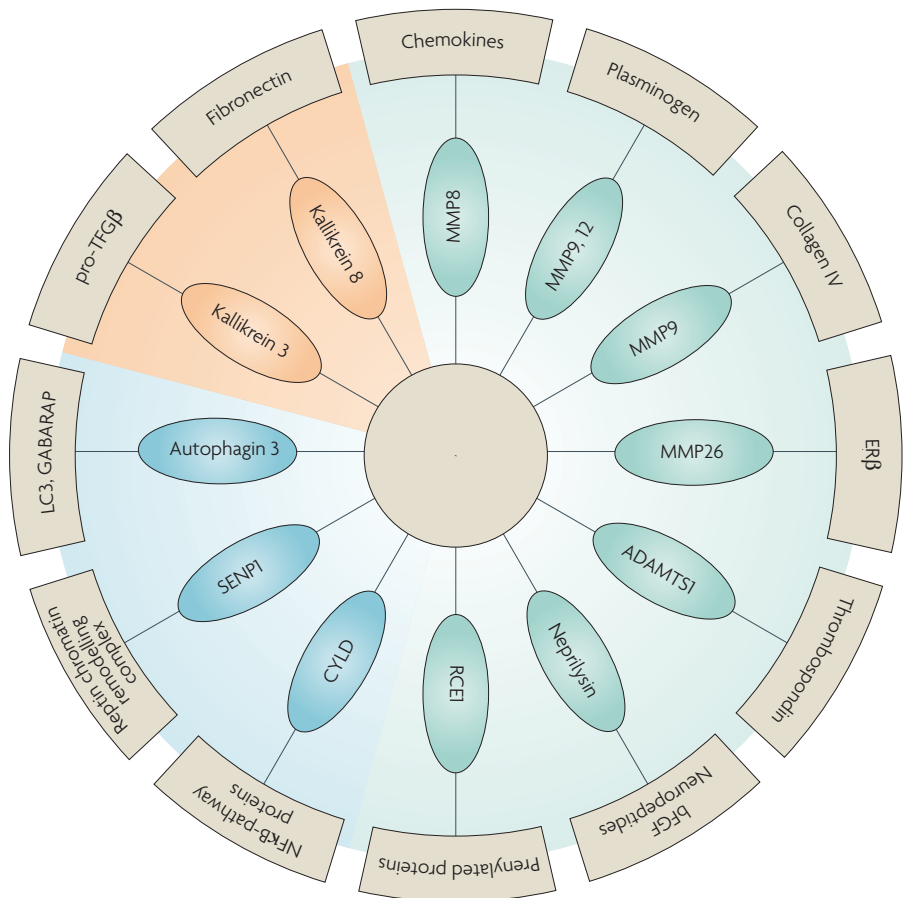


Figure 4 | **Substrates targeted by anti-tumour proteases.** The indicated proteases develop their tumour-protective functions by targeting the diverse substrates shown in the outer ring.

of metalloproteinases, resulting in the loss of the invasive potential of ovarian carcinoma cells⁹⁶. DPP4 may also block FGF2 signalling, altering cell adhesion properties and abrogating the malignant phenotype of prostate cancer cells⁹⁷. Nevertheless, the putative substrates targeted by DPP4 during these processes remain unknown.

Other serine proteases

Several studies have described protective functions for other enzymes belonging to the catalytic class of serine proteases. Thus, trypsinogen IV (*PRSS3*) is epigenetically silenced in oesophageal and gastric carcinomas⁹⁸ as well as in bladder and non-small-cell lung carcinomas^{99,100}, although whether this is a driver or passenger mutation is unknown. Moreover, several other serine proteases that are generally associated with cancer progression have been reported to have a protective function in specific instances. This is the case of seprase, which is localized to invadopodia and associated with cellular invasion, but abrogates melanoma cell tumorigenicity

through the regulation of cell growth and survival¹⁰¹. Hepsin, which is thought to contribute to prostate cancer progression in animal models¹⁰², has been reported to inhibit cell growth and invasion of prostate cancer cells¹⁰³. Finally, urokinase — which is a serine protease that is frequently associated with cancer progression — might also delay tumour development in specific cancer models¹⁰⁴. These examples highlight the dual roles of some tumour proteases, depending on the cellular source and cancer microenvironment.

Molecular mechanisms

The molecular mechanisms used by proteolytic enzymes to exert their tumour-suppressive functions are quite diverse and affect all stages of tumour progression (FIG. 3). These mechanisms include: negative regulation of cell growth and survival by the CYLD cysteine protease or various metalloproteinases and serine proteases^{6,8,29,101}; changes in cell adhesion by DPP4 (REF. 97); inhibition of angiogenesis by nephilysin and MMPs^{39,56,73}; stimulation and execution

of apoptosis by metalloproteinases and caspases^{6,20}; activation of autophagy by autophagin 3 (REF. 34); and modulation of host inflammatory responses by MMP8 and other metalloproteinases¹⁴. These protective effects result from the ability of these enzymes to selectively cleave a variety of substrates such as growth factors, growth factor receptors, precursors of bioactive peptides, proapoptotic ligands, cell-adhesion molecules, intracellular or extracellular structural components, chemokines, cytokines and other proteases (FIG. 4). Nevertheless, our knowledge of the mechanisms underlying the anti-tumour properties of many proteases is still limited, and further studies will be required to clarify them. The identification of the *in vivo* substrates for these enzymes and the generation of animal models involving gain or loss of function of specific proteases will be essential to provide this mechanistic information and validate the relevance of these enzymes in tumour-suppression events. In these validation experiments, we must consider that many host-protective proteases are not produced by the tumour cells but by the surrounding stroma or the infiltrating inflammatory cells, thus limiting the value of approaches based on the transfection of tumour cells or those involving immunologically compromised mouse models.

Clinical implications

The identification of proteases that favour the host instead of the tumour might have important clinical consequences at several levels. First, these new findings have provided explanations for the lack of success of clinical trials based on the use of a broad range protease inhibitors for treating patients with cancer^{2,12,13}. Moreover, the description of anti-tumour proteolytic enzymes has stimulated the design of novel approaches to identify the relevant proteases that must be targeted in each individual cancer patient^{38,105}. This aspect is of special relevance in the case of proteases belonging to large families that are composed of multiple members which are structurally related but have opposite roles in cancer. This global analysis of the cancer degradome has already yielded important new information, including the identification of the protective role for MMP12 in lung cancer³⁸. Likewise, these analyses have pointed to the existence of several proteases that were not previously thought to be related to cancer, and that might be of future clinical interest as biomarkers for the diagnosis or prognosis of malignant tumours¹⁰⁵.

In relation to therapeutic implications, recombinant forms of protective proteases might have potential as tumour-suppressor agents. However, these enzyme replacement therapies aimed at substituting the defective or absent protease with its normal counterpart might have serious limitations, including the high doses that would be necessary to achieve therapeutic effects and the pharmacokinetic problems linked to their administration. Gene-therapy-based approaches might offer an alternative to express and deliver these enzymes to the appropriate locations, as has been shown for the case of lentiviral-mediated nepri-lysin gene transfer to block prostate cancer growth⁷⁶. The rapid development of nanotechnology might also provide valuable options for targeted delivery of proteases with tumour-suppressive properties. Additionally, strategies like bone-marrow transplantation, substrate-deprivation and enzyme-enhancement therapies, which are currently explored for treating different diseases of proteolysis^{3,106,107}, could also offer some therapeutic options for tumours involving specific protease deficiencies. Another interesting approach in this regard is based on the inactivation of endogenous protease inhibitors that can limit the positive action of anti-tumour proteases. This strategy has already been evaluated in studies aimed at blocking XIAP — which is an endogenous inhibitor of caspases — thereby enabling caspase activation, which in turn might sensitize cancer cells to chemotherapeutic drugs or directly induce apoptosis¹⁰⁸.

It might also be possible to upregulate the expression of protective proteases through the use of agents that can reverse the gene modifications that result in the decreased expression of these enzymes in human malignancies. The finding that promoter hypermethylation is the basis for loss of expression of most of these genes in tumours provides a good opportunity for using DNA-demethylating drugs that can reactivate the expression of tumour-suppressive proteases and restore their protective function. Finally, it is very stimulating that the precise definition of the underlying mechanism of tumour suppression by some proteases has allowed the development of novel and unexpected therapeutic approaches for some specific tumours. This is the case of cylindromatosis, in which anti-inflammatory drugs restoring the negative regulation of the NF κ B pathway — which is lost because of mutations in *CYLD* — could be an effective therapeutic intervention¹⁰⁹.

The realization that a significant number of proteolytic enzymes might contribute to tumour suppression introduces a cautionary note into current and future strategies aimed at targeting proteases in cancer patients. Accordingly, any anti-protease therapy must now carefully evaluate the characteristics of the enzyme to be targeted, its cellular source and its specific function in the type and stage of the tumour in which it is to be used. The increased knowledge of the structure, function and regulation of these enzymes should facilitate the evaluation of whether redundant members of protease families can be used as drug targets in cancer as well as the identification of novel protective proteases. The design of protease chips for the comprehensive and systematic analysis of expression and activity of all human proteases will also be helpful for this purpose^{1,38}. Additionally, the identification of proteases with anti-tumour properties might also have direct clinical applications in terms of providing new prognostic markers of the disease. Thus, and in complete agreement with their tumour-suppressive role, overexpression of these enzymes predicts good clinical prognosis, whereas their loss heralds poor clinical outcome in cancer patients⁸.

The increased expression of proteases in malignant compared with normal tissues, whether they are pro- or anti-tumorigenic, can also be exploited as a means of increasing drug delivery using protease-activated prodrugs. For example, the cleavage of peptide conjugates of the chemotherapeutic agent doxorubicin by the secreted serine prostate-specific antigen (PSA) into active metabolites has been tested in clinical trials for prostate cancer¹¹⁰. Also in relation to this, recent work has shown the feasibility of using protease-activated molecular beacons as a method for quantifying the response to therapy^{111,112}. Finally, the finding of tumour-protective proteases might also lead to more effective cancer therapeutics, paradoxically aimed at increasing those proteolytic activities that are lost during tumour progression rather than blocking them, which has been the paradigm that has traditionally guided the work in this field.

Perspectives and conclusions

The evidence discussed above shows that proteases with anti-tumour properties do not represent rare exceptions to the well-established rule that protease upregulation in cancer is synonymous with tumour progression and poor clinical prognosis. More than 30 proteolytic enzymes with the ability to negatively regulate some aspects of cancer have been annotated in this work (TABLE 1).

Nevertheless, these numbers are not definitive and it is likely that the census of tumour-suppressor proteases will grow in the near future. We must also emphasize that evidence for the assignment of tumour-suppressive roles to some of these enzymes is still limited or restricted to specific situations, and further analysis will be required for their functional and clinical validation. Finally, the fact that several proteases have opposing effects in cancer, depending on tissue type and tumour microenvironment, introduces an additional level of difficulty in the elaboration of this inventory of anti-tumour proteases.

The functional exploration of the proteolytic systems that are associated with cancer has led to a change in the way we view this complex field. We have steadily moved from our consideration of tumour-associated proteases as nonspecific and late-acting pro-metastatic enzymes, to their recognition as key proteins involved in early stages of cancer, and finally to the identification of their dual roles as pro-tumorigenic or anti-tumorigenic enzymes. There are many challenges ahead before translating this new information into clinical benefits for cancer patients but, hopefully, this first attempt to catalogue the tumour-suppressor proteases may facilitate the building of a conceptual framework to separate the pro- and anti-tumorigenic activities of these enzymes and, finally, to distinguish friend and foe proteases in our war on cancer.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

Atg4c | BECN1 | CASP8 | CASP10 | CYLD | DPP4 | MYCN |

PRSS3

OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>

familial cylindromatosis

UniProtKB: <http://ca.expasy.org/sprot>

ADAM23 | ADAMTS1 | ADAMTS8 | ADAMTS9 | ADAMTS15 |

ADAMTS18 | BCL3 | CD82 | CNDP2 | CTS1L1 | FGF2 | FOLH1 |

HAUSP | IKBKG | KLK3 | KLK8 | KLK9 | KLK10 | KLK13 | KLK14 |

MME | MMP3 | MMP8 | MMP9 | MMP11 | MMP12 | MMP19 |

MMP26 | p53 | PRSS8 | PRSS21 | PTEN | RCE1 | SENP1 | SLUG |

SREBP2 | TRAF2 | TRAF6

FURTHER INFORMATION

Carlos López-Otin's homepage: <http://www.uniovi.es/degradome/>

Lynn M. Matrisian's homepage: <http://www.vicc.org/research/display.php?id=3912>

MEROPS — the peptidase database: <http://merops.sanger.ac.uk/>

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