1-1-2008

Revisiting the biological roles of PAI2 (SERPINB2) in cancer

Darren Saunders
University of British Columbia

David Croucher
Cancer Research Program Sydney, dc43@uow.edu.au

Sergei A. Lobov
University of Wollongong, sergei@uow.edu.au

Marie Ranson
University of Wollongong, mranson@uow.edu.au

Revisiting the biological roles of PAI2 (SERPINB2) in cancer

Abstract

Tumour expression of the urokinase plasminogen activator correlates with invasive capacity. Consequently, inhibition of this serine protease by physiological inhibitors should decrease invasion and metastasis. However, of the two main urokinase inhibitors, high tumour levels of the type-1 inhibitor actually promote tumour progression, whereas high levels of the type-2 inhibitor decrease tumour growth and metastasis. We propose that the basis of this apparently paradoxical action of two similar serine protease inhibitors lies in key structural differences controlling interactions with components of the extracellular matrix and endocytosis/signalling co-receptors.

Keywords

Revisiting, biological, roles, PAI2, SERPINB2, cancer

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Croucher, D., Saunders, D., Lobov, S. A. and Ranson, M. (2008). Revisiting the biological roles of PAI2 (SERPINB2) in cancer. Nature Reviews Cancer, 8 (7), 535-545.

Revisiting the Biological Roles of PAI-2 (SerpinB2) in Cancer

David R. Croucher[1], Darren N Saunders[2,3], Sergei Lobov[4] and Marie Ranson[4].

1. Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, NSW, Australia 2010.
2. Dept. of Pathology and Laboratory Medicine, University of British Columbia.
3. Molecular Oncology and Breast Cancer Program, British Columbia Cancer Research Centre, Vancouver BC, V5Z 1L3, Canada.
4. School of Biological Sciences, University of Wollongong, NSW, Australia 2522.

Preface

Tumour expression of the urokinase plasminogen activator correlates with invasive capacity. Consequently, inhibition of this serine protease by physiological inhibitors should decrease invasion and metastasis. However, of the two main urokinase inhibitors, high tumour levels of the type-1 inhibitor actually promote tumour progression, whereas high levels of the type-2 inhibitor decrease tumour growth and metastasis. We propose that the basis of this apparently paradoxical action of two similar serine protease inhibitors lies in key structural differences controlling interactions with components of the extracellular matrix and endocytosis/signalling co-receptors.

Metastasis is intrinsically linked to the ability of tumour cells to escape the constraining extracellular matrix (ECM)[1]. The broad spectrum serine-protease plasmin facilitates this process by degrading components of the ECM[2]. Plasmin is generated by the plasminogen activation system, a tightly regulated network of protease activators, receptors and inhibitors (Figure 1) that becomes dysregulated during tumour progression[2]. Accordingly, components of this system are potent biomarkers for cancer progression and patient survival. Numerous studies have identified co-expression of the serine protease urokinase plasminogen activator (uPA) and one of its inhibitors, plasminogen activator inhibitor type-1 (PAI-1, SerpinE1, see Box 1), as an independent marker of poor prognosis in many cancer types[3]. Significantly, uPA and PAI-1 were recently included in the 2007 update of the American Society of Clinical Oncology recommendations for prognosis of node negative breast cancer[4].

The link between PAI-1 and poor patient prognosis may reflect dynamic interactions with ECM components and endocytosis/signalling co-receptors that ultimately promote tumour growth and metastasis, which are supplementary to its classical biochemical activity as a uPA inhibitor. Paradoxically, tumour-associated expression of another classical uPA inhibitor, plasminogen activator inhibitor type-2 (PAI-2, SerpinB2), is associated with increased survival in breast cancer patients[3,5], and recent novel data have highlighted key structural and functional differences between these serpins[6,7]. These differences suggest that PAI-2 does not possess the additional functions attributed to PAI-1 and acts predominantly as a protease inhibitor *in vivo*. In this Perspective, we incorporate these novel structural and functional data with a thorough review of the available prognostic data for PAI-2 in multiple cancer types, and propose a hypothesis for the mechanism underlying differential prognosis of high PAI-1 versus PAI-2 levels in cancer.

Cellular and tissue expression of PAI-2

As receptor bound plasmin is protected from inhibition by α_2-antiplasmin[8], direct inhibition of uPA and tissue plasminogen activator (tPA) by PAI-1 and PAI-2 are key regulatory mechanisms of pericellular plasminogen activation (Figure 1). In comparison to PAI-2, the role of PAI-1 in the plasminogen activation system has been studied in depth[9,10]. *In vivo*, PAI-1 expression can be highly induced in both endothelial cells and activated platelets[9] and its role in inhibiting thrombolysis through the rapid inhibition of tPA is especially well documented[11]. PAI-1 is also an established regulator of diverse plasmin-dependent and independent physiological processes involving vascular remodelling and angiogenesis[12]. This includes effects on cell adhesion and migration via an interaction with the ECM protein vitronectin, and subsequent modulation of integrin–uPAR–uPA interactions with the ECM[12]. In addition, inhibition of uPA by PAI-1 induces secondary high affinity interactions with the low density lipoprotein receptor (LDLR) family of endocytosis receptors[13-17], with further effects on migration, adhesion and proliferation. These processes will be addressed in detail below.

PAI-2 can be considered a stress protein as it is one of the most up-regulated proteins of activated monocyte/macrophages and differentiating keratinocytes, and its

expression is also highly inducible in fibroblasts and endothelial cells[10,18]. PAI-2 gene expression is stimulated by a variety of inflammatory mediators, and by viral or bacterial infection[18], so biological roles in the regulation of inflammation and wound healing have been proposed[18]. However, attempts at defining the precise physiological functions of PAI-2 have been somewhat confounded by its bi-topological existence in both a predominant cytosolic (47 kDa) form and an extracellular, glycosylated (60 kDa) form[10,19-21]. The reason for the intracellular accumulation of PAI-2 is not entirely clear but may be linked to an inefficient, mildly hydrophobic internal signal peptide[19,21-24], as increasing the hydrophobicity of the signal peptide results in enhanced PAI-2 secretion[22].

The prevalence of the cytosolic form of PAI-2 has fostered some debate in the field regarding potential extra/pericellular patho-physiological role(s), and more recent research has focussed on the somewhat contentious intracellular functions of PAI-2. Nevertheless, extracellular PAI-2 does exist *in vivo* and mediates important serpin-related biological functions.

Extracellular roles for PAI-2 and the serpin inhibitory mechanism.

Under physiological conditions PAI-2 is not usually detectable in human plasma, except during pregnancy when trophoblasts produce high levels of PAI-2[10,25]. As decreased plasma levels of PAI-2 correlate with intrauterine growth retardation and preeclampsia in humans, a role for PAI-2 in human placental maintenance and foetal development has been suggested[25]. However, PAI-2 is not required for normal murine development, survival, or fertility[26], though a phenotype for adipose tissue development in PAI-$2^{-/-}$ mice was recently reported[27]. Dougherty et al[26] suggested that as PAI-2 mRNA is only detected at significant levels in the murine placenta very late in gestation[28], the lack of obvious developmental phenotypes in PAI-$2^{-/-}$ mice does not preclude a role for PAI-2 in human development. Unfortunately, studies investigating spontaneous or xenograft tumour growth and metastasis in PAI-$2^{-/-}$ mice have not been performed to date, but such experiments would yield invaluable data on the role of PAI-2 in these processes.

PAI-2 is also detectable in other human bodily fluids, including; gingival fluid[29], saliva[30], peritoneal fluid[31] and infectious pleural effusions[32]. Furthermore, the ratio of intracellular:extracellular PAI-2 can be altered by various factors *in vitro*[33,34]. These

findings suggest that the secretion of PAI-2 is a highly regulated event that is not solely controlled by an inefficient secretion signal. Additionally, non-glycosylated PAI-2 has been observed in plasma taken from pregnant women, amniotic fluid and cord blood, and in the conditioned medium of U-937 cells exposed to phorbol ester[10]. As phorbol esters induce PAI-2 expression and the presence of cytosolic proteins in the extracellular environment is often predicated by cell death, it has been suggested that cell death (tissue necrosis or apoptosis) may be one route enabling non-glycosylated PAI-2 to reach the extracellular environment[10]. There is also evidence of non-glycosylated PAI-2 secretion by viable primary human monocytes via an ER–Golgi-independent pathway[33]. Hence, the normally low circulating levels of PAI-2 in the blood are not necessarily reflective of locally secreted PAI-2 levels in tissues. Finally, despite PAI-2 being approximately 10- and 50-fold slower than PAI-1 at inhibiting uPA and tPA, respectively, *in vitro*[35], tPA–PAI-2 complexes have been detected in both saliva[30] and gingival crevicular fluid[36], while uPA–PAI-2 complexes have been detected in human gestational tissues[37]. These observations provide clear evidence of uPA and tPA inhibition by PAI-2 *in vivo*.

Extracellular PAI-2 inhibits uPA through the unique serpin 'suicide' trapping mechanism. Serpins form covalent complexes with their target proteases, distinct from the classical 'lock and key' mechanism utilized by other small molecule protease inhibitors. The reactive centre loop (RCL) of the serpin acts as a bait for the protease active site but before completion of the proteolysis reaction can occur, cleavage of the RCL induces a large conformational change in the serpin. This so-called stressed (S) to relaxed (R) transition is critical to the inhibitory activity of serpins, involving insertion of the RCL into the body of the serpin molecule (as an extra strand of β-sheet A) and a dramatic increase in the stability of the molecule. Elegant structural studies (such as Huntington et al 2000 *Nature*[38]) have been performed on this transition showing that the protease, which is still covalently bound to the RCL, moves some 70 angstroms to the opposite pole of the serpin molecule during the S to R transition. This effectively crushes the protease, distorting the active site and preventing hydrolysis of the acyl-enzyme intermediate, effectively trapping the protease in a stable serpin-protease complex (such as uPA–PAI-2). Further, the structural transitions associated with the inhibitory action of serpins form the basis for selective recognition by cellular receptors such as by members of the LDLR

family of endocytosis receptors, with important implications for functions in cell signaling and migration (see below and Figures 2 and 3).

Potential functions of intracellular PAI-2.

Several novel functions of the intracellular form of PAI-2 have been proposed[18], which appear to be independent of serpin function. Interactions with a variety of cytosolic proteins have been implicated in these functions, including retinoblastoma protein (Rb)[39], interferon regulatory factor–3[40], proteasome subunit beta type 1[41], pre-mRNA processing factor 8[42], annexins (I, II, IV and V)[43], and fusion kinase ZNF198/FGFR1[44]. Furthermore, intranuclear expression of PAI-2 has been observed[39,45,46], where it is thought to interact with Rb, preventing Rb degradation[39], in addition to modulating its own expression[46]. An emerging theme in these studies is resistance to apoptosis (induced, for example, by TNF-α) following over-expression of PAI-2[44,47-50]. These effects appear, however, to be cell type or context dependant as PAI-2 knockdown in monocytes had no effect on apoptosis induced by serum withdrawal, hydrogen peroxide or a monoclonal antibody to CD95[51]. Additionally, a recent study reported that while TNF-α stimulation increased PAI-2 expression in HT-1080 and Isreco-1 cells, over-expression of PAI-2 in these and other cells lines conferred no protection against TNF-α induced apoptosis[52]. Importantly, this study used lentiviral-mediated delivery of PAI-2 to maintain heterogeneity of PAI-2 overexpressing cell lines and thereby avoid any potential clonal bias introduced by selection of transfected cells. A role for intracellular PAI-2 in regulation of papilloma virus replication and cytopathic effect has also been reported[39]. These effects were linked to the ability of PAI-2 to inhibit papilloma virus induced degradation of Rb and so maintain Rb levels[39]. However, in addition to affording no protection from apoptosis, lentiviral-mediated overexpression of PAI-2[52] affected Rb levels in only one of the three cell lines tested and this effect was independent of its protease inhibitory activity.

Due to these conflicting results, the exact function of intracellular PAI-2 remains unclear. Given the observations of non-glycosylated PAI-2 in the extracellular milieu, it is possible that intracellular non-glycosylated PAI-2 is released under inflammatory or other conditions that result in acute cell death/damage. This, and/or conditions that enhance locally secreted glycosylated PAI-2 in tissues could thereby limit peri/extracellular proteolysis during tissue remodelling processes.

Structural and functional differences between PAI-1 and PAI-2

Differential vitronectin binding

At supraphysiological levels, PAI-1 interacts with the ECM component vitronectin, and completely blocks the interaction of vitronectin with uPAR and integrins[53]. However, at physiological levels of PAI-1 a more dynamic process takes place, in which PAI-1 acts as a 'molecular switch', switching its affinity between vitronectin and endocytosis receptors following uPA inhibition[54]. Vitronectin binding also stabilises the active (S) conformation of PAI-1 (Refer to Box 1 and 2), preventing it from adopting a latent, non-inhibitory conformation[55]. Consequently, inhibition of uPA by vitronectin-bound PAI-1 stimulates directed cell migration partially via facilitation of an interaction between vitronectin and co-localised uPAR–integrins (Figure 2A)[12]. The ability of PAI-1 to direct vitronectin-dependent cell adhesion and migration is not emulated by PAI-2 as it does not bind to vitronectin[56] (Figure 2B). Additionally, despite high (but still physiological) PAI-1 levels in metastatic breast tumours, uPA activity is still detectable[57] and available for inhibition and/or targeting by exogenous inhibitors such as recombinant PAI-2[58-62]. So, although PAI-1 and PAI-2 have similar inhibitory biochemical properties, these additional interactions of PAI-1 in the pericellular environment may have a large influence on its actual inhibitory capability. Thus, in the context of the tumour microenvironment, it is likely that secreted/released PAI-2 may be the *bona fide* uPA inhibitor, a hypothesis supported by other researchers[63-68].

Structural differences affecting interactions with endocytosis receptors

Following inhibition of uPA at the cell surface, uPA–PAI-1 complexes are internalised via interactions with at least three members of the LDLR family of endocytosis receptors; LRP[6,69,70], VLDLr[7,71-73] and LRP-2[74]. Internalisation of uPA–PAI-2 complexes by LRP[6] and VLDLr[7] has been demonstrated, but PAI-2 endocytosis by LRP-2 has not yet been addressed. Importantly, unlike PAI-1[75], PAI-2 is unable to bind directly to these endocytosis receptors[6,7] (Figure 2B). Consequently, uPA–PAI-2 binds with lower affinity than uPA-PAI-1 to both LRP and VLDLr as determined by surface plasmon resonance[6,7], a method of direct, real-time measurement of protein-protein interactions. Comparison of structural characteristics of PAI-1 and PAI-2 in their relaxed

conformations provides a clear explanation for the differential binding of PAI-1 and PAI-2 to VLDLr and LRP (Figure 3)[7]. Structural studies have previously identified positively charged residues within the helix D of PAI-1 that contribute significantly to the high affinity binding of the uPA–PAI-1 complex with LDLR family members[76-79]. Accordingly, these residues conform with the proposed common binding motif for high affinity LDLR family ligands of two basic residues separated by 2-5 residues and N-terminally flanked by hydrophobic residues[80]. Interestingly, this motif is not conserved within the helix D of PAI-2[7] (Figure 3E), explaining the lower affinity of uPA–PAI-2 for this receptor family. Whilst these biochemical differences may seem trivial, the biological consequences of this differential receptor binding are quite striking.

PAI-2 does not mediate cell signaling

As uPAR is a GPI-anchored protein, with no transmembrane region, signaling events initiated by uPAR are mediated via integrins and co-receptors (such as epidermal growth factor receptor (EGFR) and FPRL1) that interact with uPAR or the uPAR signaling complex[17,81-83]. The binding of uPA to uPAR induces a variety of cell type specific responses, including the activation of p56/p59^{hck}[84], the Jak-Stat pathway[85,86], focal adhesion kinase[87-89], protein kinase Cε[90], casein kinase 2[85] and extracellular signal-regulated kinases 1/2 (ERK)[91-93]. The interaction of components of the plasminogen activation system with members of the LDLR family can indirectly effect signaling activity by regulating levels of uPA–uPAR on the cell surface[94] and also by directly transmitting signals through adaptor proteins attached to the cytoplasmic domains of the LDLRs[95,96]. On MCF-7 breast cancer cells, the ligation of uPA to uPAR stimulates transient ERK phosphorylation and vitronectin dependent cell migration[17,97]. The inhibition of uPA by PAI-1 sustains the phosphorylation of ERK, stimulating enhanced cell proliferation[7,17]. These events are facilitated by an interaction with VLDLr (Figure 2A) and mediated through the high affinity binding site within PAI-1[17] (Figure 3), via an ill-defined mechanism that possibly involves an interaction with β3-integrin[17] and trans-activation of EGFR[98]. PAI-1 is also capable of stimulating cell migration independently of uPA, tPA and vitronectin, as the direct interaction between PAI-1 and LRP increases motility through activation of the Jak-Stat pathway[99] (Figure 2A).

The absence of a high affinity LDLR binding site within PAI-2 precludes binding of uPA–PAI-2 to VLDLr with sufficient affinity to induce sustained mitogenic cell signaling events in MCF-7 cells[7] (Figure 2B). Furthermore, PAI-2 is not able to bind LRP independently of uPA[6] and is therefore unlikely to activate the Jak-Stat pathway and stimulate cell migration mediated by direct binding of PAI-1 to LRP[99]. Together, these data suggest that PAI-2 may be able to inhibit and clear cell surface uPA, and therefore inhibit plasmin formation *in vivo*, without initiating the cell signaling events and subsequent increased metastatic potential associated with PAI-1 (Figure 2). Indeed, an anti-proliferative effect mediated by the protease inhibitory capacity of extracellular PAI-2 has been observed with the THP-1 monocyte cell line, though the mechanism underlying this effect was not determined[100]. Direct *in vivo* experimental evidence of these effects would provide a simple explanation for the disparate relationships observed between PAI-1 and PAI-2 expression and disease outcome in various cancers.

Prognostic significance of PAI-2 expression in cancer

Experimental tumour model systems

The contribution of PAI-2 to improved patient outcome by decreasing tumour growth and metastasis is supported by several experimental tumour models. For example, PAI-2 has been shown to modulate xenograft metastasis in rodent models using uPA-expressing cell lines transfected with a PAI-2 expression vector[101-103]. Both intra- and extracellular expression of PAI-2 was observed in these cells, along with the complete inhibition of cell surface uPA and significantly decreased ECM degradation *in vitro*[101,102]. In all cases, xenograft tumours were formed in the presence of PAI-2, but were consistently surrounded by a dense collagenous capsule, and metastases were reduced or completely absent. In separate studies, intraperitoneal or intratumoural injection of recombinant PAI-2 also resulted in decreased tumour size[10]. The comparable physiological outcomes obtained by administration of exogenous PAI-2 and transfection of implanted tumour cells with PAI-2 cDNA, suggest that the inhibition of extracellular uPA activity is the mechanism underlying this reduction in tumour size and metastasis. Additionally, there are multiple *in vitro* studies that correlate anti-tumourigenic phenomena (such as the expression of tumour suppressor genes, anti-angiogenic factors

or infection with a tumour suppressing E1A adenovirus) with an increase in PAI-2 expression[104-107], or pro-tumorigenic stimuli (such as oncogene expression or treatment with phorbol esters) with a subsequent decrease in PAI-2 levels[108,109].

Overview of clinicopathological evidence

Concurrent increased protein expression of uPA and PAI-1 is a powerful marker of poor prognosis in many different types of solid tumour[3,110-112]. For breast cancer patients, uPA–PAI-1 is predictive of outcome independent of the classical prognostic factors and outperforms other biological markers such as estrogen receptors, ERBB2 (also known as HER-2), p53 and cathepsin D[113]. In this context, and in light of experimental evidence for PAI-2 mediated inhibition of tumour growth and metastasis, the prognostic relevance of PAI-2 expression is of significant interest. To this end, we have collated the findings of all published data investigating the prognostic value of PAI-2 expression, which encompasses 50 separate studies covering 15 tumour types (Table 1 and Table S1). Of those studies that analysed tumour samples against matched normal tissue, all found that PAI-2 expression was increased in the tumour over normal tissue, as was expression of uPA, uPAR, PAI-1, and occasionally tPA (though the role of tPA in cancer is less clear than that of uPA). It is important to note that the arbitrarily assigned levels of 'high expression' for PAI-2 are consistently much lower than those defined for PAI-1 [PAI-1, mean = 32.2 ± 32.1 ng/mg (n = 7 studies), median = 9.0 ± 6.3 ng/mg (n = 5 studies); PAI-2, mean = 7.4 ± 9.6 ng/mg (n = 9 studies), median = 2.5 ± 1.1 ng/mg (n = 8 studies)][5,64,65,114-125], suggesting that a small increase in PAI-1 expression may be able to overwhelm the effects of a concurrent increase in PAI-2 levels. However, it must also be noted that a proportion of PAI-1 may be in the inactive, latent form and that these values reflect antigen levels (as measured by ELISA), which may or may not relate to protease inhibitory capacity.

Whether these observed increases in uPA, uPAR and PAI-1 expression are predominantly due to specific polymorphisms or tumour-specific effects of various growth factors have not been determined[126-129]. To our knowledge no tumour-specific polymorphisms causing changes in expression of PAI-2 have been identified. As PAI-2 expression is strongly up-regulated by many inflammatory and/or stress related mediators[10], increases in tumour-associated PAI-2 may reflect a host response to a

rapidly growing and/or invasive tumour and not necessarily increased expression by tumour cells. Indeed, where analysed, PAI-2 (as well as uPA, uPAR and PAI-1) within tumour sections are often localised to tumour associated stromal cells such as fibroblasts, macrophages and endothelial cells[63,66,130-139] (Table 1 and Table S1). In some cases differential cell type expression of PAI-1 and PAI-2 may potentially contribute to the opposing prognoses associated with these two serpins. For example, in one lung cancer study PAI-2 expression by the fibroblasts correlated with the absence of lymph node involvement, while uPA and PAI-1 in the tumour cells and fibroblasts correlated with lymph node involvement[139]. Additionally, PAI-2 expression in esophageal squamous cell carcinoma associated fibroblasts correlated with increased patient survival time[137]. However, in a separate bladder cancer study, no association between stromal PAI-2 and patient outcome was found, although only a very small proportion of samples contained PAI-2 positive stroma[132].

Breast cancer

Breast cancer is the most frequently studied cancer type in which the prognostic value of PAI-2 expression has been assessed. Strikingly, all of the studies published (Table 1) demonstrate a significant association between PAI-2 expression and prognosis. Specifically, relatively high tumour-associated PAI-2 expression is linked with prolonged survival, decreased metastasis, or decreased tumour size. Conversely, relatively low PAI-2 expression was associated with the opposite effect. Two studies which found high PAI-2 expression to be favourable, also found that *very low* PAI-2 expression was associated with a favourable outcome[64,140], although these findings may actually reflect the concomitant low expression of uPA and PAI-1 in these tumours. Another study suggested that high PAI-2 expression was associated with increased sensitivity to tamoxifen treatment, in contrast to uPA–PAI-1 expression [122]. However, in this study, no link was found between estrogen receptor and uPA, PAI-1 or PAI-2 expression, so the mechanism of this modulation in tamoxifen resistance is unknown.

Importantly, multivariate analysis from several studies revealed further sub-groups of tumours where the combination of high PAI-1 and low PAI-2 had increased significance for poor prognosis and vice versa[5,65,115,141,142]. Furthermore, in a study of 2780 patients, high PAI-2 expression was an indicator of positive prognosis only in

primary invasive tumours that also expressed uPA and PAI-1, and was independent of all other clinicopathological parameters[5]. This study is also corroborated by others in breast[5,65], head and neck[137,143], oral[63] and lung[144] cancer which demonstrate the importance of uPA expression for the significance of PAI-2 expression (Table S1). These findings are supported by experimental evidence described above for a role for PAI-2 in the inhibition of tumour-associated uPA *in vivo*.

Other cancer types

The results of the relatively few studies conducted into the prognostic value and functional role of PAI-2 expression in other cancer types (head and neck, oral, colorectal, gastric, lung and pancreatic carcinomas) are not as clear compared with breast cancer, however the general trend is towards a positive or neutral outcome associated with PAI-2 expression (Table S1). Interestingly, all three studies conducted into endometrial cancer concluded that increased PAI-2 expression was associated with increased disease recurrence, local invasion, or more aggressive tumour stage. These differences may reflect functional disparity in the biochemistry of progression and metastasis of other tumour types. Ovarian cancer provides an illustration of this concept, where the ability of colony stimulating factor-1 (CSF-1) to induce secretion of PAI-2 has been investigated with respect to the poor prognosis associated with the high levels of soluble PAI-2 in ascites, and the good prognosis associated with high levels of cell associated (intracellular) PAI-2[34,130]. As CSF-1 is also known to up-regulate the expression of both uPA and PAI-1[131] and stimulate tumour cell invasion in a uPA-dependent manner[145], it seems likely that this effect of CSF-1 is responsible for the poor outcome, and not the presence of high levels of secreted PAI-2. Indeed, in these studies, high PAI-1 levels were significantly associated with CSF-1 expression by the tumour epithelium[131]. It is also worth noting that CSF-1 is often over-expressed in endometrial tumours[145] and this may be related to the observation of consistently high PAI-2 expression in more invasive endometrial tumours and the shorter survival time for these patients. This observation is also consistent with the significant link between high PAI-1 and PAI-2 expression observed in the largest study of endometrial cancer patients[146].

In summary, it appears that the significance of PAI-2 expression on prognosis in other cancer types is heavily context dependant, generally relies upon uPA expression, and

is inversely related to PAI-1 levels. Whilst a possible role for intracellular PAI-2 in regulating apoptosis cannot be excluded, any extracellular PAI-2 found in the tumour microenvironment may be able to compete for the binding of PAI-1 to uPA. Extracellular PAI-2 could thus limit plasmin generation while possibly neutralizing the alternative actions of PAI-1.

Concluding remarks

Even though PAI-1 can inhibit receptor bound uPA *in vivo* and *in vitro*, the mechanism/s linking PAI-1 expression to tumour malignancy may be distinct from a direct role in inhibition of cell surface plasminogen activation. These mechanisms promote cell proliferation, migration and/or de-adhesion and involve interactions between PAI-1 and vitronectin or integrins–uPAR–uPA–PAI-1 and LDLRs (Figure 2). Critical structural differences in PAI-2 preclude direct high affinity binding to vitronectin or members of the LDLR family[6,7] and hence PAI-2 does not possess the capability to induce these additional cellular responses. Rather, high levels of PAI-2 in the tumour microenvironment would facilitate cell surface uPA inhibition and clearance and may also counteract PAI-1 stimulatory actions on tumour invasion and metastasis (Figure 2). From a clinicopathological perspective, these structural and functional differences may thus explain, at least in part, the paradoxical biomarker data for PAI-1 versus PAI-2 in cancer prognosis. Therefore, inclusion of PAI-2 expression in clinical analyses would be expected to increase the prognostic power of measuring uPA–PAI-1 expression. Further animal model studies aimed at directly measuring the relative contributions of PAI-1 and PAI-2 to tumour progression are also needed (e.g. measurement of growth and metastasis of spontaneous or xenografted tumours in PAI-2 or PAI-1/PAI-2 knockout mice). Detailed understanding of the functional differences between PAI-1 and PAI-2 will facilitate improved design of uPA-targeted therapies aimed at specifically inhibiting uPA activity while avoiding mitogenic and motogenic signaling through LDLRs.

Only two studies on the prognostic impact of PAI-2 have attempted to distinguish between the two topological localizations of PAI-2[130,132], and none have determined the effect of glycosylation. In the tumour microenvironment, conditions such as hypoxia and inflammation can lead to phenotypic changes of the tumour associated stroma, (e.g.

cancer associated fibroblasts[147]) potentially inducing PAI-2 expression and secretion or release of PAI-2 protein. It is also possible that the contentious role of intracellular PAI-2 in the regulation of apoptosis may be of some prognostic influence, but this process is currently poorly understood. Hence, it is clear that further studies need to discriminate between the functions of the two topologically different forms of PAI-2.

In conclusion, the emerging evidence for the existence of peri/extracellular PAI-2 and the clear anti-tumour benefits of inhibition of uPA by PAI-2, as opposed to PAI-1, all suggest that PAI-2 plays an important role as an inhibitory serpin in the tumour microenvironment.

1. Hanahan, D. & Weinberg, R.A. The hallmarks of cancer. *Cell* **100**, 57-70 (2000).
2. Dano, K. et al. Plasminogen activation and cancer. *Thromb Haemost* **93**, 676-81 (2005).
3. Duffy, M.J. The urokinase plasminogen activator system: role in malignancy. *Curr Pharm Des* **10**, 39-49 (2004).
4. Harris, L. et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol* **25**, 5287-312 (2007).
5. Foekens, J.A. et al. The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. *Cancer Res* **60**, 636-43 (2000).
6. Croucher, D., Saunders, D.N. & Ranson, M. The urokinase/PAI-2 complex: a new high affinity ligand for the endocytosis receptor low density lipoprotein receptor-related protein. *J Biol Chem* **281**, 10206-13 (2006).
7. Croucher, D.R., Saunders, D.N., Stillfried, G.E. & Ranson, M. A structural basis for differential cell signalling by PAI-1 and PAI-2 in breast cancer cells. *Biochem J* **408**, 203-10 (2007).
8. Mullertz, S., Thorsen, S. & Sottrup-Jensen, L. Identification of molecular forms of plasminogen and plasmin-inhibitor complexes in urokinase-activated human plasma. *Biochem J* **223**, 169-77 (1984).
9. Agirbasli, M. Pivotal role of plasminogen-activator inhibitor 1 in vascular disease. *Int J Clin Pract* **59**, 102-6 (2005).
10. Kruithof, E.K., Baker, M.S. & Bunn, C.L. Biological and clinical aspects of plasminogen activator inhibitor type 2. *Blood* **86**, 4007-24 (1995).
11. Huber, K. Plasminogen activator inhibitor type-1 (part one): basic mechanisms, regulation, and role for thromboembolic disease. *J Thromb Thrombolysis* **11**, 183-93 (2001).
12. Stefansson, S., McMahon, G.A., Petitclerc, E. & Lawrence, D.A. Plasminogen activator inhibitor-1 in tumor growth, angiogenesis and vascular remodeling. *Curr Pharm Des* **9**, 1545-64 (2003).
13. Czekay, R.P., Aertgeerts, K., Curriden, S.A. & Loskutoff, D.J. Plasminogen activator inhibitor-1 detaches cells from extracellular matrices by inactivating integrins. *J Cell Biol* **160**, 781-91 (2003).
14. Czekay, R.P. & Loskutoff, D.J. Unexpected role of plasminogen activator inhibitor 1 in cell adhesion and detachment. *Exp Biol Med (Maywood)* **229**, 1090-6 (2004).
15. Kjoller, L. The urokinase plasminogen activator receptor in the regulation of the actin cytoskeleton and cell motility. *Biol Chem* **383**, 5-19 (2002).
16. Loskutoff, D.J., Curriden, S.A., Hu, G. & Deng, G. Regulation of cell adhesion by PAI-1. *Apmis* **107**, 54-61 (1999).
17. Webb, D.J., Thomas, K.S. & Gonias, S.L. Plasminogen activator inhibitor 1 functions as a urokinase response modifier at the level of cell signaling and thereby promotes MCF-7 cell growth. *J Cell Biol* **152**, 741-52 (2001).
18. Medcalf, R.L. & Stasinopoulos, S.J. The undecided serpin. The ins and outs of plasminogen activator inhibitor type 2. *Febs J* **272**, 4858-67 (2005).
19. Belin, D., Wohlwend, A., Schleuning, W.D., Kruithof, E.K. & Vassalli, J.D. Facultative polypeptide translocation allows a single mRNA to encode the

secreted and cytosolic forms of plasminogen activators inhibitor 2. *Embo J* **8**, 3287-94 (1989).
20. Genton, C., Kruithof, E.K. & Schleuning, W.D. Phorbol ester induces the biosynthesis of glycosylated and nonglycosylated plasminogen activator inhibitor 2 in high excess over urokinase-type plasminogen activator in human U-937 lymphoma cells. *J Cell Biol* **104**, 705-12 (1987).
21. Wohlwend, A., Belin, D. & Vassalli, J.D. Plasminogen activator-specific inhibitors produced by human monocytes/macrophages. *J Exp Med* **165**, 320-39 (1987).
22. von Heijne, G., Liljestrom, P., Mikus, P., Andersson, H. & Ny, T. The efficiency of the uncleaved secretion signal in the plasminogen activator inhibitor type 2 protein can be enhanced by point mutations that increase its hydrophobicity. *J Biol Chem* **266**, 15240-3 (1991).
23. Wohlwend, A., Belin, D. & Vassalli, J.D. Plasminogen activator-specific inhibitors in mouse macrophages: in vivo and in vitro modulation of their synthesis and secretion. *J Immunol* **139**, 1278-84 (1987).
24. Ye, R.D., Wun, T.C. & Sadler, J.E. Mammalian protein secretion without signal peptide removal. Biosynthesis of plasminogen activator inhibitor-2 in U-937 cells. *J Biol Chem* **263**, 4869-75 (1988).
25. Astedt, B., Lindoff, C. & Lecander, I. Significance of the plasminogen activator inhibitor of placental type (PAI-2) in pregnancy. *Semin Thromb Hemost* **24**, 431-5 (1998).
26. Dougherty, K.M. et al. The plasminogen activator inhibitor-2 gene is not required for normal murine development or survival. *Proc Natl Acad Sci U S A* **96**, 686-91 (1999).
27. Lijnen, H.R., Frederix, L. & Scroyen, I. Deficiency of plasminogen activator inhibitor-2 impairs nutritionally induced murine adipose tissue development. *J Thromb Haemost* **5**, 2259-65 (2007).
28. Kawata, Y., Mimuro, J., Kaneko, M., Shimada, K. & Sakata, Y. Expression of plasminogen activator inhibitor 2 in the adult and embryonic mouse tissues. *Thromb Haemost* **76**, 569-76 (1996).
29. Kinnby, B. The plasminogen activating system in periodontal health and disease. *Biol Chem* **383**, 85-92 (2002).
30. Virtanen, O.J. et al. Plasminogen activators and their inhibitors in human saliva and salivary gland tissue. *Eur J Oral Sci* **114**, 22-6 (2006).
31. Scott-Coombes, D., Whawell, S., Vipond, M.N. & Thompson, J. Human intraperitoneal fibrinolytic response to elective surgery. *Br J Surg* **82**, 414-7 (1995).
32. Aleman, C. et al. Association between inflammatory mediators and the fibrinolysis system in infectious pleural effusions. *Clin Sci (Lond)* **105**, 601-7 (2003).
33. Ritchie, H. & Booth, N.A. Secretion of plasminogen activator inhibitor 2 by human peripheral blood monocytes occurs via an endoplasmic reticulum-golgi-independent pathway. *Exp Cell Res* **242**, 439-50 (1998).

34. Chambers, S.K., Gertz, R.E., Jr., Ivins, C.M. & Kacinski, B.M. The significance of urokinase- type plasminogen activator, its inhibitors, and its receptor in ascites of patients with epithelial ovarian cancer. *Cancer* **75**, 1627-33 (1995).
35. Thorsen, S., Philips, M., Selmer, J., Lecander, I. & Astedt, B. Kinetics of inhibition of tissue-type and urokinase-type plasminogen activator by plasminogen-activator inhibitor type 1 and type 2. *Eur J Biochem* **175**, 33-9 (1988).
36. Brown, J.M., Watanabe, K., Cohen, R.L. & Chambers, D.A. Molecular characterization of plasminogen activators in human gingival crevicular fluid. *Arch Oral Biol* **40**, 839-45 (1995).
37. Tsatas, D., Baker, M.S. & Rice, G.E. Tissue-specific expression of the relaxed conformation of plasminogen activator inhibitor-2 and low-density lipoprotein receptor-related protein in human term gestational tissues. *J Histochem Cytochem* **45**, 1593-602 (1997).
38. Huntington, J.A., Read, R.J. & Carrell, R.W. Structure of a serpin-protease complex shows inhibition by deformation. *Nature* **407**, 923-6 (2000).
39. Darnell, G.A. et al. Inhibition of retinoblastoma protein degradation by interaction with the serpin plasminogen activator inhibitor 2 via a novel consensus motif. *Mol Cell Biol* **23**, 6520-32 (2003).
40. Zhang, Y.Q. et al. Identification of interaction between PAI-2 and IRF-3. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* **35**, 661-5 (2003).
41. Fan, J. et al. Interaction of plasminogen activator inhibitor-2 and proteasome subunit, beta type 1. *Acta Biochim Biophys Sin (Shanghai)* **36**, 42-6 (2004).
42. Fan, J. et al. Interaction between plasminogen activator inhibitor type-2 and pre-mRNA processing factor 8. *Acta Biochim Biophys Sin (Shanghai)* **36**, 623-8 (2004).
43. Jensen, P.H. et al. The exon 3 encoded sequence of the intracellular serine proteinase inhibitor plasminogen activator inhibitor 2 is a protein binding domain. *J Biol Chem* **271**, 26892-9 (1996).
44. Kasyapa, C.S., Kunapuli, P., Hawthorn, L. & Cowell, J.K. Induction of the plasminogen activator inhibitor-2 in cells expressing the ZNF198/FGFR1 fusion kinase, that is involved in atypical myeloproliferative disease. *Blood* (2006).
45. Bird, C.H. et al. Nucleocytoplasmic distribution of the ovalbumin serpin PI-9 requires a nonconventional nuclear import pathway and the export factor Crm1. *Mol Cell Biol* **21**, 5396-407 (2001).
46. Macaluso, M. et al. Cytoplasmic and nuclear interaction between Rb family proteins and PAI-2: a physiological crosstalk in human corneal and conjunctival epithelial cells. *Cell Death Differ* **13**, 1515-22 (2006).
47. Greten, F.R. et al. NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. *Cell* **130**, 918-31 (2007).
48. Kumar, S. & Baglioni, C. Protection from tumor necrosis factor-mediated cytolysis by overexpression of plasminogen activator inhibitor type-2. *J Biol Chem* **266**, 20960-4 (1991).

49. Dickinson, J.L., Bates, E.J., Ferrante, A. & Antalis, T.M. Plasminogen activator inhibitor type 2 inhibits tumor necrosis factor alpha-induced apoptosis. Evidence for an alternate biological function. *J Biol Chem* **270**, 27894-904 (1995).
50. Zhou, H.M., Bolon, I., Nichols, A., Wohlwend, A. & Vassalli, J.D. Overexpression of plasminogen activator inhibitor type 2 in basal keratinocytes enhances papilloma formation in transgenic mice. *Cancer Res* **61**, 970-6 (2001).
51. Ritchie, H. & Fragoyannis, A. Thrombin inhibits apoptosis of monocytes and plasminogen activator inhibitor 2 (PAI-2) is not responsible for this inhibition. *Exp Cell Res* **260**, 20-9 (2000).
52. Fish, R.J. & Kruithof, E.K. Evidence for serpinB2-independent protection from TNF-alpha-induced apoptosis. *Exp Cell Res* **312**, 350-61 (2006).
53. Stefansson, S. & Lawrence, D.A. The serpin PAI-1 inhibits cell migration by blocking integrin alpha V beta 3 binding to vitronectin. *Nature* **383**, 441-3 (1996).
54. Deng, G., Curriden, S.A., Wang, S., Rosenberg, S. & Loskutoff, D.J. Is plasminogen activator inhibitor-1 the molecular switch that governs urokinase receptor-mediated cell adhesion and release? *J Cell Biol* **134**, 1563-71 (1996).
55. Whisstock, J.C. & Bottomley, S.P. Molecular gymnastics: serpin structure, folding and misfolding. *Curr Opin Struct Biol* **16**, 761-8 (2006).
56. Mikus, P., Urano, T., Liljestrom, P. & Ny, T. Plasminogen-activator inhibitor type 2 (PAI-2) is a spontaneously polymerising SERPIN. Biochemical characterisation of the recombinant intracellular and extracellular forms. *Eur J Biochem* **218**, 1071-82 (1993).
57. Chappuis, P.O. et al. Functional evaluation of plasmin formation in primary breast cancer. *J Clin Oncol* **19**, 2731-8 (2001).
58. Allen, B.J., Rizvi, S., Li, Y., Tian, Z. & Ranson, M. In vitro and preclinical targeted alpha therapy for melanoma, breast, prostate and colorectal cancers. *Crit Rev Oncol Hematol* **39**, 139-46 (2001).
59. Allen, B.J., Tian, Z., Rizvi, S.M., Li, Y. & Ranson, M. Preclinical studies of targeted alpha therapy for breast cancer using 213Bi-labelled-plasminogen activator inhibitor type 2. *Br J Cancer* **88**, 944-50 (2003).
60. Li, Y., Rizvi, S.M., Ranson, M. & Allen, B.J. 213Bi-PAI2 conjugate selectively induces apoptosis in PC3 metastatic prostate cancer cell line and shows anti-cancer activity in a xenograft animal model. *Br J Cancer* **86**, 1197-203 (2002).
61. Ranson, M., Tian, Z., Andronicos, N.M., Rizvi, S. & Allen, B.J. In vitro cytotoxicity of bismuth-213 (213Bi)-labeled-plasminogen activator inhibitor type 2 (alpha-PAI-2) on human breast cancer cells. *Breast Cancer Res Treat* **71**, 149-59 (2002).
62. Stutchbury, T.K. et al. Preclinical evaluation of 213Bi-labeled plasminogen activator inhibitor type 2 in an orthotopic murine xenogenic model of human breast carcinoma. *Mol Cancer Ther* **6**, 203-12 (2007).
63. Nozaki, S. et al. Immunohistochemical localization of a urokinase-type plasminogen activator system in squamous cell carcinoma of the oral cavity: association with mode of invasion and lymph node metastasis. *Oral Oncol* **34**, 58-62 (1998).
64. Bouchet, C. et al. Dissemination risk index based on plasminogen activator system components in primary breast cancer. *J Clin Oncol* **17**, 3048-57 (1999).

65. Bouchet, C. et al. Prognostic value of urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitors PAI-1 and PAI-2 in breast carcinomas. *Br J Cancer* **69**, 398-405 (1994).
66. Umeda, T., Eguchi, Y., Okino, K., Kodama, M. & Hattori, T. Cellular localization of urokinase-type plasminogen activator, its inhibitors, and their mRNAs in breast cancer tissues. *J Pathol* **183**, 388-97 (1997).
67. Zhao, E.M., Han, D.M., Yu, Z.K., Fan, E.Z. & Li, Y. [Expression and significance of the urokinase-type plasminogen activator and its inhibitors in squamous cell carcinoma of human larynx]. *Zhonghua Er Bi Yan Hou Ke Za Zhi* **38**, 39-42 (2003).
68. Nakamura, M. et al. Possible role of plasminogen activator inhibitor 2 in the prevention of the metastasis of gastric cancer tissues. *Thromb Res* **65**, 709-19 (1992).
69. Herz, J., Clouthier, D.E. & Hammer, R.E. LDL receptor-related protein internalizes and degrades uPA-PAI-1 complexes and is essential for embryo implantation. *Cell* **71**, 411-21 (1992).
70. Kounnas, M.Z., Henkin, J., Argraves, W.S. & Strickland, D.K. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor mediates cellular uptake of pro-urokinase. *J Biol Chem* **268**, 21862-7 (1993).
71. Argraves, K.M. et al. The very low density lipoprotein receptor mediates the cellular catabolism of lipoprotein lipase and urokinase-plasminogen activator inhibitor type I complexes. *J Biol Chem* **270**, 26550-7 (1995).
72. Rettenberger, P.M. et al. Ligand binding properties of the very low density lipoprotein receptor. Absence of the third complement-type repeat encoded by exon 4 is associated with reduced binding of Mr 40,000 receptor-associated protein. *J Biol Chem* **274**, 8973-80 (1999).
73. Webb, D.J., Nguyen, D.H., Sankovic, M. & Gonias, S.L. The very low density lipoprotein receptor regulates urokinase receptor catabolism and breast cancer cell motility in vitro. *J Biol Chem* **274**, 7412-20 (1999).
74. Stefansson, S. et al. gp330 on type II pneumocytes mediates endocytosis leading to degradation of pro-urokinase, plasminogen activator inhibitor-1 and urokinase-plasminogen activator inhibitor-1 complex. *J Cell Sci* **108 (Pt 6)**, 2361-8 (1995).
75. Nykjaer, A. et al. Regions involved in binding of urokinase-type-1 inhibitor complex and pro-urokinase to the endocytic alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein. Evidence that the urokinase receptor protects pro-urokinase against binding to the endocytic receptor. *J Biol Chem* **269**, 25668-76 (1994).
76. Horn, I.R., van den Berg, B.M., Moestrup, S.K., Pannekoek, H. & van Zonneveld, A.J. Plasminogen activator inhibitor 1 contains a cryptic high affinity receptor binding site that is exposed upon complex formation with tissue-type plasminogen activator. *Thromb Haemost* **80**, 822-828 (1998).
77. Rodenburg, K.W., Kjoller, L., Petersen, H.H. & Andreasen, P.A. Binding of urokinase-type plasminogen activator-plasminogen activator inhibitor-1 complex to the endocytosis receptors alpha2-macroglobulin receptor/low-density lipoprotein receptor-related protein and very-low-density lipoprotein receptor involves basic residues in the inhibitor. *Biochem J* **329 (Pt 1)**, 55-63 (1998).

78. Skeldal, S. et al. Binding areas of urokinase-type plasminogen activator-plasminogen activator inhibitor-1 complex for endocytosis receptors of the low-density lipoprotein receptor family, determined by site-directed mutagenesis. *Febs J* (2006).
79. Stefansson, S. et al. Plasminogen activator inhibitor-1 contains a cryptic high affinity binding site for the low density lipoprotein receptor-related protein. *J Biol Chem* **273**, 6358-66 (1998).
80. Jensen, G.A. et al. Binding Site Structure of One LRP-RAP Complex:Implications for a Common Ligand-Receptor Binding Motif. *J Mol Biol* **362**, 700-16 (2006).
81. Liu, D., Aguirre Ghiso, J., Estrada, Y. & Ossowski, L. EGFR is a transducer of the urokinase receptor initiated signal that is required for in vivo growth of a human carcinoma. *Cancer Cell* **1**, 445-57 (2002).
82. Preissner, K.T., Kanse, S.M. & May, A.E. Urokinase receptor: a molecular organizer in cellular communication. *Curr Opin Cell Biol* **12**, 621-8 (2000).
83. Resnati, M. et al. The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. *Proc Natl Acad Sci U S A* **99**, 1359-64 (2002).
84. Konakova, M., Hucho, F. & Schleuning, W.D. Downstream targets of urokinase-type plasminogen-activator-mediated signal transduction. *Eur J Biochem* **253**, 421-9 (1998).
85. Dumler, I. et al. Urokinase activates the Jak/Stat signal transduction pathway in human vascular endothelial cells. *Arterioscler Thromb Vasc Biol* **19**, 290-7 (1999).
86. Koshelnick, Y., Ehart, M., Hufnagl, P., Heinrich, P.C. & Binder, B.R. Urokinase receptor is associated with the components of the JAK1/STAT1 signaling pathway and leads to activation of this pathway upon receptor clustering in the human kidney epithelial tumor cell line TCL-598. *J Biol Chem* **272**, 28563-7 (1997).
87. Nguyen, D.H. et al. Urokinase-type plasminogen activator stimulates the Ras/Extracellular signal-regulated kinase (ERK) signaling pathway and MCF-7 cell migration by a mechanism that requires focal adhesion kinase, Src, and Shc. Rapid dissociation of GRB2/Sps-Shc complex is associated with the transient phosphorylation of ERK in urokinase-treated cells. *J Biol Chem* **275**, 19382-8 (2000).
88. Tang, H., Kerins, D.M., Hao, Q., Inagami, T. & Vaughan, D.E. The urokinase-type plasminogen activator receptor mediates tyrosine phosphorylation of focal adhesion proteins and activation of mitogen-activated protein kinase in cultured endothelial cells. *J Biol Chem* **273**, 18268-72 (1998).
89. Yebra, M., Goretzki, L., Pfeifer, M. & Mueller, B.M. Urokinase-type plasminogen activator binding to its receptor stimulates tumor cell migration by enhancing integrin-mediated signal transduction. *Exp Cell Res* **250**, 231-40 (1999).
90. Busso, N., Masur, S.K., Lazega, D., Waxman, S. & Ossowski, L. Induction of cell migration by pro-urokinase binding to its receptor: possible mechanism for signal transduction in human epithelial cells. *J Cell Biol* **126**, 259-70 (1994).

91. Aguirre Ghiso, J.A., Alonso, D.F., Farias, E.F., Gomez, D.E. & de Kier Joffe, E.B. Deregulation of the signaling pathways controlling urokinase production. Its relationship with the invasive phenotype. *Eur J Biochem* **263**, 295-304 (1999).
92. Kanse, S.M. et al. Induction of vascular SMC proliferation by urokinase indicates a novel mechanism of action in vasoproliferative disorders. *Arterioscler Thromb Vasc Biol* **17**, 2848-54 (1997).
93. Nguyen, D.H., Hussaini, I.M. & Gonias, S.L. Binding of urokinase-type plasminogen activator to its receptor in MCF-7 cells activates extracellular signal-regulated kinase 1 and 2 which is required for increased cellular motility. *J Biol Chem* **273**, 8502-7 (1998).
94. Webb, D.J., Nguyen, D.H. & Gonias, S.L. Extracellular signal-regulated kinase functions in the urokinase receptor-dependent pathway by which neutralization of low density lipoprotein receptor-related protein promotes fibrosarcoma cell migration and matrigel invasion. *J Cell Sci* **113 (Pt 1)**, 123-34 (2000).
95. Herz, J. & Strickland, D.K. LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest* **108**, 779-84 (2001).
96. Strickland, D.K., Gonias, S.L. & Argraves, W.S. Diverse roles for the LDL receptor family. *Trends Endocrinol Metab* **13**, 66-74 (2002).
97. Nguyen, D.H. et al. Myosin light chain kinase functions downstream of Ras/ERK to promote migration of urokinase-type plasminogen activator-stimulated cells in an integrin-selective manner. *J Cell Biol* **146**, 149-64 (1999).
98. Jo, M. et al. Dynamic assembly of the urokinase-type plasminogen activator signaling receptor complex determines the mitogenic activity of urokinase-type plasminogen activator. *J Biol Chem* **280**, 17449-57 (2005).
99. Degryse, B. et al. The low density lipoprotein receptor-related protein is a motogenic receptor for plasminogen activator inhibitor-1. *J Biol Chem* **279**, 22595-604 (2004).
100. Yu, H., Maurer, F. & Medcalf, R.L. Plasminogen activator inhibitor type 2: a regulator of monocyte proliferation and differentiation. *Blood* **99**, 2810-8 (2002).
101. Laug, W.E., Cao, X.R., Yu, Y.B., Shimada, H. & Kruithof, E.K. Inhibition of invasion of HT1080 sarcoma cells expressing recombinant plasminogen activator inhibitor 2. *Cancer Res* **53**, 6051-7 (1993).
102. Mueller, B.M., Yu, Y.B. & Laug, W.E. Overexpression of plasminogen activator inhibitor 2 in human melanoma cells inhibits spontaneous metastasis in scid/scid mice. *Proc Natl Acad Sci U S A* **92**, 205-9 (1995).
103. Praus, M., Wauterickx, K., Collen, D. & Gerard, R.D. Reduction of tumor cell migration and metastasis by adenoviral gene transfer of plasminogen activator inhibitors. *Gene Ther* **6**, 227-36 (1999).
104. Ma, W. et al. Alteration in gene expression profile and biological behavior in human lung cancer cell line NL9980 by nm23-H1 gene silencing. *Biochem Biophys Res Commun* (2008).
105. Fernandez-Soria, V. et al. Adenovirus E1A orchestrates the urokinase-plasminogen activator system and upregulates PAI-2 expression, supporting a tumor suppressor effect. *Int J Oncol* **28**, 143-8 (2006).

106. Guan, M. et al. Adenovirus-mediated PEDF expression inhibits prostate cancer cell growth and results in augmented expression of PAI-2. *Cancer Biol Ther* **6**, 419-25 (2007).
107. Marutsuka, K. et al. Effects of suramin on metastatic ability, proliferation, and production of urokinase-type plasminogen activator and plasminogen activator inhibitor type 2 in human renal cell carcinoma cell line SN12C-PM6. *Clin Exp Metastasis* **13**, 116-22 (1995).
108. Li, P. et al. Multiple roles of the candidate oncogene ZNF217 in ovarian epithelial neoplastic progression. *Int J Cancer* **120**, 1863-73 (2007).
109. Shimizu, T., Sato, K., Suzuki, T., Tachibana, K. & Takeda, K. Induction of plasminogen activator inhibitor-2 is associated with suppression of invasive activity in TPA-mediated differentiation of human prostate cancer cells. *Biochem Biophys Res Commun* **309**, 267-71 (2003).
110. Harbeck, N., Kates, R.E. & Schmitt, M. Clinical relevance of invasion factors urokinase-type plasminogen activator and plasminogen activator inhibitor type 1 for individualized therapy decisions in primary breast cancer is greatest when used in combination. *J Clin Oncol* **20**, 1000-7 (2002).
111. Schmitt, M. et al. The urokinase plasminogen activator system as a novel target for tumour therapy. *Fibrinolysis and Proteolysis* **14**, 114-132 (2000).
112. Weigelt, B., Peterse, J.L. & van 't Veer, L.J. Breast cancer metastasis: markers and models. *Nat Rev Cancer* **5**, 591-602 (2005).
113. Duffy, M.J. & Duggan, C. The urokinase plasminogen activator system: a rich source of tumour markers for the individualised management of patients with cancer. *Clin Biochem* **37**, 541-8 (2004).
114. Abe, J. et al. Larger and more invasive colorectal carcinoma contains larger amounts of plasminogen activator inhibitor type 1 and its relative ratio over urokinase receptor correlates well with tumor size. *Cancer* **86**, 2602-11 (1999).
115. Borstnar, S., Vrhovec, I. & Cufer, T. Prognostic value of plasminogen activator inhibitors in breast cancer. *Int J Biol Markers* **17**, 96-103 (2002).
116. Foekens, J.A. et al. Plasminogen activator inhibitor-2: prognostic relevance in 1012 patients with primary breast cancer. *Cancer Res* **55**, 1423-7 (1995).
117. Ganesh, S. et al. Prognostic relevance of plasminogen activators and their inhibitors in colorectal cancer. *Cancer Res* **54**, 4065-71 (1994).
118. Ganesh, S. et al. Prognostic value of the plasminogen activation system in patients with gastric carcinoma. *Cancer* **77**, 1035-43 (1996).
119. Gleeson, N., Gonsalves, R. & Bonnar, J. Uterine fibrinolytic enzymes in endometrial cancer. *Eur J Gynaecol Oncol* **14**, 369-73 (1993).
120. Ho, C.H., Yuan, C.C. & Liu, S.M. Diagnostic and prognostic values of plasma levels of fibrinolytic markers in ovarian cancer. *Gynecol Oncol* **75**, 397-400 (1999).
121. Itoh, T. et al. Clinical significance of urokinase-type plasminogen activator activity in hepatocellular carcinoma. *J Gastroenterol Hepatol* **15**, 422-30 (2000).
122. Meijer-van Gelder, M.E. et al. Urokinase-type plasminogen activator system in breast cancer: association with tamoxifen therapy in recurrent disease. *Cancer Res* **64**, 4563-8 (2004).

123. Osmak, M. et al. Plasminogen activator inhibitor type 2: potential prognostic factor for endometrial carcinomas. *Neoplasma* **48**, 462-7 (2001).
124. Salden, M. et al. The urokinase-type plasminogen activator system in resected non-small-cell lung cancer. Rotterdam Oncology Thoracic Study Group. *Ann Oncol* **11**, 327-32 (2000).
125. Sumiyoshi, K. et al. Plasminogen activator system in human breast cancer. *Int J Cancer* **50**, 345-8 (1992).
126. Castello, R. et al. Plasminogen activator inhibitor-1 4G/5G polymorphism in breast cancer patients and its association with tissue PAI-1 levels and tumor severity. *Thromb Res* **117**, 487-92 (2006).
127. Sternlicht, M.D. et al. Prognostic value of PAI1 in invasive breast cancer: evidence that tumor-specific factors are more important than genetic variation in regulating PAI1 expression. *Cancer Epidemiol Biomarkers Prev* **15**, 2107-14 (2006).
128. Wang, J., Stockton, D.W. & Ittmann, M. The fibroblast growth factor receptor-4 Arg388 allele is associated with prostate cancer initiation and progression. *Clin Cancer Res* **10**, 6169-78 (2004).
129. Przybylowska, K. et al. Antigen levels of the urokinase-type plasminogen activator and its gene polymorphisms in colorectal cancer. *Cancer Lett* **181**, 23-30 (2002).
130. Chambers, S.K., Ivins, C.M. & Carcangiu, M.L. Expression of plasminogen activator inhibitor-2 in epithelial ovarian cancer: a favorable prognostic factor related to the actions of CSF-1. *Int J Cancer* **74**, 571-5 (1997).
131. Chambers, S.K., Ivins, C.M. & Carcangiu, M.L. Plasminogen activator inhibitor-1 is an independent poor prognostic factor for survival in advanced stage epithelial ovarian cancer patients. *Int J Cancer* **79**, 449-54 (1998).
132. Champelovier, P. et al. Plasminogen- and colony-stimulating factor-1-associated markers in bladder carcinoma: diagnostic value of urokinase plasminogen activator receptor and plasminogen activator inhibitor type-2 using immunocytochemical analysis. *Urol Res* **30**, 301-9 (2002).
133. De Vries, T.J. et al. Components of the plasminogen activation system in uveal melanoma--a clinico-pathological study. *J Pathol* **175**, 59-67 (1995).
134. Duggan, C. et al. Plasminogen activator inhibitor type 2 in breast cancer. *Br J Cancer* **76**, 622-7 (1997).
135. Ferrier, C.M. et al. High tPA-expression in primary melanoma of the limb correlates with good prognosis. *Br J Cancer* **83**, 1351-9 (2000).
136. Heiss, M.M. et al. Tumor-associated proteolysis and prognosis: new functional risk factors in gastric cancer defined by the urokinase-type plasminogen activator system. *J Clin Oncol* **13**, 2084-93 (1995).
137. Shiomi, H., Eguchi, Y., Tani, T., Kodama, M. & Hattori, T. Cellular distribution and clinical value of urokinase-type plasminogen activator, its receptor, and plasminogen activator inhibitor-2 in esophageal squamous cell carcinoma. *Am J Pathol* **156**, 567-75 (2000).
138. Smith, R. et al. High expression of plasminogen activator inhibitor-2 (PAI-2) is a predictor of improved survival in patients with pancreatic adenocarcinoma. *World J Surg* **31**, 493-502; discussion 503 (2007).

139. Robert, C. et al. Expression of plasminogen activator inhibitors 1 and 2 in lung cancer and their role in tumor progression. *Clin Cancer Res* **5**, 2094-102 (1999).
140. Spyratos, F. et al. Prognostic value of uPA, PAI-1 and PAI-2 mRNA expression in primary breast cancer. *Anticancer Res* **22**, 2997-3003 (2002).
141. Borstnar, S., Vrhovec, I., Svetic, B. & Cufer, T. Prognostic value of the urokinase-type plasminogen activator, and its inhibitors and receptor in breast cancer patients. *Clin Breast Cancer* **3**, 138-46 (2002).
142. Ishikawa, N., Endo, Y. & Sasaki, T. Inverse correlation between mRNA expression of plasminogen activator inhibitor-2 and lymph node metastasis in human breast cancer. *Jpn J Cancer Res* **87**, 480-7 (1996).
143. Zhao, E. et al. [Prognostic value of the urokinase-type plasminogen activator and its inhibitors in squamous cell carcinoma of human larynx]. *Lin Chuang Er Bi Yan Hou Ke Za Zhi* **16**, 599-602 (2002).
144. Nagayama, M. et al. Plasminogen activators and their inhibitors in non-small cell lung cancer. Low content of type 2 plasminogen activator inhibitor associated with tumor dissemination. *Cancer* **73**, 1398-405 (1994).
145. Kacinski, B.M. Tumor-stromal cytokine interactions in ovarian neoplasms. *Exs* **74**, 181-90 (1995).
146. Nordengren, J. et al. High tumor tissue concentration of plasminogen activator inhibitor 2 (PAI-2) is an independent marker for shorter progression-free survival in patients with early stage endometrial cancer. *Int J Cancer* **97**, 379-85 (2002).
147. Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. *Nat Rev Cancer* **6**, 392-401 (2006).
148. Ranson, M. & Andronicos, N.M. Plasminogen binding and cancer: promises and pitfalls. *Front Biosci* **8**, s294-304 (2003).
149. Ploug, M. Structure-function relationships in the interaction between the urokinase-type plasminogen activator and its receptor. *Curr Pharm Des* **9**, 1499-528 (2003).
150. Behrendt, N., List, K., Andreasen, P.A. & Dano, K. The pro-urokinase plasminogen-activation system in the presence of serpin-type inhibitors and the urokinase receptor: rescue of activity through reciprocal pro-enzyme activation. *Biochem J* **371**, 277-87 (2003).
151. Ra, H.J. & Parks, W.C. Control of matrix metalloproteinase catalytic activity. *Matrix Biol* **26**, 587-96 (2007).
152. Al-Ejeh, F., Croucher, D. & Ranson, M. Kinetic analysis of plasminogen activator inhibitor type-2: urokinase complex formation and subsequent internalisation by carcinoma cell lines. *Exp Cell Res* **297**, 259-71 (2004).
153. Lillis, A.P., Mikhailenko, I. & Strickland, D.K. Beyond endocytosis: LRP function in cell migration, proliferation and vascular permeability. *J Thromb Haemost* **3**, 1884-93 (2005).
154. Lund, L.R. et al. Plasminogen activation independent of uPA and tPA maintains wound healing in gene-deficient mice. *EMBO J* **25**, 2686-97 (2006).
155. Felez, J. et al. Characterization of cellular binding sites and interactive regions within reactants required for enhancement of plasminogen activation by tPA on the surface of leukocytic cells. *Thromb Haemost* **76**, 577-84 (1996).

156. Castellino, F.J. & Ploplis, V.A. Structure and function of the plasminogen/plasmin system. *Thromb Haemost* **93**, 647-54 (2005).
157. Aertgeerts, K., De Bondt, H.L., De Ranter, C.J. & Declerck, P. Mechanisms contributing to the conformational and functional flexibility of plasminogen activator inhibitor-1. *Nature Structural Biology* **2**, 891-897 (1995).
158. Jankova, L. et al. Crystal structure of the complex of plasminogen activator inhibitor 2 with a peptide mimicking the reactive center loop. *J Biol Chem* **276**, 43374-82 (2001).
159. Silverman, G.A. et al. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J Biol Chem* **276**, 33293-6 (2001).
160. Law, R.H. et al. An overview of the serpin superfamily. *Genome Biol* **7**, 216 (2006).

Acknowledgements

The work from the authors laboratories were funded by the National Health and Medical Research Council of Australia and the Cancer Institute NSW. DRC, MR and DNS are Cancer Institute NSW Fellows. The authors thank Professor David Ginsburg (Howard Hughes Medical Institute, University of Michigan Medical Center, MI, USA) for helpful discussions.

Table 1: PAI-2 expression and breast cancer prognosis.

Sample Size	Detection System	Prognostic Impact	Ref
80	ELISA	- PAI-2 expression was higher in carcinomas without lymph node involvement, in contrast to PAI-1 which was higher in carcinomas with node involvement.	125
314	ELISA	- Low levels of PAI-2 correlated with shorter disease free survival in overall population, menopausal women and node-negative patients. - Poor prognosis associated with a high PAI-1/low PAI-2 subgroup in the overall population and node-negative patients. - High uPA/low PAI-2 also indicative of poor prognosis in menopausal women.	65
1012	ELISA	- No association between PAI-2 expression and prognosis in the overall population. - Concurrently high uPA/PAI-2 expression associated with prolonged relapse free survival, metastasis free survival and overall survival.	116
50	RT-PCR	- Low PAI-2 expression was associated with lymph node involvement and correlated with high uPA/uPAR and PAI-1 levels.	142
170	ELISA, ICC	- PAI-2 expression was higher in carcinoma tissue than benign tissue. - High levels of PAI-2 expression were associated with prolonged disease free survival and overall survival. - PAI-2 expressed in both stromal and tumour cells.	134
73	ISH, IHC	- PAI-2 mRNA and protein expressed by tumour cells, fibroblasts, macrophages and lymphocytes. - PAI-2 (and PAI-1) positive cells predominantly located at the periphery of the invasive front of the tumour. - The presence of PAI-2 positive cancer cells was associated with prolonged overall survival. - PAI-2 expression in fibroblasts was associated with decreased lymph-node involvement but not associated with overall survival.	66
499	ELISA	- Correlation between PAI-2 expression and increased clinical tumour size, MSBR tumour grade and progesterone receptor expression. - High or very low PAI-2 expression was linked to prolonged disease free survival. - A dissemination risk index based on the opposing influences of PAI-1 and PAI-2 on uPA demonstrated the shorter disease free survival associated with the increasing ratio of PAI-1:PAI-2.	64
2780	ELISA	- PAI-2 expression associated with decreased lymph-node involvement and tumour size. - High PAI-2 expression is associated with prolonged relapse free survival and overall survival. - In multivariate analysis, PAI-2 is not associated with improved prognosis unless combined with uPA and PAI-1.	5
332	ELISA	- Low PAI-2 expression was associated with shorter relapse free survival in multivariate analysis. - Worst prognosis was associated with high PAI-1/low PAI-2 expression levels whilst low PAI-1/high PAI-2 was associated with favourable prognosis.	115
460	ELISA	- High levels of PAI-2 expression were associated with prolonged disease free survival. - Prognostic significance was increased when expression of PAI-1 and PAI-2 was combined.	141
130	RT-PCR, ELISA	- Low and very high levels of PAI-2 mRNA tended to be associated with prolonged disease free survival. - Levels of PAI-2 mRNA positively correlated with PAI-2 protein.	140
691	ELISA	- PAI-2 expression was associated with increased sensitivity to tamoxifen treatment, in contrast to uPA/uPAR/PAI-1,	122
148	ELISA	PAI-2 expression correlated with prolonged overall and disease free survival.	113

Figure 1. Proteolytic cascade regulating plasminogen activation at the cell surface. Schematic representation of the classical role of uPA, showing the assembly and regulation of the plasminogen activation proteolytic cascade via interactions with various cell surface co-receptors, inhibitors and ECM molecules. The serine protease uPA, bound to its specific cell surface receptor (uPAR), efficiently cleaves cell surface bound plasminogen zymogen at the Arg^{580}-Val^{581} amide bond, activating the broad spectrum serine protease plasmin[148,149]. Multiple plasmin/ogen receptor proteins have been identified[148] and uPAR is anchored to the plasma membrane outer leaflet via a glycosyl-phosphatidylinositol (GPI) moiety. In a feed-forward loop, activation of uPAR-bound pro-uPA to two-chain uPA via plasmin-mediated proteolytic cleavage facilitates further activation of additional co-localised plasminogen to plasmin. As receptor bound plasmin is refractory to inhibition by its circulating inhibitor α_2-antiplasmin (α2-AP), this cyclical positive feedback mechanism is highly effective in amplifying plasmin production[149,150]. Plasmin promotes tissue degradation and remodelling of the local extracellular environment directly, by degrading extracellular matrix molecules and activating/releasing latent growth factors[148,149]. Plasmin also potentially activates a limited sub-set of pro-matrix metalloproteinases (pro-MMPs) such as pro-MMP-2 and -9, though other activation mechanisms may be more relevant *in vivo*[151].The proteolytic activity of both soluble and receptor-bound uPA is efficiently inhibited by plasminogen activator inhibitors type-1 and -2 (PAI-1 and PAI-2)[10,152,153]. Upon uPA inhibition and formation of uPA-PAI complexes, uPAR/uPA-PAI associates with low-density lipoprotein receptor (LDLR) proteins, leading to endocytosis degradation of uPA-PAI complexes, and partial recycling of unoccupied uPAR to the cell surface[153].
Not shown: Plasminogen is also activated by plasma kallikrein[154] and tissue-type plasminogen activator (tPA)[10]. The activation of tPA is potentiated by co-binding to fibrin and several cell surface receptors/binding moieties[10,155,156].

Figure 2: The proposed mechanism of improved patient prognosis associated with high PAI-2 expression. Differing cell surface interactions may explain the disparity between PAI-1 and PAI-2 in cancer prognosis. (**A)** In tumours with low PAI-2 levels, PAI-1 contributes to poor patient prognosis through the stimulation of tumour vascularization, growth and metastasis. This is achieved through various complex interactions that increase both cell proliferation and migration. PAI-1 bound to vitronectin prevents cellular attachment via uPAR and integrins. However upon uPA inhibition, PAI-1 loses its affinity for vitronectin, freeing up vitronectin for binding by the now co-localised uPAR and integrins - initiating the rounds of cell attachment and de-attachment required for efficient cell migration[12]. Following uPA inhibition, uPA-PAI-1 binds with high affinity to members of the LDLR family, stimulating endocytosis, degradation of uPA-PAI-1, and partial recycling of the receptors. However, this interaction also generates other cell type- and receptor-specific responses. The interaction of uPA-PAI-1 with LRP causes a decrease in ERK phosphorylation and cell migration[94], although it may also cause a loss in cell adhesion due to the removal of integrins from the plasma membrane[13]. The interaction of uPA-PAI-1 with VLDLr stimulates sustained ERK phosphorylation and increases cell proliferation[17]. Additionally, PAI-1 can bind directly to LRP, inducing activation of the Jak/Stat pathway, leading to increased cell motility[99].
(B) High PAI-2 levels in tumours may contribute to good patient outcome solely via inhibition of uPA, which ultimately reduces invasive capacity by preventing plasmin-mediated ECM degradation and growth factor activation. While uPA-PAI-2 is cleared from the cell surface via interactions with both LRP and VLDLr, these are of lower affinity than uPA-PAI-1 due to the lack of a complete LDLR binding motif in PAI-2[7]. Therefore, unlike uPA-PAI-1, endocytosis of uPA-PAI-2 via VLDLr does not induce signaling events leading to cell proliferation[7]. Additionally, PAI-2 does not bind directly to LRP[6], therefore it is unable to induce cell migration through binding of this receptor. High PAI-2 levels also potentially compete with vitronectin-bound PAI-1 for uPA binding, preventing the removal of PAI-1 from vitronectin, and therefore decreasing vitronectin dependent cell migration.
Nb: Some interactions not directly involving PAI-1 or PAI-2 have been omitted for the sake of clarity.

Figure 3. Structural comparison of PAI-1 and PAI-2 receptor-binding interfaces showing position of key receptor binding residues. Comparison of structural characteristics of PAI-1 (PDB code 9PAI[157]) and PAI-2 (PDB code 1JRR[158]) in their relaxed conformations (i.e. mimicking the conformation in uPA–serpin complexes). Arg_{76}, Lys_{80} and Lys_{88} within and adjacent to helix D, along with Arg_{118} and/or Lys_{122} mediate binding of uPA:PAI-1 to LRP and VLDLr[77,78], with Arg_{76} forming part of a cryptic high-affinity binding site for LRP exposed by complex formation with uPA[79]. These residues conform with the proposed common binding motif for LRP ligands[80] but this motif is not conserved in PAI-2. The corresponding residue to Arg_{76} in PAI-1 is conserved in PAI-2 (Arg_{108}) but the residue corresponding to Lys_{80} is replaced by Ser_{112} in PAI-2 and the adjacent hydrophobic residue is not conserved. Further, there are clear differences in the surface topography and overall electrostatic charge between PAI-1 and PAI-2. (**A and B**) Ribbon diagram showing secondary structure and key binding residues around α-helix D of PAI-1 and PAI-2. (**C** and **D**) Surface representation showing regions of positive electrostatic potential in blue, negative potential in red, and neutral regions in white. (**E**) Alignment of helix D amino acid sequence from PAI-1 and PAI-2. The putative minimal binding motif[80] in PAI-1 is underlined with basic and hydrophobic residues highlighted in yellow and blue respectively.

Box 1. Nomenclature and structure of serpin genes and proteins.

The serpins are a large, broadly distributed family of structurally similar but functionally diverse proteins, with over 1500 members in many phyla (including animals, plants, bacteria and virus). A comprehensive review and phylogenetic analysis of the serpin gene family led to the identification of 16 clades (A-P) and construction of a systematic nomenclature that is now becoming more widely used[159].

Most serpins function as inhibitors of serine proteases but some have activity against cysteine proteases and there are rare examples of non-inhibitory functions including hormone transport, molecular chaperone activity and chromatin condensation. Demonstrated physiological roles of serpins are diverse and include regulation of fibrinolysis, apoptosis, tumour suppression, inflammation, development, and blood pressure regulation. Numerous examples of mutation or altered expression of serpins have been described with various pathological consequences (so-called "serpinopathies"), including emphysema, hypertension, thrombosis, liver disease, metastasis, and dementia. A comprehensive database[1] of serpin mutations is available at the Structural Medicine Lab at the Cambridge Institute for Medical Research.

The structural biology of serpins is quite unique (refer Figure 3) and has been studied intensely (over 70 solved structures in the RCSB Protein Data Bank[2]). The native structure of serpins is highly conserved (consisting of 3 β-sheets and 7-9 α-helices) (Smart:SM00093[3], Pfam:PF00079[4]) and instead of folding into the most stable conformation, serpins folding into a metastable state that has been likened to a form of "molecular mousetrap" (refer Box 2). In this state, the flexible reactive centre loop (RCL) is extended as a kind of "bait" for the target protease. Many of the pathological serpin mutations have been shown to render the inhibitors inactive by causing misfolding or polymerization of mutant proteins (for detailed review see Whisstock and Bottomley, 2006 *Current Opinion in Structural Biology*[55]). For a more detailed overview of serpin biology, refer to Law et al 2006 *Genome Biology*[160] and the Whisstock Lab serpin page[5]

[1] http://www-structmed.cimr.cam.ac.uk/Serpins/serp_regions/table2.html

[2] http://www.pdb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb53_report.html

[3] http://smart.embl.de/smart/do_annotation.pl?DOMAIN=SM00093

[4] http://pfam.sanger.ac.uk/family?acc=PF00079

[5] http://en.wikipedia.org/wiki/Serpin

Box 2.

For a detailed description of the serpin inhibitory mechanism refer to Huntington et al 2000 *Nature*[38]; Whisstock & Bottomley 2000 *Current Opinion in Structural Biology*[55]) and this Movie[6] of serpin inhibitory mechanism from the Structural Medicine Lab at the Cambridge Institute for Medical Research.

[6] http://huntingtonlab.cimr.cam.ac.uk/Movies/serpin_mech05.mov

Figure 1

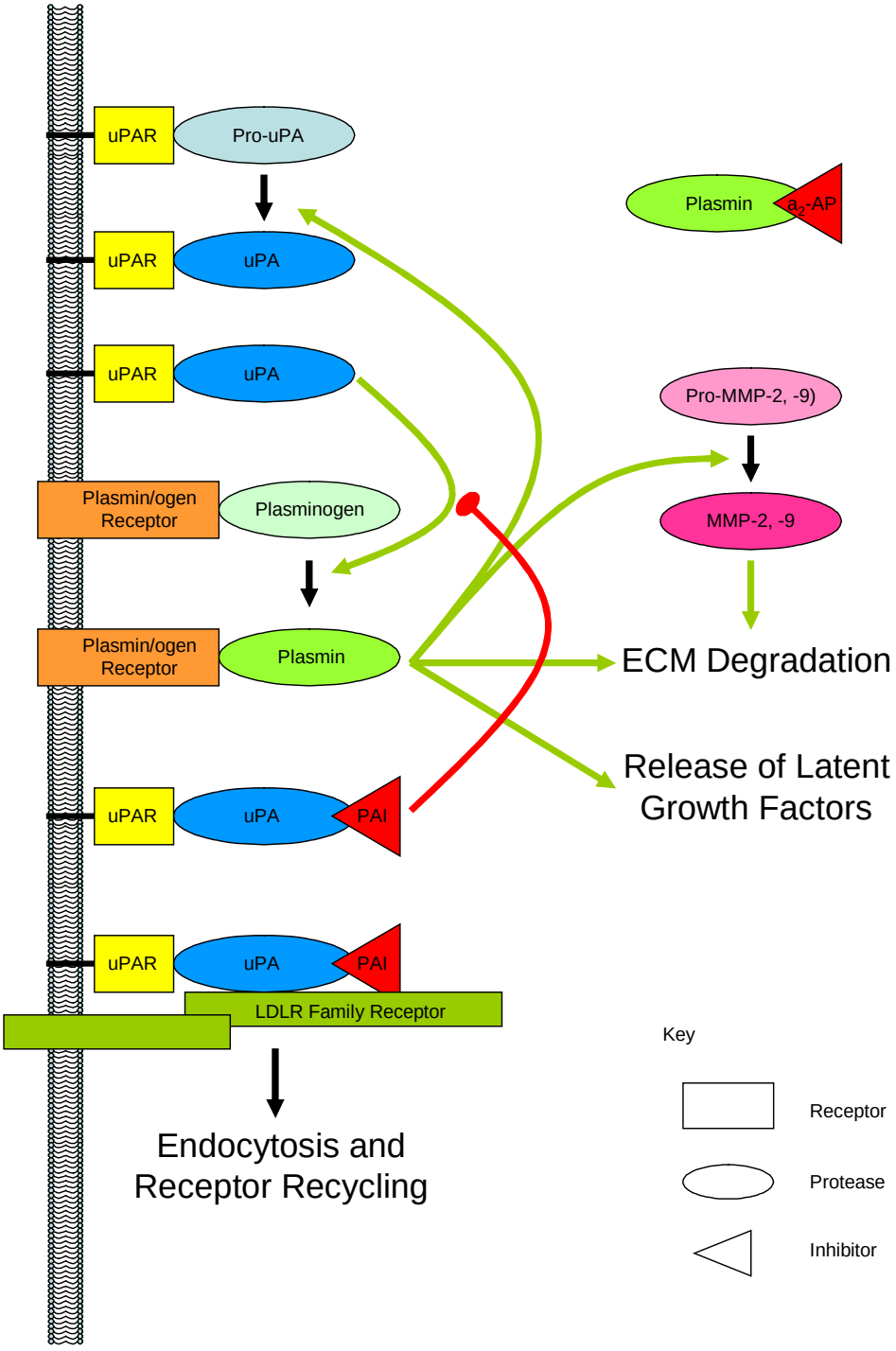
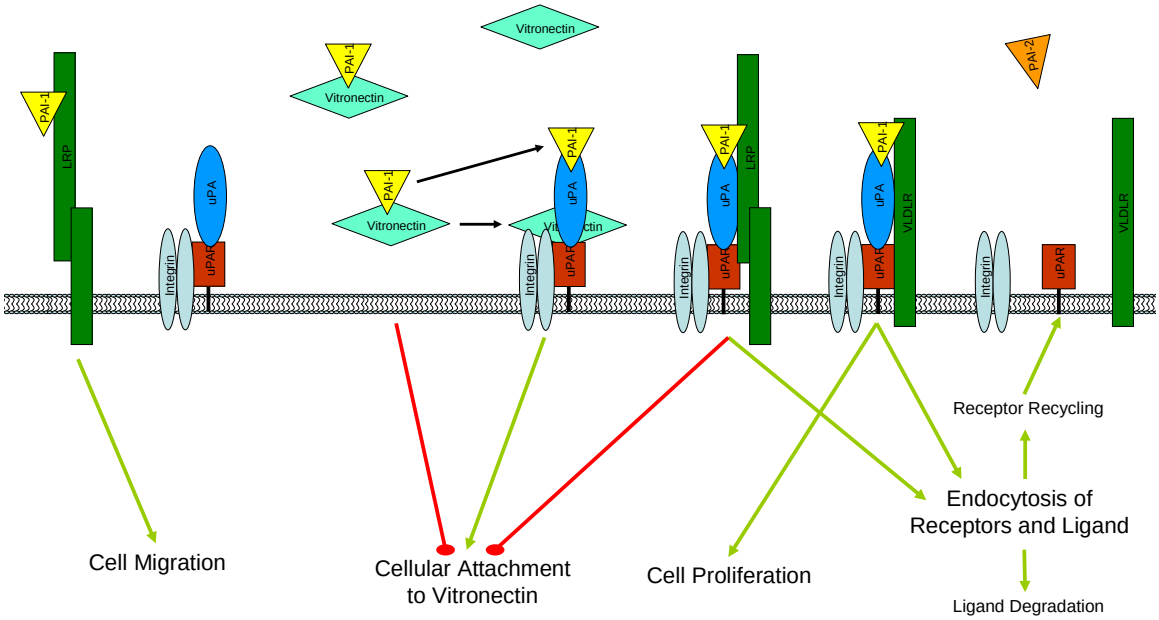

uPAR
Pro-uPA
uPAR
uPA
uPAR
uPA
Plasmin/ogen Receptor
Plasminogen
Plasmin/ogen Receptor
Plasmin
uPAR
uPA
PAI
uPAR
uPA
PAI
LDLR Family Receptor
Endocytosis and Receptor Recycling
Plasmin
a2-AP
Pro-MMP-2, -9)
MMP-2, -9
ECM Degradation
Release of Latent Growth Factors
Key
Receptor
Protease
Inhibitor

Figure 2

a Low PAI2



(**B**) High PAI-2

Overall effect – uPA inhibition and clearance, reduced invasion and metastasis

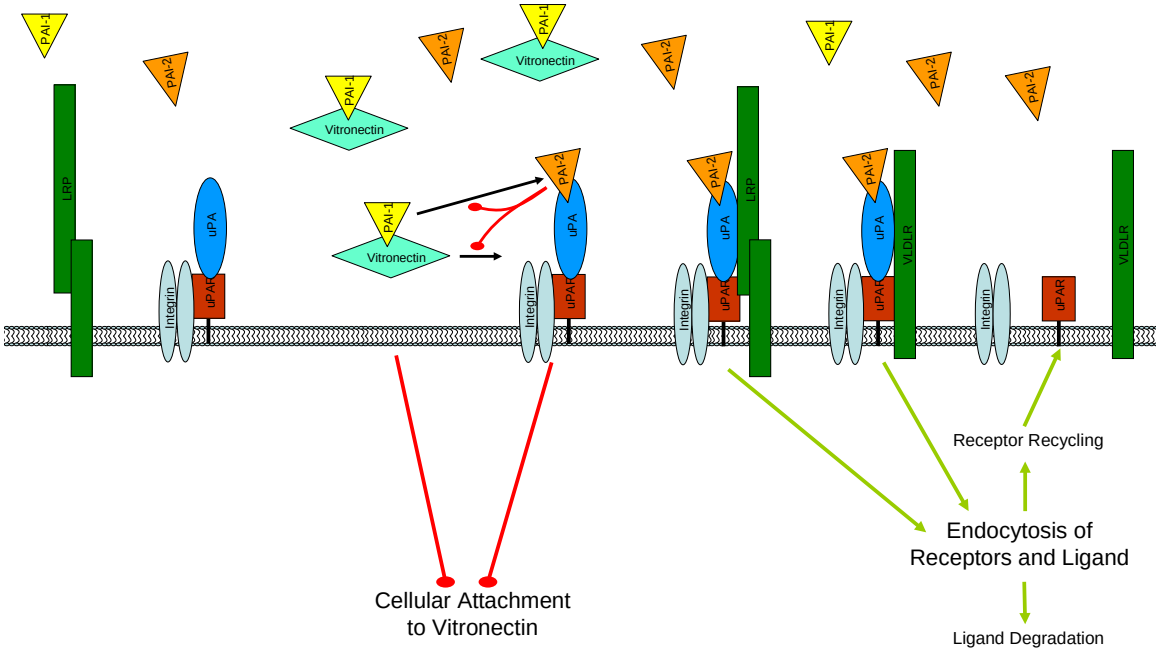
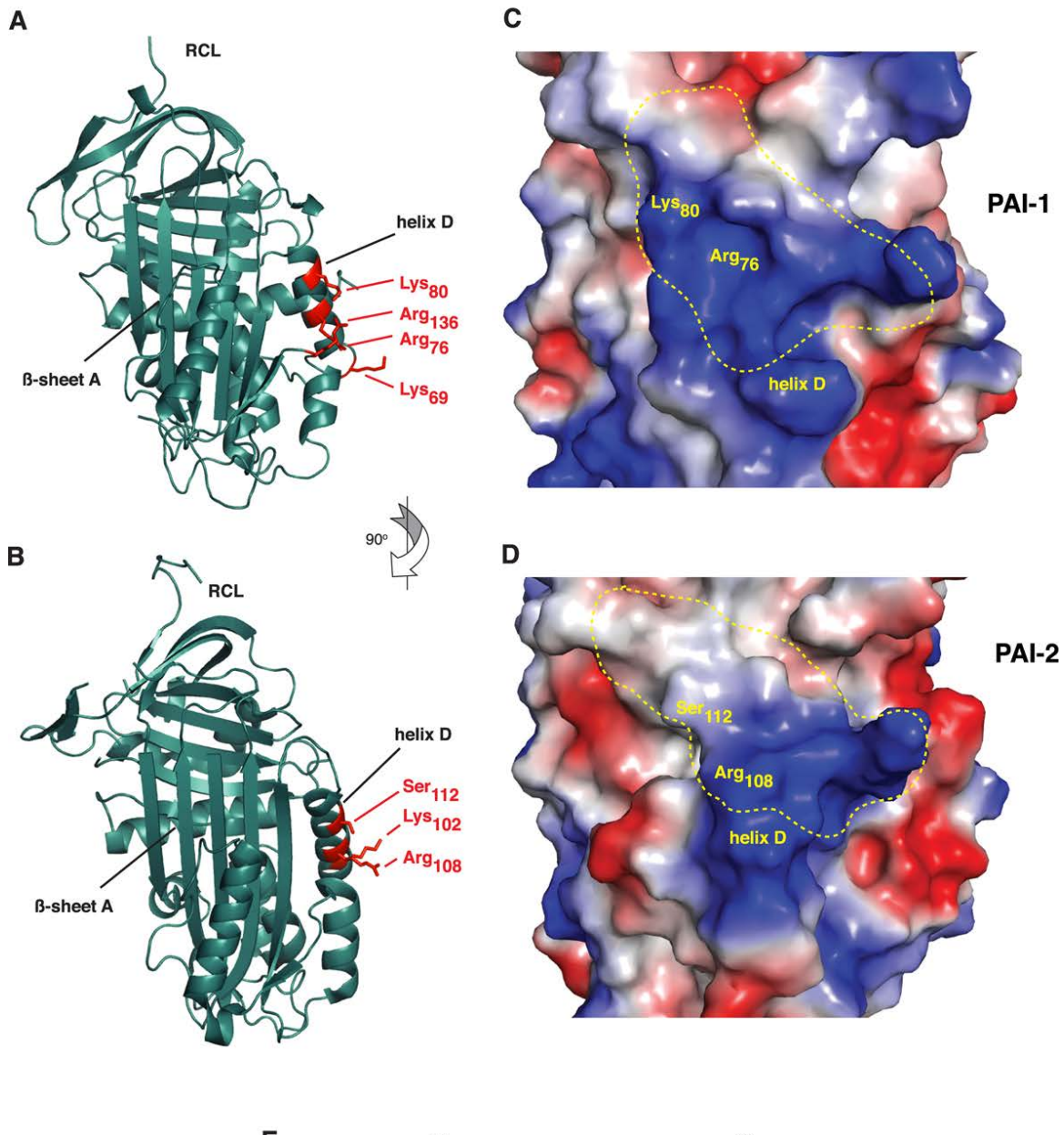


Figure 3



E

```
          69                  88
PAI-1   KGMAPALRHLYKELMGPWN
PAI-2   DKIHSSFRSLSSAINASTG
        101                 119
```