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Theme Issue Article

Structure and function of the plasminogen/plasmin system

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Summary

Activation of the fibrinolytic system is dependent on the conversion of the plasma zymogen, plasminogen (Pg), to the serine protease plasmin (Pm) by the physiological activators urokinase-type Pg activator (uPA) or tissue-type plasminogen activator (tPA). The primary *in vivo* function of Pm is to regulate vascular patency by degrading fibrin-containing thrombi. However, the identification of Pg/Pm receptors and the ability of Pm to degrade other matrix proteins have implicated Pm in other functions involving degradation of protein barriers, thereby mediating cell migration, an important event in a number of normal e.g., embryogenesis, wound healing, angiogenesis, and pathological, e.g., tumor growth and dissemination, processes. Prior to the development of Pg-deficient mice, much of the evidence for its role in other biological events was based on indirect studies. With the development and characterization of these mice, and ability to apply challenges utilizing a number of animal models that mimic the human condition, a clearer delineation of Pg/Pm function has evolved and has contributed to an understanding of mechanisms associated with a number of pathophysiological events.

Keywords

Plasminogen, fibrinolysis, kringle domains, gene targeting, cell invasion

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Introduction

Activation of the fibrinolytic system is dependent on the conversion of the plasma zymogen, plasminogen (Pg), to the serine protease plasmin (Pm) by the physiological activators urokinase-type Pg activator (uPA) or tissue-type plasminogen activator (tPA). The primary *in vivo* function of Pm is to regulate vascular patency by degrading fibrin-containing thrombi. However, the identification of Pg/Pm receptors and the ability of Pm to degrade other matrix proteins have implicated Pm in other functions involving degradation of protein barriers, thereby mediating cell migration, an important event in a number of normal ones e.g., embryogenesis, wound healing, angiogenesis, and pathological, e.g., tumor growth and dissemination, processes. Prior to the development of Pg-deficient mice, much of the evidence for its role in other biological events was based on indirect studies. With the development and characterization of these mice, and ability to apply challenges utilizing a number of animal models that mimic the human condition, a clearer delineation of Pg/Pm function has evolved and has contributed to an understanding of mechanisms associated with a number of pathophysiological events.

Structure and activation of plasminogen

While human Pg is synthesized as a 810-amino acid polypeptide protein, the mature form of this protein is 791-amino acids, due to cleavage of a 19-amino acid leader peptide during secretion (1). The liver is the primary tissue that synthesizes Pg (2–4); however, other sources have been identified that include adrenal glands, kidney, brain, testis, heart, lung, uterus, spleen, thymus, and gut (5). Posttranslational modification of this protein consists of Asn^{289}-(6), Thr^{346}-(7), Ser^{248}-(8), Thr^{339}-(9) linked glycosylation, and O-linked phosphorylation (10).

A view of the human Pg molecule is presented in Figure 1, sequentially from the amino-terminus to the carboxyl-terminus, with emphasis on its domain organization. Within the heavy chain of human Pm (residues Glu^{1}-Arg^{561}) are 5 kringle (K) domains, which are triple-disulfide linked peptides of approximately 80 amino acids (11). These modules, which interact with

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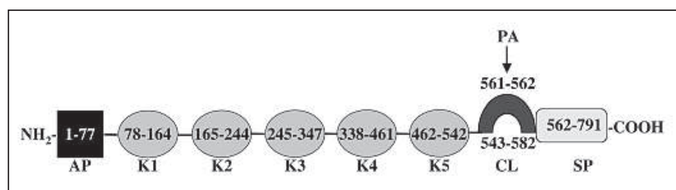

Figure 1: The modular structure of human Pg. The structural and functional domains of human Pg are highlighted. From the amino-terminus of the mature protein, the residue numbers of the domains are represented. The 77-residue activation peptide (AP) is followed by 5 consecutive kringle (K) domains, with introns serving as the boundary determinants. These regions are also known to fold independently. Similarly, the cleavage (activation) loop (CL) is depicted (residues 543–582), bounded by its gene introns. Pg activators catalyze cleavage at the peptide bond of residues Arg^{561}-Val^{562}, leading to Pm formation. The functional serine protease (SP) domain (562–791) overlaps with the CL since its amino-terminus begins at residue Val^{562}.

lysine-like ligands, are not unique to Pg, and have been identified in other proteins associated with hemostasis (12–15), as well as apolipoprotein (a) (16). The kringle domains facilitate Pg binding to large substrates, e.g., fibrinogen (17), bacterial proteins (18–20), and mammalian cell surfaces (21), as well as small molecule ligands, e.g., Cl^- and α,ω-amino acids, interactions that also regulate the activation of this protein (22, 23). Combinations of Pg kringles also serve independent functions, especially as angiogenic agents (24) and inhibitors of cell migration (25, 26).

The presence of distinct carboxyl-terminal lysine-residues, and cryptically-conformed pseudo-lysine residues, on human Pg binding proteins in solution and on cells, greatly influences the activation rates of Glu^1-Pg. Because many nonsubstrate ligands that interact with Pg kringles are differentially inhibited by lysine and many of its analogues, and the nature of these inhibitions are believed to reflect the specificity of the binding pocket of the kringles, a large investment of efforts by several laboratories has been focused on interactions of isolated kringle domains with a variety of α,ω-amino acids. Interactions of human Pg or its isolated kringles with lysine-type ligands takes place with all kringles, except for K3. The K1 and K4 modules exhibit the strongest affinities for this class of ligands (27–30), and K2 possesses the weakest affinity (31). The nature of the ligand is critical to its binding strength. K5 presents the tightest binding kringle to alkylamine-type ligands, providing a different specificity to Pg binding interactions (32). On the other hand, a strong specificity for K2 exists in the binding of Pg to a endo-polypeptide (VEK-30) that is derived from a bacterial cell wall Pg binding M-protein (PAM) (20).

The conversion of human Pg to Pm involves cleavage of Arg^{561}-Val^{562} resulting in the generation of Glu^1-Pm, which contains an N-terminal heavy chain of 561-amino acids and a disulfide-linked carboxy-terminal light chain of 230-amino acids. The catalytic triad is located in the light chain and consists of His^{603}, Asp^{646}, and Ser^{741}. Another proteolytic reaction that has physiological relevance is the Glu^1-Pm and Lys^{78}-Pm-catalyzed hydrolysis of the N-terminal 77 amino acids of Glu^1-Pg or Glu^1-Pm, converting Glu^1-Pg to Lys^{78}-Pg and Glu^1-Pm to Lys^{78}-Pm (33). The properties of the different types of Pg activators are described in another chapter of this series.

Tight (T)- and relaxed (R)-conformations of Glu^1-Pg and Lys^{78}-Pg and the role of the AP in maintenance of the conformational states of Pg

Dramatic differences exist in the conformations of Glu^1-Pg and Lys^{78}-Pg that are highly influential to their functions. Glu^1-Pg exists in a closed T-conformation that is regulated by the presence of the AP region of Pg. Somewhat different compact conformations of human Glu^1-Pg have been proposed, based on electron microscopy (34) and small angle electron scattering (35). Additional structural models of Glu^1-Pg have been forwarded that differ in detail (36), and the discrepancies will not be resolved until the X-ray crystal structure of the protein is determined. All models taken together suggest that Glu^1-Pg in Cl^--containing buffers exists in the T-conformation. On the other hand, Lys^{78}-Pg presents a more open R-conformation, and models have also been proposed for these structures based on chemical cross-linking and electron microscopy (34), and low-resolution transport methods (37). This more open conformation of Lys^{78}-Pg is also associated with increases in the intrinsic fluorescence and differences in sedimentation velocity behavior of the protein (38). The secondary structures of both Glu^1-Pg and Lys^{78}-Pg are very similar (39), suggesting that little change in the secondary structure is associated with large tertiary conformational changes. Thus, differences exist in the spatial relationships of the individual domains between the T- and R-forms of Pg, while the structures of the domains themselves remain constant. This model makes much sense considering the rather large alterations that occur by single site-specific binding of small ligands at low concentrations. The domains, themselves, are not conformationally altered, but their interactions are substantially changed so as to affect the overall conformation in a major way.

This laboratory has studied relationships between Pg conformation and activation for a number of years, and the following summary of the T- and R-conformations of Pg and their activation properties is offered. The T-conformation is adopted only in Glu^1-Pg in the presence of Cl^-, and the R-state occurs in Glu^1-Pg + ω-amino acids of the lysine class, e.g., EACA, as well as in Lys^{78}-Pg ± EACA. An intact sample of Glu^1-Pg, in the presence of Cl^- and absence of EACA derivatives (the T-state), is severely attenuated in its activation by Pg activators, but the R-state is highly activatible (38).

Organization of the plasminogen gene

The human Pg gene has been mapped to chromosome 6q26–6q27 and is 52 kb (40). The gene consists of 19 exons (75–387 bp) and 18 introns. There is a 57 bp signal sequence and the remaining coding sequence (2,373 nucleotides) comprises the mature protein. A nucleotide sequence (CTGGGA) that is upstream of the signal initiation codon (Met^{-19}) is similar to that for acute phase reactant proteins e.g., fibrinogen (41). Recognition sites for hepatocyte-enriched HNF-1 and AP-3 have also been identified and these sequences regulate liver specificity and transcription of the gene (42). Other potential regulatory sites are

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also evident, e.g., IL-6 (43). This was later confirmed and a 1,067 bp sequence proposed as the IL-6 responsive site (44).

Plasminogen receptors on cells

The expression of Pg receptors on a number of cell surfaces allows for localized activation of Pg, which can facilitate cell migration by degrading surrounding barrier matrix proteins. This is evident in processes involving macrophage recruitment during inflammation (45), tissue remodeling and wound healing (46–48), as well as tumor invasion and metastasis (49). Other functions mediated by the interaction of Pg with cell surface-expressed receptors involve localized clot lysis, prohormone processing (50), and stimulation of cell signaling pathways (51–54).

Pg receptors are expressed on a broad range of prokaryotic and eukaryotic cells (55–59). Additionally, enhanced binding is also observed on apoptotic and nonviable/necrotic monocytoid cells (60, 61), nonviable epithelial cells (62), necrotic breast cancer cells (49), and damaged amniotic epithelial cells (63). The Kd associated with this interaction is approximately 1 μM (64), and, based on the concentration of Pg in plasma (2.2 μM) (65), there is more than 50% occupancy of receptor in plasma and interstitial fluid (21, 66). Pg receptors are abundant on cell surfaces (37,000 sites/platelet – >10^7 sites/endothelial cell) (21), and are not limited to a single class of molecules (21, 67). Lysine, lysine analogues, and peptides with carboxyl-terminal lysines effectively block Pg interactions with cell surfaces (67). Therefore, free lysine binding sites within the kringle domains of Pg support these interactions.

The specific class of Pg receptor is cell-type specific and 5 subsets have been identified. One type has carboxyl-terminal lysines when synthesized and promotes Pg activation e.g., α-enolase (67–69). A second subset promotes Pg activation, does not endogenously express carboxyl-terminal lysines when synthesized, but does express them when exposed to the cell surface, e.g., annexin II (70). Another subset of sites promotes Pg activation, does not express carboxyl-terminal lysines when synthesized, and expression once exposed to the cell surface has not been investigated, e.g., $\alpha_M\beta_2$ on neutrophils (71) and actin on endothelial cells (72). A fourth subset of cellular receptors binds Pg but does not promote its activation, e.g., Tissue Factor (73). A final identified subset of Pg receptors, e.g., integrins $\alpha_M\beta_2$ and $\alpha_5\beta_1$, interact in a lysine-dependent fashion with Glu^1-Pg deposited in the extracellular matrix, and plays a role in cellular-adhesion (74).

As a result of binding of Glu^1-Pg to cells, its activation is substantially enhanced compared to the reaction in solution (55, 75–78). The majority of cells that bind Pg also express cell-surface receptors for the Pg activators, uPA (uPAR) and tPA (contains a number of cellular binding sites) (71, 79).

Colocalization of Pg activator and Pg on cell surfaces serves to increase the local concentrations of reactants. A requirement for the interaction of Pg with the cell surface in the enhancement of Pg activation has been demonstrated in studies with lysine analogues, which interact with the Pg kringles to block its binding to many types of cells. Further, inactivation of cellular binding sites by treatment of cell surfaces with carboxypeptidase-B reduces the enhancement of Pg activation on cell surfaces (77).

Human Pg activation on cells

When the binding modality of human Glu^1-Pg to cells is *via* lysine binding sites of kringles, as is the usual case, Glu^1-Pg could be placed in a highly activatible R-conformation, and the same mechanisms as above could be responsible for this stimulation, since Glu^1-Pg would adopt both the conformation and increased activatibility of Lys^{78}-Pg. It is known that the lysine binding sites of human Pg, *viz.,* K1, K4, and K5, control the change of Glu^1-Pg to a Lys^{78}-Pg-like conformer (80), and these kringle domains, as well as the K1–3 motif, also modulate the interactions of human Pg with cells (81). One possible mechanism for enhanced activation rates of Pg on cells is that the lysine binding sites within the kringle domains of Glu^1-Pg interact with carboxyl-terminal lysine residues of proteins on cell surfaces to place Glu^1-Pg in the activatible R-conformation. However, it is unlikely that the situation is quite so simple since Pg binding to at least one bacterial cell protein is controlled by the K2 domain (20), which has not been studied in terms of its ability to modulate the T- to R-alterations in human Pg.

Another mechanism that functions in cell-based stimulation of human Pg activation is the enhancement of the proteolytic conversion of Glu^1-Pg to Lys^{78}-Pg on cells, as compared to solution-based rates, followed by the more rapid conversion of Lys^{78}-Pg to Lys^{78}-Pm, relative to that of Glu^1-hPg. Thus, in this mechanism, the cell surface promotes the release of the AP by Pm catalysis, perhaps *via* concentration of reagents and/or by providing a R-conformation susceptible to plasminolysis of the Lys^{77}-Lys^{78} peptide bond in Glu^1-Pg. Some solid evidence has been obtained with certain cells for this latter mechanism (82), but there is no reason to believe that mechanism 1, above, would not be functional under certain conditions with certain cells and/or with soluble effector proteins.

Plasminogen deficiency in humans

The first documented abnormal human Pg (Pg-Tochigi) was reported over 25 years ago in a patient heterozygous for a Pg deficiency with a history of thrombotic events. Normal Pg antigen levels were present in the plasma of this individual and the functional Pm activity was approximately 40% (83). Additional studies of the purified Pg from this patient indicated an active site defect, the result of a transition in exon XV of Pg, generating an $Ala^{601}\rightarrow$Thr substitution near the active site His^{603} of the catalytic triad (84, 85). This protein can be converted to two-chain Pm, but is functionally inactive. *In vitro* studies involving the generation of recombinant human Pg with a $Pro^{611}\rightarrow$Ile mutation near the active His^{603} amino acid resulted in a similar effect (personal observation). This 601 Type-1 mutation appears at a high frequency (2%) in the Japanese population. Many of these patients suffer from a history of venous thrombosis but some remain asymptomatic. Within the past 5 years a few patients with homozygous Type-1 Pg deficiency have been reported (86, 87). A common pathology in these patients is ligneous conjunctivitis or pseudomembranous disease. Patients with other homozygous or compound heterozygous mutations also present with ligneous conjunctivitis and in some cases occlusive hydrocephalus (88, 89). Since there is a limited number of reported human Pg de-

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fects and clinical manifestations are variable, defining a role for Pg in a number of physiological and pathophysiological processes is limited. Therefore, mice with a deficiency of this protein have become valuable resources for delineating, *in vivo*, its relative importance in a number of biological processes.

Spontaneous phenotypes associated with a total plasminogen deficiency in mice

Two separate approaches were utilized to generate mice with a total deficiency in Pg (*PG$^{-/-}$*). In one, a segment of the catalytic domain (intron 14–16) was replaced with the neomycin resistance gene (90). The other approach involved replacement of proximal promoter sequences, the signal peptide, and 43 residues of the activation peptide with the phosphoglycerate kinase promoter -*HPRT* gene (91). A complete Pg deficiency in mice was not lethal and these mice survived well into adulthood. Some spontaneous phenotypes that were observed included diminished growth rates, spontaneous fibrin deposition in a number of organs, gastric and colonic ulcerations, rectal prolapse, and fibrin-dependent ligneous conjunctivitis. This latter pathology was strain and age-sensitive.

Phenotypes observed after challenge in mice deficient for Pg

Fibrinolytic capacity

Pulmonary clot lysis in *PG$^{-/-}$* mice was determined by measuring the spontaneous lysis of a radiolabeled plasma clot after its injection into the jugular vein. Relative to WT mice, clot lysis was severely compromised in *PG$^{-/-}$* mice, with an intermediate reduction of fibrinolytic capacity in *PG$^{+/-}$* mice (90). When *PG$^{-/-}$* mice were administered an i.v. bolus of Pg, normal clot lysis and dissolution of spontaneous fibrin deposits in the liver were observed, indicating that *in vivo* clot lysis is dependent on the presence of Pg (92). Additional studies have indicated that polymorphonuclear leukocytes may compensate for a lack of Pg in the degradation of fibrin(ogen) (93).

Vascular injury/repair

A number of indirect investigations have indicated that Pg plays a critical role in events involved in vascular remodeling. With the availability of mice deficient for components of the fibrinolytic pathway, several vascular injury challenges in these gene deficient mice have been developed (94–96). In two separate studies, arterial injuries utilizing mechanical and electrical inducers in *PG$^{-/-}$* mice resulted in a delay in removal of necrotic tissue and fibrin, impaired wound healing, and reduced inflammatory cell and smooth muscle cell accumulation relative to WT mice (97, 98). Other more clinically relevant models of vascular injury/repair have been performed in *PG$^{-/-}$* mice. As an example, a model of arterial graft-*versus*-host disease demonstrated a similar phenotype in *PG$^{-/-}$* mice (99). However, in a vein patch model, no differences were observed in neointima formation between WT and *PG$^{-/-}$* mice (100). Finally, in a model of atherosclerosis, accelerated lesion formation in the proximal and distal aorta was observed in *APOE$^{-/-}$/PG$^{-/-}$* double deficient mice, relative to mice with a single deficiency of apoE (101). The method of injury, as well as the location and local environment, may be contributing factors to the discrepancies observed in these various models.

Pathogen susceptibility and inflammation

Based primarily on a number of *in vitro* studies, the Pg system has been implicated in events associated with the inflammatory process. Pm can release interleukin-1 from macrophages and activate transforming growth factor-β (TGF-β) (102, 103). In *PG$^{-/-}$* mice a significant attenuation of macrophage recruitment was observed after peritoneal stimulation with thioglycollate (45). Additionally, a recent study assessing the inflammatory response to implanted biomaterials demonstrated that neutrophil and macrophage recruitments were attenuated in *PG$^{-/-}$* mice compared to WT mice (104). Additionally, while recruitment to the biomaterial implant site was attenuated in these mice, adhesion was not.

Host Pm associated with the surface of a pathogen can facilitate invasion by mediating tissue barrier degradation and dissemination of the invading organism. Several studies have utilized *PG$^{-/-}$* mice in pathogen-challenge models and have compared the responses to WT mice. *PG$^{-/-}$* mice infected with *Yersinia pestis,* the causative pathogen for plague, demonstrated an enhanced resistance to this organism relative to WT mice (105). Additionally, a lack of host Pg during relapsing *Borrelia* fever and infection by *Borrelia crocidurae* resulted in decreased spirochetal burden in the hearts, brains, and kidneys of these mice compared to WT mice (106, 107). Mice infected with the malaria strains, *Plasmodium falciparum* and *Plasmodium chabaudi,* showed no difference on the erythrocytic life cycle in the absence or presence of Pg (108). These results were surprising since uPA has been identified in erythrocytes that have been infected with these two strains of parasites and inhibitors and antibodies to uPA attenuate the parasite-mediated rupture of erythrocytes. This would implicate a role for uPA that is not Pg activation-dependent (109). However, *PG$^{-/-}$* mice infected with Group A *streptococcus* (CS101) demonstrated a reduced virulence relative to WT mice (110). Therefore, Pg plays a role in the virulence of this pathogen and supports *in vitro* observations which demonstrated that human plasma incubated CS101 acquires Pm activity on its surface (19). It was also demonstrated that the a1a2 repeats of PAM, a M-like surface protein present in Group A *streptococci*, is the interactive component for human Pg and its binding is mediated through the K2 domain of Pg (20). Murine Pg is insensitive to streptokinase and recently, a transgenic line of mice was developed that express, exclusively, human Pg (111). These mice, when infected with *streptococci*, demonstrated increased mortality and increased bacterial dissemination, strongly implicating the Pg system in this process.

Infection of *PG$^{/-}$* mice, as well as mice deficient in Pg activators, with *Mycobacterium avium* showed that granulomas in *PG$^{-/-}$* mice had increased contents of fibrinogen and fibronectin, as well as neutrophils, suggesting that Pm functioned in turnover of the extracellular matrix within granulomas, likely limiting the fibrotic response in these lesions (112).

These *in vivo* studies support *in vitro* observations related to the role of Pg in pathogen invasion and dissemination.

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Glomerulonephritis

One of the hallmark features of severe proliferative and crescentic glomerulonephritis is deposition of fibrin in the glomerulus, which can ultimately result in renal failure (113). Additionally, alterations in the fibrinolytic system have been demonstrated in both experimental and human forms of this disease (114–116). In order to more directly determine a role for Pg in GN, proliferative glomerulonephritis was induced in WT and *PG*$^{-/-}$ mice (117). Results from this study indicated that progressive glomerulonephritis with renal impairment developed in both WT and *PG*$^{-/-}$ mice but was severely exacerbated in deficient mice. *PG*$^{-/-}$ mice presented with enhanced extracellular matrix and fibrin deposition in the glomerulus, implicating a protective function for Pg in suppressing an acute inflammatory injury to the glomerulus.

Rheumatoid arthritis

Rheumatoid arthritis is an autoimmune disease involving chronic inflammation of the synovia which ultimately progresses to the destruction of cartilage and bone. By regulating inflammation, fibrin deposition, synovial cell infiltration into the surrounding cartilage, as well as cartilage remodeling in the joint (118, 119), Pg could play a critical role in this and other inflammatory joint diseases. To more directly assess the relative importance of Pg in inflammatory joint disease, *PG*$^{-/-}$ and WT mice were challenged utilizing a murine model of antigen-induced arthritis (120). During the early stages of the disease, inflammation in the joint was comparable between these mice. However, at a later time (30 days), inflammation was significantly elevated in *PG*$^{-/-}$ mice. Synovial thickness subsided in WT mice but remained elevated up to 2 months in *PG*$^{-/-}$ mice. At 30 days, *PG*$^{-/-}$ mice developed bone erosion and fibrin deposits in the arthritic joints. By regulating fibrin accumulation in the joints and resulting bone degradation, Pg plays a protective role in arthritis.

Pulmonary fibrosis

Impaired fibrinolytic activity is an underlying feature in the development of pulmonary diseases (121–123). The chemotherapeutic agent, bleomycin, has been used to induce pulmonary fibrosis in animal models, and a number of these studies evaluated a role for fibrinolysis in this disease. Mice that overexpress PAI-1 and are administered bleomycin demonstrate enhanced collagen deposition relative to WT mice; however, collagen deposition in *PAI-1*$^{-/-}$ mice was similar to WT mice (124). Administration of uPA to bleomycin-treated rats and mice demonstrated a beneficial effect that resulted in attenuated pulmonary collagen accumulation (125, 126). In *PG*$^{-/-}$ mice, enhanced collagen deposition was observed after bleomycin-treatment relative to control WT mice (127). However, while fibrin deposition is a feature of pulmonary disease, similar studies in fibrinogen-deficient (*FG*$^{-/-}$) mice indicated that its presence was not critical for fibrotic lesion formation (128).

Wound healing

Some of the key events associated with skin wound healing are the formation of a temporary fibrin-rich matrix, recruitment of inflammatory cells, proliferation and migration of keratinocytes at the edges of the wound, and the formation of granulation tissue. Components of the fibrinolytic system have been implicated in a number of these events. Functional overlap of this proteolytic system and the matrix metalloprotease system has been identified in skin wound healing processes and inhibition of both pathways leads to a complete arrest of the wound healing process (129). Studies with *PG*$^{-/-}$ mice demonstrated impaired keratinocyte migration resulting in a delay in skin wound healing relative to WT mice (46), while investigations with *PG*$^{-/-}$/*FG*$^{-/-}$ mice indicated that altered migration of these cells from the wound edges was associated with an inability to degrade the provisional fibrin matrix associated with the wound (130). Other wound healing studies in *PG*$^{-/-}$ mice (131, 132) support observations made in the skin wound healing studies. These phenotypes were also resolved in *PG*$^{-/-}$/*FG*$^{-/-}$ mice. *PG*$^{-/-}$ mice also demonstrated inability to efficiently remove necrotic tissue and heal wounds in myocardial infarction (48) and liver injury/repair (133). In the liver injury model, the wound healing process was not fibrinogen-dependent. However, an inability to effectively eliminate protein matrix barriers was a common observation in these studies and underscores the importance of a balanced fibrinolytic system in wound healing events.

Neuronal and axonal degeneration and other neurologic-related processes

The hippocampus is a region of the brain that is particularly sensitive to neuronal death. A co-localization of Pg and its activator, tPA, in the hippocampus has been identified, and, therefore, these proteins could potentially mediate neuronal degeneration (134). In order to determine a role for Pg in regulating neuronal degeneration, an excitoxin-induced neuronal injury model was used in *PG*$^{-/-}$ mice to determine if these mice were protected (135). For this study, a glutamate analog, kainite, was injected into the hippocampus and neuronal survival was determined after 5 days. The results from this study indicated that *PG*$^{-/-}$ mice were resistant to excitoxin injury compared to WT controls. A similar resistance to excitotoxin-induced injury was observed in kainate-treated WT mice that were injected with α_2-antiplasmin, confirming the role of Pm in neuronal survival (135). Studies with *PG*$^{-/-}$/*FG*$^{-/-}$ indicated that laminin is the target substrate involved in excitoxin-induced neuronal death and that Pm disrupts critical neuron-laminin interactions (136, 137). Another neurological injury model involved injury to the sciatic nerve with resultant axonal demyelination (138). In *PG*$^{-/-}$ mice, axonal demyelination was enhanced, compared to *WT* control mice, paralleled by an increase in fibrin deposition. In contrast to neuronal survival studies with kainite, fibrin was the Pm substrate that affected the axonal degenerative process (138).

Other studies have indicated that *PG*$^{-/-}$ mice demonstrate altered acoustic startle reflex response relative to *WT* mice (139). This startle reflex response is modulated by hormones associated with the hypothalamic-pituitary axis (140, 141). Processing of hormones derived from the pro-opiomelanocortin precursor in the pituitary in *PG*$^{-/-}$ mice was significantly reduced and a reversal of the altered response was accomplished by administration of β-endorphin and α-melanocyte stimulating hormone into the brain of *PG*$^{-/-}$ mice (142).

Amyloid-β plaques are hallmark features of Alzheimer's disease and Pm has been implicated in the degradation of these plaques (143). Studies in *PG*$^{-/-}$ mice indicated that the level of en-

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dogenous amyloid-β is not increased relative to *WT* mice and suggests that, under nonpathological conditions, Pm does not regulate steady state levels of this protein (144).

Tumor development, metastasis and angiogenesis

A number of clinical studies demonstrated that enhanced plasma levels of proteins associated with the fibrinolytic system in patients with cancer positively correlated with an overall poor prognosis for the patient (145). The first study in $PG^{-/-}$ mice utilized the Lewis lung carcinoma model and demonstrated that the rate of appearance of the tumor was similar to *WT* mice but the primary tumors were smaller, less hemorrhagic, and there was reduced skin ulceration in $PG^{-/-}$ mice (146). There were no significant differences in the number of lung metastases between the two genotypes, but dissemination to the lymph nodes was delayed in $PG^{-/-}$ mice. Another model utilizing $PG^{-/-}$ mice involved cross-breeding these mice with transgenic mice expressing polyoma middle T antigen under the control of the mouse mammary tumor virus long terminal repeat (147). In this model, well-differentiated and histologically similar adenocarcinomas developed in both *WT* and $PG^{-/-}$ mice. However, unlike the Lewis lung carcinoma study, lung metastases were significantly reduced in $PG^{-/-}$ mice. Finally, implantation of the fibrosarcoma cell line, T241, into $PG^{-/-}$ and *WT* mice demonstrated growth in both genotypes but a significant reduction in tumor burden was observed in $PG^{-/-}$ mice (148). Additionally, tumor infiltrating macrophage density was significantly higher in the stroma of $PG^{-/-}$ mice and, while there was no difference in the initial vessel density, there was a 40% reduction at a later timepoint in $PG^{-/-}$ mice. Attenuated angiogenesis was also observed in a mouse corneal model in nontumor bearing $PG^{-/-}$ mice (149).

These studies indicate that primary tumor growth and dissemination can be affected by dominance of other proteolytic pathways, i.e., matrix metalloproteases, the genetic composition of both host and tumor tissue, and tumor microenvironment. A deficiency of Pg may dictate the pathology of the disease by altering these and other regulatory factors.

Summary and perspectives

Components of the fibrinolytic system, which include Pg, Pg activators, Pm, cellular receptors of Pm, inhibitors of Pm and Pg activators, as well as fibrinogen and fibrin, have undergone intense study in the past 3 decades and have provided fascinating models of *in vitro* and *in vivo* structure-function relationships. The modular structure of Pg, in which its components function as independent domains, has revealed much knowledge regarding efficient evolution of complex structures by exon swapping, contributions of specific modules to the various activities, and the nature of one of the largest ligand-induced conformational changes described in the biochemical literature. Not only is the Pg-Pm system of obvious importance in control of fibrin-containing clot degradation under physiological and pathophysiological conditions, but this system functions as the most important extracellular protease system *in vivo*. Pm bound to various types of cells can degrade extracellular matrix and fibrin barriers and provide potent protease activity on cells that functions in pathways that range from tumor metastasis to bacterial invasion and dissemination. These properties reveal that fibrinolytic components are important drug targets for a wide range of pathologies.

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