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Extracellular proteases as targets for drug development

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Abstract

Proteases constitute one of the primary targets in drug discovery. In the present review, we focus on extracellular proteases (ECPs) because of their differential expression in many pathophysiological processes, including cancer, cardiovascular conditions, and inflammatory, pulmonary, and periodontal diseases. Many new ECP inhibitors are currently under clinical investigation and a significant increase in new therapies based on protease inhibition can be expected in the coming years. In addition to directly blocking the activity of a targeted protease, one can take advantage of differential expression in disease states to selectively deliver therapeutic or imaging agents. Recent studies in targeted drug development for the metalloproteases (matrix metalloproteinases, adamalysins, pappalysins, neprilysin, angiotensin-converting enzyme, metallocarboxypeptidases, and glutamate carboxypeptidase II), serine proteases (elastase, coagulation factors, tissue/urokinase plasminogen activator system, kallikreins, tryptase, dipeptidyl peptidase IV), cysteine proteases (cathepsin B), and renin system are discussed herein.

Keywords

drug targets; extracellular proteases; metalloproteases; serine proteases; cysteine proteases; protease inhibitors

1. Introduction

A number of potential targets for the development of new therapeutics to treat human disease are now readily provided by genomics and proteomics [1, 2]. Proteins are the main targets for drug or vaccine discovery and amongst them proteases constitute one of the main classes [3, 4]. Out of the roughly 500 drug targets that are currently known, about 200 are enzymes, and more than 60% of the total enzyme market is made up of proteases. Proteases became economically important drug targets upon the realization that, besides their primary function in the hydrolysis of peptide bonds, they were extremely important signaling molecules involved in numerous vital processes. According to bioinformatics analysis of the human genome, 566 proteases had been identified so far. Of these, 273 have been found in

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extracellular compartments or in the lumen of secretory compartments, 277 in intracellular compartments, and 16 in the cell membrane at the surface [5]. The focus of this review will be on extracellular proteases (ECP), a complex and heterogenous family of enzymes.

The two primary and the best-characterized ECP groups are metalloproteases and serine proteases. Cysteine proteases have been recently added to ECPs since they may be secreted and function at or near the cell surface. ECPs have been widely explored as potential drug targets since regulated extracellular proteolysis is critical for physiological processes including tissue remodeling, wound healing, and embryogenesis. Extracellular proteolysis also plays a key role in pathophysiological processes including cancer, cardiovascular conditions, and inflammatory, pulmonary, and periodontal diseases. In addition to directly blocking the activity of the targeted protease, protease inhibitors also block activation of downstream proteases or affect protease cofactor complexes. The heparin and vitamin K analog of warfarin, the first drug that affected protein signaling, or more precisely, the blood coagulation cascade, was brought to clinical practice 50 years ago. Many new ECP inhibitors are currently under clinical investigation and a significant increase in new therapies based on protease inhibition can be expected in the coming years. Because of their differential expression in disease, proteases and their inhibitors could be used as biomarkers for early diagnostic and, in certain cases, as prognostic markers. As well, one can take an advantage of disease-associated proteases to selectively deliver therapeutic or imaging agents. Protease-activated prodrugs, nanotechnology-based drug delivery systems, hydrogels, gene delivery systems, and imaging systems have been described for disease applications [6].

2. Metalloproteases

2.1. Matrix Metalloproteinases

MMPs are a large family of zinc-dependent neutral endopeptidases involved in the degradation of ECM components, and thus they play a crucial role in the homeostasis of normal tissue remodeling, Fig. (1) [7, 8]. The activity of MMPs is controlled and finely balanced at many levels: RNA transcription, protein translation, secretion, localization, activation of zymogen, inhibition by endogenous proteins, and degradation. MMPs are usually minimally expressed in normal physiological conditions. Overexpression of MMPs results in an imbalance between the activity of MMPs and endogenous inhibitors of MMPs, TIMPs, leading to tissue degradation and consequently facilitating a variety of pathological disorders, including arthritis and cancer [9–11]. Accordingly, MMPs became important pharmaceutical targets for treatment of these diseases [12, 13]. MMPs were the first proteases seriously considered as targets to combat cancer because of their role in ECM degradation. The compelling results of preclinical studies on MMP inhibition in tumor models raised the idea that the development of strategies to inhibit MMPs may prove to be a powerful tool to fight cancer. However, the results of MMP inhibitor clinical trials have been disappointing. MMP inhibitors such as the hydroxamates batimastat (British Biotech), marimastat (British Biotech), and prinomastat (Aguron) and the non-hydroxamates neovastat (Aeterna), rebimastat (Bristol-Myers Squibb), and tanomastat (Beyer) have failed clinical trials because of severe side effects and/or offering no significant therapeutic advantage. Only one product,

periostat (CollaGenex), a tetracycline analog which inhibits both the activity and synthesis of MMPs, is currently on the market (for treatment of adult periodontitis). The failure of MMP inhibitors can be attributed to numerous factors, but one is certainly a lack of enzyme specificity. Altogether, the critical examination of previous results has promted serious re-evaluation of MMP inhibition strategies focusing attention on the identification of specific MMP targets at different stages of tumor progression, both in order to improve efficacy and to reduce side effects. It seems that the direction to go is to design selective, yet potent, MMP inhibitors, to seek weaker rather than stronger zinc binding ligands, and look for alternative ways to increase inhibitory potency by exploiting the differences between the various enzyme subtypes [14, 15]. Triple-helical phosphinate transition state analogs have been described that are highly selective for the gelatinase members (MMP-2 and MMP-9) of the MMP family [16]. In addition, progress has been made in the development of MMP selective inhibitors based on novel zinc binding groups [17].

Another important area of future research would be the development of novel applications of MMP inhibitors. This would allow the use of broad-spectrum inhibitors in clinical treatment. One possible approach would be to apply MMP inhibitors to treatment of skin diseases, where both MMPs and members of the ADAM family are implicated in the processing of cytokines and growth factors. In fact, CollaGenex has recently filed an NDA for a doxycycline derivative, Oracea, which is the first orally administered, systemically delivered drug to treat rosacea, a skin disease.

2.2. Adamalysins

ADAM family members belong to the sub-clan of zinc-dependent metalloproteinases also known as the metzincins and display sequence similarities with the reprolysin family of snake venomases, Fig. (1) [18]. Two groups are distinguished in the adamalysin family: the membrane-anchored ADAMs and the secreted ADAMTSs. Although ADAMTSs are soluble proteins, many appear to bind ECM through their thrombospondin motifs or their spacer region [19]. In contrast to MMPs, ADAM function is more focused, regulating growth signaling and tumor cell adhesion [9]. ADAMs play a crucial role in protein ectodomain shedding. Many cell surface molecules such as cytokines, cytokine receptors, cell adhesion molecules, and growth factors are processed to convert them into physiologically active soluble derivatives [20, 21]. Proteases responsible for releasing cell surface molecules are classified as sheddases or secretases. ADAM 9 has been reported to shed the heparin-binding EGF-like receptor [22, 23]. The best-characterized ADAM sheddase is ADAM 17 (TNFα converting enzyme; TACE) [24]. ADAM 17 is a sheddase of membrane-bound pro-TNFα. The association of TNFα with adverse inflammatory states such as rheumatoid arthritis, Chron's disease, and multiple sclerosis has prompted the development of TACE inhibitors. Design of specific TACE inhibitors has been problematic due to the strong homology with the MMPs [25, 26]. Reports that TIMPs are capable of inhibiting ADAM protease activity further suggests a common mechanism for substrate recognition and cleavage by ADAMs and MMPs.

ADAM and ADAMTS family members have been implicated in several pathologies [19, 27–32]. ADAMTS-13 deficiency is responsible for thrombotic

thrombocytopenic purpura, a rare disorder of the blood coagulation system, characterized by the formation of microvascular vWF and platelet-rich thrombi. ADAM 17 expression and activity are increased in inflammatory bowel diseases [33]. A strong association has been established between ADAM 33 and asthma-related bronchial hyperresponsiveness in humans [34, 35]. Another ADAM with apparent role in the immune system is ADAM 8. ADAM 8 expression is increased in an animal model of asthma following allergen exposure [36] and in the bronchi of human asthmatics [35]. The possibility that ADAM 8 is a CD23 sheddase is quite intriguing and deserves further investigation. A specific inhibition of this activity would be important in the treatment of allergic reactions. ADAM 9 and ADAM 15 are upregulated in atherosclerosis [37].

ADAMTS-4 and ADAMTS-5 are involved in the turnover of aggrecan resulting in loss of functionality of tissue and joint disability [38, 39]. Studies have indicated that ADAMTS-5 is likely the major aggrecanase in cartilage metabolism and pathology [40]. Furthermore, its aggrecanase activity is 1000-fold greater than that of ADAMTS-4 under physiological conditions [41]. Interest in the ADAMTS family role in cancer stem from reports that highly invasive CNS-1 glioma cells utilize ADAMTS-4 to cleave brevican [42], while high expression levels of ADAMTS-8 in combination with low expression levels of ADAMTS-15 is prognostic of poor clinical outcome in breast carcinoma patients [43]. Indeed, the architecture of ADAMs and ADAMTSs, with domains that confer proteolytic activities and the ability to bind to diverse cells and ECM-associated molecules, suggests that these enzymes may be functionally relevant to steps involved in cancer development and in metastatic dissemination of tumor cells. The active metalloproteinase domain might be needed to degrade ECM components and to shed growth factors and cytokines, contributing in this way to the control of cell proliferation, migration, and angiogenesis [31]. Adhesion and migration of cells might be regulated by the disintegrin or cysteine-rich domains [44, 45].

Each of the physiological mechanisms employed to regulate ADAMTS proteinases represents a potential opportunity for therapeutic intervention. There is increasing evidence that ADAMTS proteinases are regulated at multiple levels: initiation of transcription, RNA splicing, translation, protein processing, and inhibition/activation through interaction with inhibitors/cofactors. One of the most direct ways of modulating activity, both physiologically and pharmacologically, is with inhibitors. The most significant of the identified endogeous inhibitors of ADAMTS proteinases is TIMP-3 [46]. ADAMTS proteinase activity could be theoretically modulated by its administration. However, biological macromolecules are relatively expensive to produce compared with low molecular weight synthetic compounds. The majority of the reported low molecular weight, non-peptidic compounds effective as ADAMTS proteinase inhibitors are hydroxamate- or carboxylate-based and function by co-ordinating with the catalytic zinc ion central to the enzyme reaction mechanism. Because of evolutionary similarity in the catalytic site, many of these compounds also inhibit other metalloproteinases including MMPs and ADAMs [47, 48]. In clinical trials, use of broad-spectrum metalloproteinase inhibitors has had associated musculoskeletal side effects including joint stiffness and pain (see above) [49]. It is therefore desirable to develop inhibitors specific to the metalloproteinase family of interest in order to minimize the likehood of such effects.

2.3. Pappalysins

The pappalysin metalloproteinase family has been recent addition to the traditional four families within the metzincin sub-clan and consists of PAPP-A1 and -A2 [50]. PAPP-A1 was originally identified in the early 1970s as an antigen present in human plasma during pregnancy, but its characteristic metalloproteinase zinc-binding motif and its role in regulation of the bioavailability of IGFs by specific proteolytic inactivation of IGFBPs was only discovered recently. Using mass spectrometric microsequencing, PAPP-A1 was identified as the IGFBP-4 degrading enzyme in fibroblast conditioned medium in the presence of IGFs [51]. PAPP-A2, a second member of the pappalysin family, shares ~45% homology with PAPP-A1, specifically cleaves IGFBP-5 at one site, and is IGF-independent [52]. While the TIMPs appear to be the major physiologic inhibitors of both MMPs and possibly the ADAMs, the major inhibitor of pappalysins is the proform of eosinophil major basic protein (pro-MBP), which is normally associated with PAPP-A1 in serum through a covalent complex via two disulfide bonds [53]. This association is dependent on reducing and oxidizing agents and may have implications for PAPP-A1 function under pathological conditions, where redox potential is altered. The potential clinical consequences of IGFBP proteolysis by PAPP-A1 has been explored by Bayes-Genis *et al*. [54] who have demonstrated that PAPP-A1 was abundantly expressed in arteriolar plaques and ECM of ruptured or unstable plaques, but not in stable plaques. In addition, circulating PAPP-A1 levels were significantly higher in patients with unstable angina or acute myocardial infarction than in patients with stable angina and controls [54]. Furthermore, PAPP-A1 levels correlated with levels of free IGF-1, suggesting that it may release IGFs from their binding proteins acutely after a major cardiac event [54]. Control of bioavailability of IGFs could lead to better therapeutic intervention in the regulation of this growth factor and its actions in conditions such as cancer and fibrosis [55, 56].

2.4. Neprilysin or Neutral Endopeptidase

NEP belongs to the family of zinc-dependent endopeptidases. The catalytic properties of NEP resemble thermolysin, a zinc dependent bacterial endopeptidase. It is located at the cell surface with the bulk of the protein, including the active site, facing the extracellular space, and therefore functions as an ectoenzyme, catalyzing peptide hydrolysis at the surface of the plasma membrane [57, 58]. NEP has been implicated in the regulation of opioid peptide action through the degradation of endogenously released enkephalins [59]. NEP is involved in the physiological degradation of the peptides modulating blood pressure, such as the cardiac hormone ANP, bradykinin, and endothelin [60]. More recently, NEP has been implicated in the degradation of amyloid β peptide (Aβ1–22) [61, 62], the primary pathogenic agent in Alzeheimer's disease, and has been shown to play a role in the degradation of the incretin hormone GLP-1, which is a potent stimulator of insulin secretion. Potent inhibitors of NEP produce a pharmacological response through an increase in opioid or vasoactive peptide levels, indicating their therapeutic potential as novel analgesics or antihypertensive agents [63–65].

2.5. Angiotensin-Converting Enzyme

ACE is a zinc metalloproteinase that acts as a carboxy dipeptidase. It is a pivotal component of the renin-angiotensin system and consequently plays a key role in blood pressure and electrolyte homeostasis. ACE catalyses the conversion of angiotensin I into angiotensin II, a step required for angiotensin receptor activation, Fig. (2) [66]. Thus, ACE inhibitors are widely used in the treatment of cardiovascular disease. ACE inhibitors also inhibit the inactivation of bradykinin and substance P. These peptides mediate some of the side-effects of ACE inhibitors, such as cough and angioedema [67].

In the year 2002, ACE inhibitors were the most commonly prescribed drugs for the treatment of hypertension in the USA and are definitively the major protease inhibitor success story [3]. Current generation ACE inhibitors are widely used for cardiovascular diseases, including high blood pressure, heart failure, heart attack, and kidney failure, and have combined annual sales in excess of US $6 billion [67]. Thirteen ACE inhibitors are currently approved for clinical use and several others are in clinical trials. However, the use of these ACE inhibitors, which were developed in the late 1970s and early 1980s, are hampered by common side effects. The side effects could be explained by the fact that first generation of ACE inhibitors were designed based on the structure of carboxypeptidase A, which we now know is considerably different from ACE. Once the crystal structure of ACE was solved it revealed that ACE consists of N- and C-domains that have different functions and specificities [68]. Early ACE inhibitors were relatively non-selective and inhibited both domains with similar activities. The C-terminal domain seems to be primarily responsible for the conversion of angiotensin I to angiotensin II, with the major effect on blood pressure regulation. Therefore, the design of domain-selective ACE inhibitors is expected to produce safer and more effective drugs.

Based on large body of experimental evidence it has become apparent that RAS, the kallikrein-kinin pathway, and the natriuretic peptides are important modulators of cardiovascular homeostasis. These findings have provided the impetus to develop inhibitors that simultaneously block angiotensin II and increase ANP, which are regulated by endothelial, membrane-bound ACE and NEP, Fig. (2) [69]. The concept of dual inhibition of the two enzymes by a single molecule has shown major benefits and potential superiority versus other agents in various experimental models of hypertension, heart failure, and renal diseases. The underlying presumed rationale for the combined inhibition of ACE and NEP is to block the vasoconstrictor angiotensin II and simultaneously increase the vasodilator ANP by decreasing its enzymatic degradation. However, it remains controversial as to whether dual ACE/NEP inhibitors confer superior cardiovascular effects when compared to ACE inhibition alone. A major impediment for recommendation of routine use of these new agents remains the potentially life threatening side effect of angioedema. Omapatrilat (Bristol-Meyers Squibb) [70], the most advanced dual ACE/NEP inhibitor, although shown to be superior over existing agents in reducing hypertension, was halted by the USA Food and Drug Administration in Phase III clinical trials because of increased side effects, such as severe angioedema [67].

2.6. Metallocarboxypeptidases

MCPs are zinc-containing exopeptidases that catalyze the removal of C-terminal amino acids from proteins and peptides. Two main groups of MCPs can be defined with respect to the type and location of their physiological function: the digestive (or pancreatic) MCPs, which act on the degradation of intake proteins, and the regulatory MCPs, hydrolyzing biologically active peptides and hormones in non-digestive tissues and fluids [71–73]. The regulatory MCPs are becoming important emerging drug targets in biomedicine due to possessing a wide range of physiological roles [74]. The MCP TAFI [75, 76], also known as plasma procarboxypeptidase B [77] or procarboxypeptidase U [78], provides an important link between coagulation and fibrinolysis [79, 80]. TAFI down-regulates fibrinolysis presumably by removing C-terminal lysines from partially degraded fibrin, and therefore constitutes an important drug target for thrombolytic therapies. Numerous *in vivo* studies in rabbit have demonstrated that inhibition of TAFI activity with a carboxypeptidase inhibitor enhanced tPA-induced thrombolysis as well as endogenous fibrinolysis [81, 82]. Many recent reports show that the function of this enzyme goes beyond the fibrinolytic system. TAFI might also play a potentially important role in processes like inflammation, blood pressure regulation, and wound healing [83].

2.7. Glutamate Carboxypeptidase II

GCPII is a membrane associated zinc metalloenzyme with the bulk of the protein located in the extracellular space [84, 85]. The enzyme catalyzes the hydrolysis of the neurotransmitter *N*-acetyl-L-aspartyl-L-glutamate (NAAG) to *N*-acetyl-L-aspartate and L-glutamate [86, 87] and increases the concentration of glutamate in the extracellular space [88]. Interfering with glutamate production through GCPII has been discovered as a promising alternate approach for the treatment of stroke, and other neurological disorders associated with glutamate excitotoxicity [89, 90]. Potent and selective GCPII inhibitors have been shown to decrease brain glutamate and provide neuroprotection in preclinical models of stroke, amyotropic lateral sclerosis, and neuropathic pain [85].

GCPII is identical to prostate-specific membrane antigen [91, 92], a tumor marker in prostate cancer [93]. It is also found in the membrane brush border of the small intestine where it acts as a folate hydrolase [94, 95]. Therefore, GCPII inhibitors could be helpful in the imaging and treatment of tumors where folate is required for their growth. Overall, GCPII inhibitors could be useful in the treatment of neuronal diseases and prostate cancer [88].

3. Serine Proteases

The serine proteases are a heterogenous enzyme group [96]. Coagulation factors (thrombin, protein C, factor VII, IX, X and XII), Fig. (3), and the fibrinolytic system, Fig. (4), represent important parts of human vascular biology. The fibrinolytic system deserves special attention because plasminogen conversion to the active serine protease plasmin occurs through two serine proteases: uPA and tPA, Fig. (4). Despite their common enzymatic activities, the two plasminogen activators play distinct roles through two different biological behaviors. tPA has high affinity for fibrin, resulting in a potent fibrinolytic process and clot dissolution. uPA, however, is recruited to the cell surface immediately after its secretion via

a specific uPA receptor. This plays a central role in localizing uPA to cell associated proteolysis. Thus, in addition to a major role in clot dissolution, the fibrinolytic system plays a defining role in many important vascular biological processes through pericellular proteolysis.

3.1. Elastase

hNE is a serine protease that is produced in the most abundant of white blood cells, the granulocytes. Its function in the immune system lies in the defense against pathogens and foreign protein material. The activity of hNE released from granulocytes is tightly regulated by several inhibitors [97], but in tissues massively infiltrated by neutrophils this regulation can be insufficient. High levels of unregulated hNE can cause degradation of healthy tissues and result in the development of diseases such as pulmonary emphysema, CF, or rheumatoid arthritis [98, 99]. Thus, new inhibitors of hNE are of considerable interest as anti-inflammatory drugs [100, 101]. EPI-hNE4 is a highly specific and potent inhibitor of hNE, currently under development at Debiopharm for the treatment of CF [102]. It is the first representative of a new therapeutic class in CF treatment.

3.2. Coagulation Factors

Thrombin, factor VIIa, factor IXa, and factor Xa are four of the key serine proteases in the coagulation cascade, Fig. (3). Inhibition of any of these enzymes may prevent the formation of fibrin clots and thus be useful in the management of thrombotic diseases [103]. Despite the enormous efforts of industrial and academic laboratories world-wide, the long lasting aim of developing a safe, orally effective anticoagulant with an acceptable pharmacokinetic profile and without the need for regular monitoring remains an unmet challenge. Thrombin is still a promising anticoagulant target [104, 105]. Direct thrombin inhibitors desirudin (Novartis), lepirudin (Aventis), and bivalirudin (Biogen) were developed based on hirudin, a very potent and selective thrombin inhibitor isolated from the European medicinal leech *Hirudo medicinalis* [106]. Argatroban (Mitsubishi-Tokyo Pharmaceuticals) was the first clinically approved low molecular weight thrombin inhibitor [107]. Ximelagatran (Astra-Zeneca) was the first orally available low molecular weight thrombin inhibitor with profound antithrombotic efficacy [108]. Unfortunately, this product has been removed from the market because of hepatotoxicity [109].

Another promissing target is factor Xa [110]. Factor Xa inhibitors could effectively prevent the generation of thrombin without affecting existing thrombin levels. Therefore, sufficient thrombin might remain to allow platelet activation and normal haemostasis, while preventing pathological thrombus formation. Indirect factor Xa inhibitors such as low molecular weight heparins have been extensively used [111]. Fondaparinux (Sanofi/Aventis) was launched in 2002 for the treatment of deep vein thrombosis and venous thromboembolism [112].

A less characterized target is the TF:VIIa complex. Many research programs have focused on the discovery of a low molecular weight TF:VIIa inhibitor that would result in an antithrombotic effect with minimized bleeding. The most extensively studied inhibitors of the TF:VIIa complex include endogenous coagulant TFPI [113] and a nematode-derived

anticoagulant protein [114]. Factor IXa inhibitors can be of superior efficacy in reducing coronary or cerebral thrombosis events, and in addition safer with respect to bleeding over factor Xa and thrombin inhibitors.

The concept of anticoagulant therapy employing simultaneous inhibition of various targets of the blood coagulation system in order to achieve an efficient anticoagulant effect has emerged recently. Many research groups have reported results in the development of dual anticoagulants agents simultaneously targeting thrombin/Xa, Xa/VIIa, thrombin/VIIa, or IXa/Xa [115]. The main challenge in the development of dual coagulation cascade inhibitors is to design structural elements that will optimally bind to the active site/subsites in order to reach potency against desired coagulation factors and enable selectivity away from other serine proteases.

3.3. Tissue-Type Plasminogen Activator

tPA is found on the surface of veins, capillaries, the pulmonary artery, heart, and uterus and is secreted after vascular injury. Increased enzymatic activity of tPA causes hyperfibrinolysis which manifests in excessive bleeding, Fig. (4). On the other hand, decreased activity leads to hypofibrinolysis which can result in thrombosis or embolism. Recombinant tPA is used in diseases which feature blood clots, such as myocardial infarction and stroke [116]. However, in order to be effective, tPA must be administered within the first 3 h of the event. Since tPA increases the risk of symptomatic intracranial haemorrhage, it is administered to less than 5% of stroke patients.

3.4. Urokinase-Type Plasminogen Activator

uPA/uPAR mediate a variety of biological activities at the cell surface, including plasminogen activation, ECM remodeling, growth-factor activation, and the initiation of intracellular signaling [117–119]. The uPA system plays an important role in cell adhesion, migration, invasion, and tissue remodeling [120]. The importance of uPA/uPAR in tumor biology and metastasis has been well established. Elevated levels of soluble uPARs in cancer cells usually indicates a poor prognosis for patient survival [121]. Inhibition of uPAR expression has been shown to decrease tumor cell invasiveness, prevent metastasis, and increase the duration of tumor latency [122]. These findings suggest that uPAR antagonists may be useful therapeutically as inhibitors of tumor progression.

3.5. Kallikreins (Tissue and Plasma)

The hKs are a more recently described family of secreted serine proteases with diverse expression patterns and physiological roles [123]. They are primarily known for their clinical applicability as cancer biomarkers. PSA/hK3 has long been an effective biomarker for prostate cancer. Recent evidence implicates hKs in many cancer-related processes, including cell-growth regulation, angiogenesis, invasion, and metastasis. Emerging data also indicate that kallikreins might be directly involved in neoplastic progression, although they exert diverse and often contrasting effects on the tumor and its environment. hKs act individually and/or in cascades with other hKs and proteases such as the uPA/uPAR system and MMPs, and therefore represent an attractive target for therapeutic interventions in the early stage of cancer initiation and progression [123]. On the basis of numerous reports that incriminate hKs

as factors that promote tumor growth through their proteolytic activity, the design of hKs inhibitors is a potential anticancer strategy. Recently, a novel approach for designing kallikrein-specific inhibitors based on inhibitor protein-protease reactive site interactions has been described [124]. Other therapeutic strategies exploit hK activity and/or tissue specificity in the development of the hK3 activated prodrugs [125–128], cytoreductive gene-therapy approaches for the selective destruction of prostate tumors [129], and active immunotherapy using hK-based vaccines [130–132].

3.6. Tryptase

Tryptase are trypsin-like serine proteases found in the secretory granules of mast cells mainly in active form, in complex with proteoglycans. The best-characterized protease from this family is tryptase β, primarily because it was the first tryptase identified and it is the predominant protease and protein component of mast cells. Tryptase β has become a very useful diagnostic marker for the identification of mast cells and their activation [133, 134]. More importantly, tryptase β contributes to the pathogenesis of allergic inflammatory disorders, most notably asthma. Convincing evidence exist that tryptases released in immediate hypersensitivity reactions are important mediators of allergic inflammation [135]. Further evidence is emerging that tryptases also contribute to the pathogenesis of chronic inflammation in other mast cell-mediated disorders [135]. The application of chemically diverse tryptase inhibitors *in vivo* has provided evidence that they have therapeutic potential, particularly in asthma [136].

3.7. Dipeptidyl Peptidase IV

A significant and rapidly growing fraction of the human population is affected by type II diabetes, a disease characterized by elevated blood glucose levels and relative insulin insufficiency. The activity of the potent stimulator of the insulin secretion, GLP-1, is rapidly abolished by truncation mediated by the serine protease DPP-IV [137, 138]. Since GLP-1 based therapy is a promising treatment for type II diabetes [139], strategies to inhibit DPP-IV have been explored. *In vivo* administration of synthetic inhibitors of DPP-IV prevent *N*-terminal degradation of GLP-1, resulting in higher plasma concentrations of this hormone, increased insulin secretion, and improved glucose tolerance [140]. Such results have led to an elevated interest in inhibitors of DPP-IV for the treatment of type II diabetes [141]. Consequently, several potent DPP-IV inhibitors with a remarkably low degree of adverse events were discovered and are presently in clinical study [3, 139]. The DPP-IV inhibitor sitagliptin was approved by the Food and Drug Administration in 2006 and is marketed in the USA as Januvia® by Merck & Co. Vildagliptin has been submitted to the USA Food and Drug Administration for approval, and will be marketed as Galvus by Novartis. Because of their efficiency, safety, and tolerability in association with their oral mode of administration, it is expected that DPP-IV inhibitors will be a first-line treatment for the early stages of type II diabetes, particularly in combination with metmorfin and thiazolidinediones [139]. Dual inhibition strategy had been explored in this case as well. NEP is involved in inactivation of GLP-1 along with other bioactive peptides (enkephalins, substance P, endothelin, bradykinin, and atrial natriuretic factor) [60, 142]. An improvement in GLP-1 stability has been demonstrated by a combined inhibition of NEP and DPP-IV in anaesthetized pigs [143]. Plambock and co-workers [143] have shown that treatment of diabetic rats with a combination

of a DPP-IV inhibitor and an NEP inhibitor results in glucose-lowering effects that are superior to those observed using only a DPP-IV inhibitor. It is proposed that mixed inhibition of NEP and DPP-IV offers an alternative strategy for the treatment of type II diabetes.

4. Cysteine Proteases

In general, cysteine proteases function optimally in acidic conditions, such as acidic lysosomes where they degrade intracellular proteins. However, extracellular cysteine proteases may be secreted and function at or near the cell surface through mechanisms that are incompletely understood [96, 144]. Macrophages, smooth muscle cells, and endothelial cells can mobilize cathepsin B, L, S, and K into the extracellular space where they may participate in plaque proteolysis [145, 146]. Cathepsin B is known to be overexpressed in a number of cancers. Secretion and relocalization of cathepsin B is believed to be important in tumor progression and clinical outcome for patients [147]. It is of interest that the activity of this enzyme is highest in the invasive edge of the tumor. It is not clear whether cathepsin B is directly involved in degradation of ECM or if its role is primarily through activation of the other proteases implicated in cancer.

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List of Abbreviations

ACE	angiotensin-converting enzyme
ADAM	a disintegrin and metalloproteinase
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
ANP	atrial natriuretic peptide
CF	cystic fibrosis
DPP-IV	dipeptidyl peptidase IV
ECM	extracellular matrix
ECP	extracellular proteases
GCPII	glutamate carboxypeptidase II
GLP-1	glucagon-like peptide 1
hK	human kallikrein
hNE	human neutrophil elastase
IGF	insulin-like growth factor
IGFBP	IGF-binding protein
MCP	metallocarboxypeptidase

MMP	matrix metalloproteinase
NEP	neprilysin or neutral endopeptidase
PAPP	pregnancy-associated plasma protein
PSA	prostate specific antigen
RAS	renin-angiotensin system
TACE	TNFα converting enzyme
TAFI	thrombin-activable fibrinolysis inhibitor
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TIMP	tissue inhibitor of metalloproteinse
tPA	tissue-type plasminogen activator
uPA	urokinase-type plasminogen activator
uPAR	urokinase plasminogen activator receptor system
vWF	von Willebrand factor

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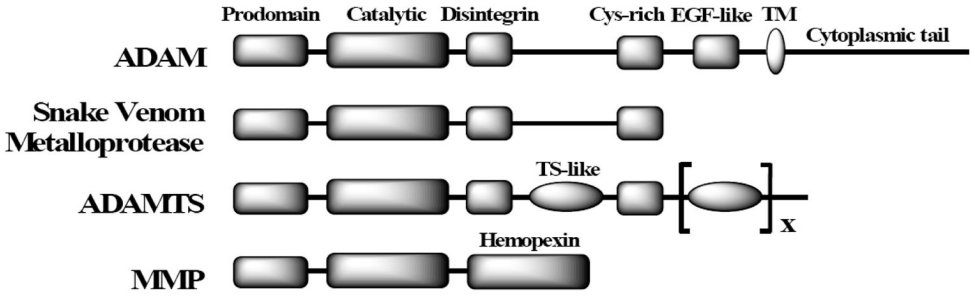

Figure 1.
The metzincin sub-clan of proteases.

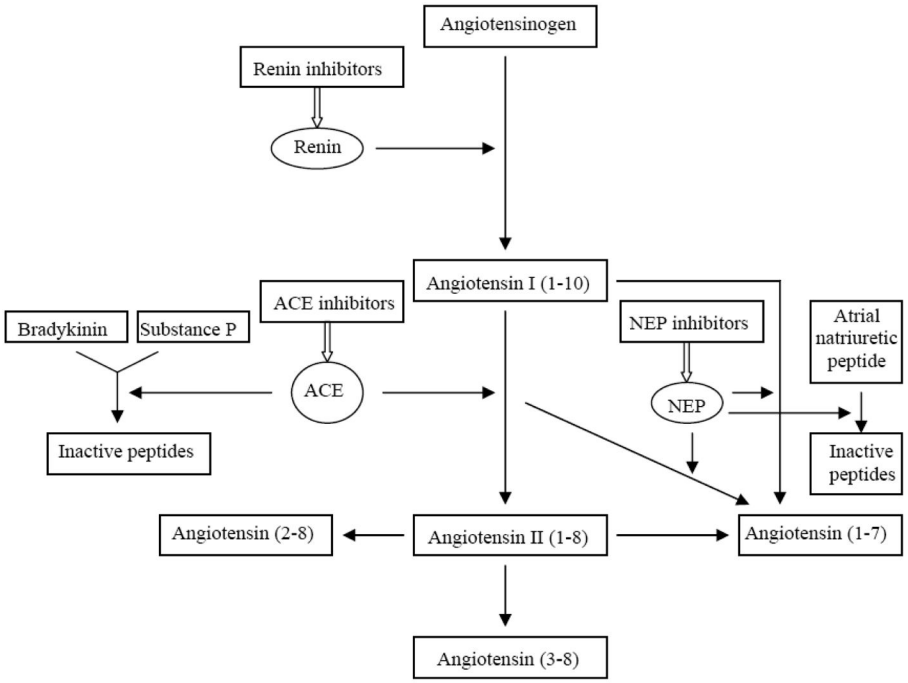

Figure 2.
The renin-angiotensin system.

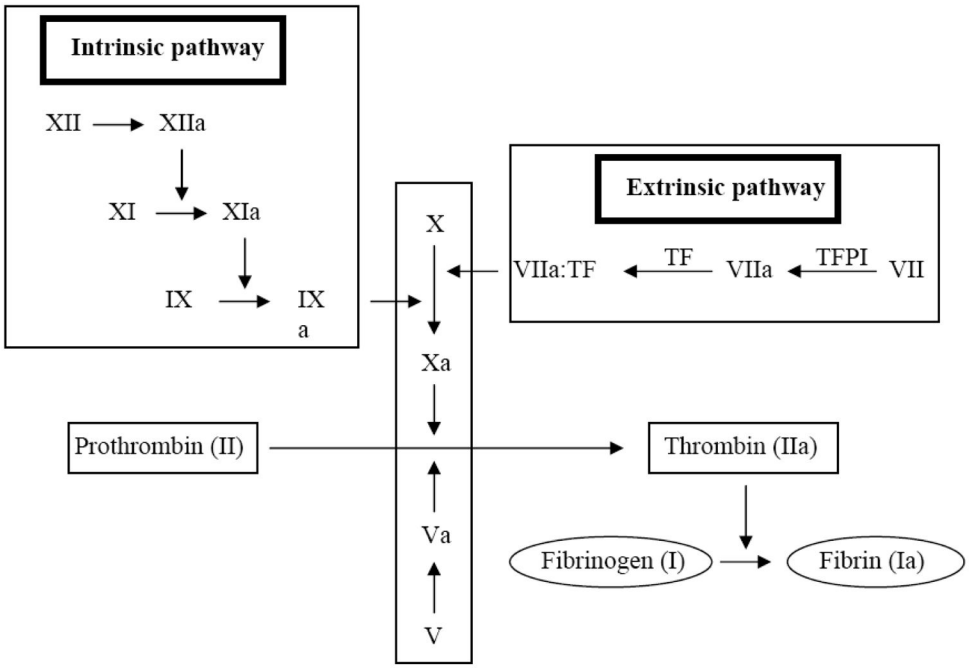

Figure 3.
The coagulation cascade.

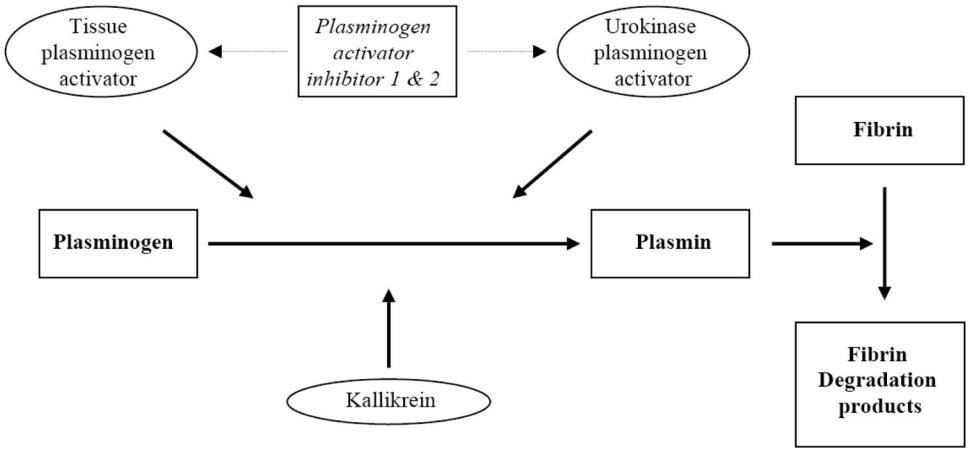

Figure 4.
The fibrinolytic system.