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GENE TARGETING IN HEMOSTASIS. PLASMINOGEN

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1. ABSTRACT

A number of *in vitro* and *in vivo* observations have implicated plasminogen in contributing to events associated with diverse physiological and pathophysiological processes. The development of gene knockout technology has led to the generation of plasminogen deficient mice. These mice survive to adulthood and are thus a valuable resource for directly assessing its role in these processes. As a result, fibrinolytic and nonfibrinolytic functions have been identified from studies in which these mice were challenged utilizing a number of models that mimic both normal biological and pathological events.

2. INTRODUCTION

2.1. Physiological and pathophysiological roles of the plasminogen dependent pathway

Activation of the primary fibrinolytic system ultimately results in the proteolytic conversion of the plasma zymogen, plasminogen (Pg), to the serine protease, plasmin (Pm) (1). The formation of Pm and regulation of its proteolytic activity are mediated by a number of plasma- and cell-associated components. The fibrinolytic capacity of the Pg system is manifested in its ability to bind to fibrin-rich thrombi and interact with the fibrin-specific Pg activator, tissue-type plasminogen activator (t-PA) (2). The

localization of Pm activity at the site of a developing thrombus, as well as temporal protection of these proteases from circulating inhibitors, are critical events in the regulation of fibrinolysis (1). Cell surface expression of specific receptors for Pg and the Pg activators, both urokinase-type plasminogen activator (uPA) and t-PA, implicate Pm expression in cell-mediated proteolytic processes, such as extracellular matrix degradation and directional cell migratory events (3-6). These events are potentially involved in physiological processes associated with wound healing, embryogenesis, and angiogenesis (7-9). The observation that neoplastic cells express elevated levels of u-PA, t-PA, and/or uPA receptor (uPAR) suggests that this pathway is involved in pathophysiological events, such as tumor growth and metastasis (10-13). Indeed, elevated plasma levels of uPA and/or uPAR in cancer patients have been shown to be prognostic markers for poor overall survival (14). On the other hand, the identification and characterization of the anti-angiogenic protein, angiostatin, a protein potentially derived from host Pg, implicates this protein as a negative regulator of tumor growth and dissemination (15). Another molecule strikingly similar to Pg, apo(a), a protein associated with the pro-atherogenic lipoprotein, lipoprotein(a), suggests that Pg may also play a role in regulating atherosclerotic lesion development (16,17). Indeed, components of the fibrinolytic system have been identified in human atherosclerotic lesions (18,19). Therefore, aside from the obvious involvement in controlling thrombus formation and dissolution, plasmin(ogen) may play a broad and diverse role in a number of physiological and pathophysiological events. Direct analyses of the diverse functions of Pg have recently been initiated through the development and characterization of Pg-deficient mice.

2.2. Plasminogen structure and activation

The cDNAs for human Pg (HPg) and mouse Pg (MPg) have been cloned and sequenced (20,21). There is 76% conservation at the DNA level between HPg and MPg. Part of the HPg genomic DNA, which encompasses 52.5 kb, has also been cloned and sequenced (22). The HPg consists of 19 exons, 75-387 bp in length, and 18 introns of type I, type II and type O (23). Regulatory elements of the HPg gene are contained in the nucleotide sequences 5' and 3' of the coding sequence and have been partially identified (22). The HPg gene is located on chromosome 6q26-q27, while MPg is located on chromosome 17.

HPg is synthesized as an 810 amino acid residue polypeptide chain (Figure 1). MPg contains 2 additional amino acid residues at positions 543 and 587. During secretion, a 19 amino acid residue leader peptide is excised generating the mature form of Pg, which contains 791 amino acid residues for the human form and 793 amino acid residues for the murine form. The only known post-translational modifications that occur on HPg are N- and O- linked glycosylation (24,25) and phosphorylation (26). For example, an Asn-linked glycosylation sequence, Asn^{289}-Arg-Thr, is present on approximately one-half of the HPg molecules, while an additional site containing O-linked glycan at Thr^{346} occurs on all HPg molecules. Other sites of O-linked glycosylation have been identified at positions Ser^{249} (27) and Ser^{339} (28). There are 2 potential sites for N-linked glycosylation on MPg, *viz.*, Asn^{117} and Asn^{289}.

The conversion of Glu^{1}-Pg to the serine protease Glu^{1}-Pm is the result of activator-catalyzed cleavage of the Arg^{561}-Val^{562} peptide bond, for HPg, and Arg^{562}-Val^{563} for MPg, resulting in the generation of a 561 (562 for MPg) amino acid heavy chain, from the amino-terminus of Pg, linked by 2 disulfide bonds to a light chain of 230 (231 for MPg) amino acids (29). This latter chain, from the carboxy-terminus of Pg, is homologous to other serine proteases such as trypsin and chymotrypsin, and contains the catalytic triad of amino acids (His^{603}, Asp^{646}, and Ser^{741}) that is the hallmark of a serine protease. Additional sites of hydrolysis that are functionally significant are the result of catalysis by HPm and occur between residues Lys^{77} and Lys^{78} (30), as well as sites within the 77 amino acid polypeptide itself (31).

Five homologous triple-disulfide-linked peptide regions, *ca,* 80 amino acid residues, termed kringles (32), occur on the heavy chain of HPg and exist in several other proteins, many of which are clotting and fibrinolytic proteins (33-37). The Pg kringles have been shown to be involved in facilitating protein-protein interactions between Pg and fibrin(ogen), bacterial cell surfaces (38-40), and small molecule activation effectors, such as Cl^- and omega-amino acids (41,42). The specific kringles in Pg implicated in interactions with effector molecules are kringles 1, 2, 4, and 5. These interactions have been shown to be inhibited by lysine and lysine analogues, with kringles 1 and 4 demonstrating the strongest omega-amino acid binding sites (43-45). Further studies, utilizing site-directed mutagenesis of isolated kringles, have facilitated the elucidation of the nature of the interactions between the kringles and lysine-type ligands (46-48).

2.3. Plasminogen defects in humans

The first documented abnormal HPg (HPg-Tochigi) was reported in 1978 (49). This patient suffered from a history of thrombotic occurrences with 37% functional Pg activity. Further studies indicated an active site defect in which a G→A transition in exon XV of Pg resulted in an Ala^{601}→Thr substitution near the active site His^{603} of the catalytic triad (50,51). While this protein can be converted to two-chain Pm, it was found to be inactive. *In vitro* studies, utilizing recombinant HPg with a Pro^{611}→Ile mutation near the active His^{603} residue, resulted in a similar effect (F.J. Castellino personal communication). The Ala^{601}→Thr type 1 mutation appears at a high frequency in the Japanese population (2%) and may be a genetic marker. While many of these patients have clinical histories of venous thrombosis, some remain asymptomatic. Recently, a few patients with type I Pg deficiency have been identified (52,53). These patients present with ligneous conjunctivitis or pseudomembranous disease. Other patients with different homozygous or compound heterozygote mutations also have been reported to present with ligneous conjunctivitis and in some cases occlusive hydrocephalus (54,55). Due to the limited number of identifiable patients who have Pg defects leading to deficiencies of Pm activity and to the variability in the extent to which these defects are clinically manifested, the ability to define the role of Pg in a number of physiological

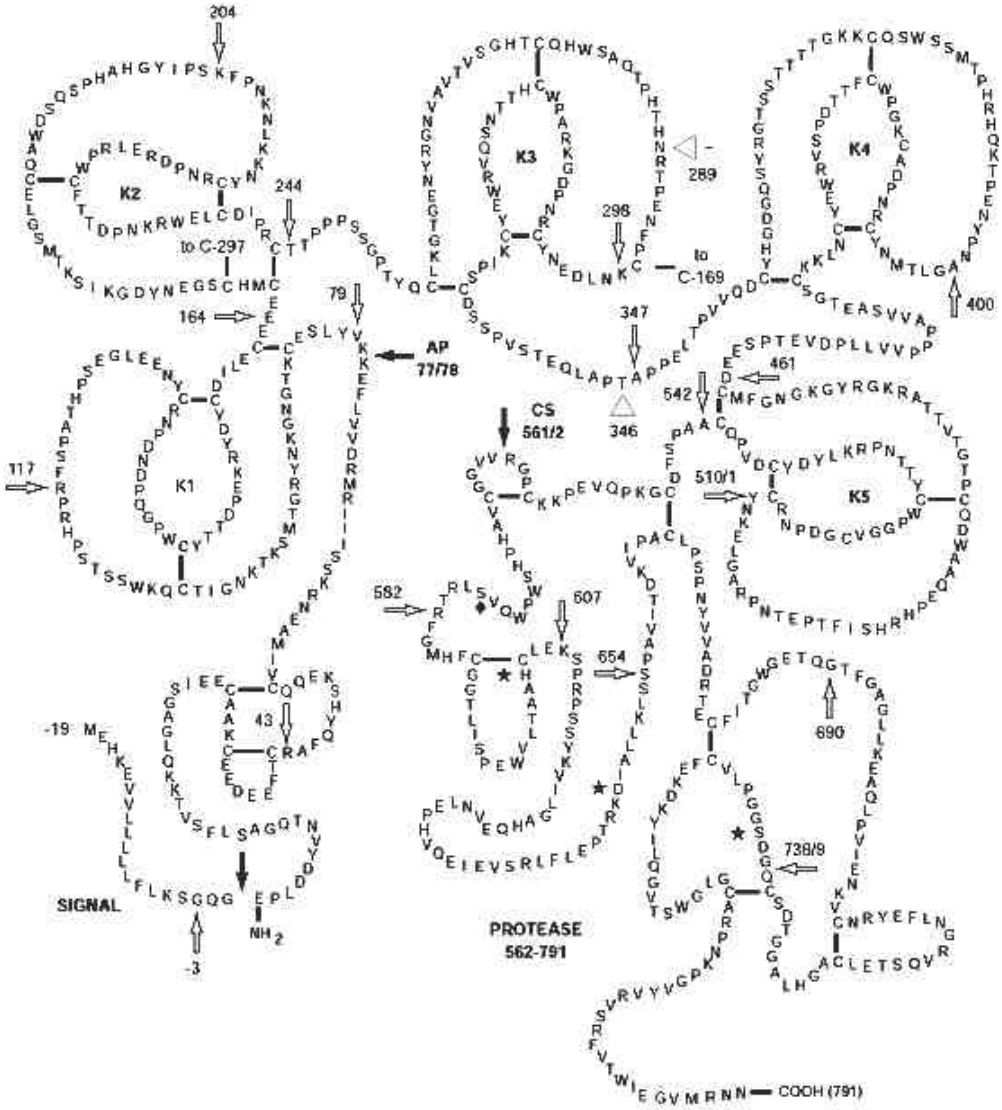

Figure 1. Primary structure of human plasminogen (HPg). Plasmin-mediated cleavage of Glu^{1}-Pg at position Lys^{77}-Lys^{78} results in the conversion of Glu^{1}-Pg to Lys^{78}-Pg and the release of the activation peptide (AP). Cleavage by plasminogen activators occurs at Arg^{561}-Val^{562} (CS) and results in the conversion of single chain Pg to the two-chain disulfide-linked serine protease, plasmin (Pm). The active site of Pm consists of the catalytic triad His^{603}/Asp^{646}/Ser^{741}(*).

processes has been severely limited. Therefore, mice deficient for Pg have become a valuable resource for delineating the role of Pg in the initiation and progression of a number of biological processes.

3. DEVELOPMENT AND CHARACTERIZATION OF PLASMINOGEN DEFICIENT MICE

3.1. Targeted Inactivation of the Murine Plasminogen Gene

3.1. Targeted Inactivation of the Murine Plasminogen Gene

Two research groups have successfully inactivated the murine plasminogen gene utilizing two separate strategies. One approach (56), replaced a portion of the 3' end of intron 14 to intron 17, with the neomycin resistance gene (neo), resulting in the elimination of exons 15 and 16, which encode His605 and Asp648 (two of the three amino acids which make up the catalytic triad) and exon 17 (Figure 2A). Another approach (57) replaced a 9 kb portion of the gene, which included 5' flanking sequences and the first 2 exons, with the phosphoglycerate kinase promoter (PGK) – HPRT gene (Figure 2B). This replacement resulted in the deletion of the proximal promoter sequences, exon 1 (the signal peptide), and exon 2 (43 residues of the preactivation peptide).

Deletion of the gene was confirmed in offspring from the F1 generation utilizing Northern blot of liver mRNA and plasminogen immunohistochemistry of liver tissue. Additional confirmation came from studies assessing plasminogen antigen and activity in plasma

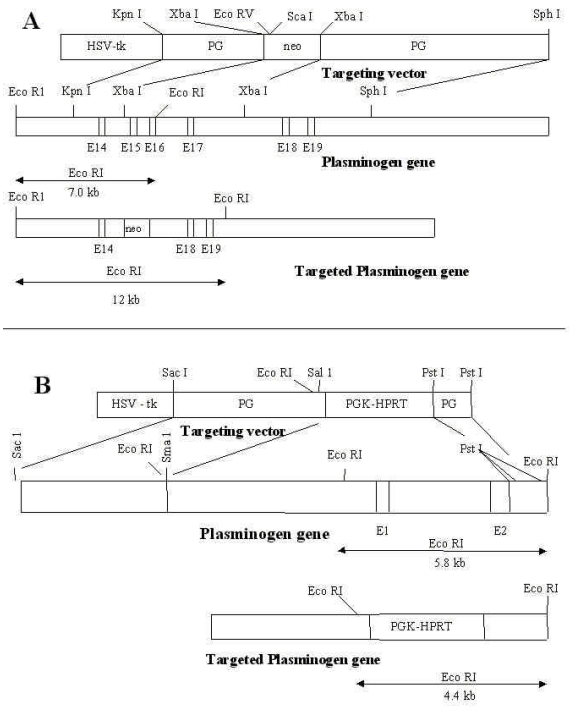

Figure 2. Strategies for the targeted inactivation of the murine plasminogen gene. Panel A. Replacement a portion of the 3' end of intron 14 to intron 17 with the neomycin resistance gene (neo), resulting in the elimination of exon 15 (E15) and exon 16 (E16), which encode His605 and Asp648 (two of the three amino acids which make up the catalytic triad) and exon 17 (E17). Panel B. Replacement of a 9 kb portion of the murine plasminogen gene, including 5' flanking sequences and the first 2 exons, with the phosphoglycerate kinase promoter (PGK)-HPRT gene resulting in deletion of the proximal promoter sequences, exon 1 (E1), the signal peptide, and exon 2 (E2), 43 residues of the preactivation peptide.

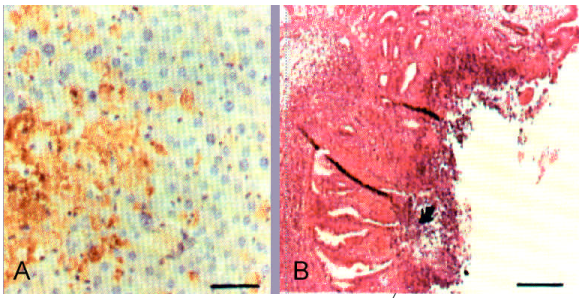

Figure 3. Histology of tissue from $PG^{-/-}$ mice. Panel A. Liver section from an 11 week old $PG^{-/-}$ mouse immunostained with a murine fibrin(ogen)-specific antiserum revealing fibrin deposits. Panel B. H&E stain of stomach from a 7 week old $PG^{-/-}$ mouse demonstrating a gastric ulcer. Loss of normal architecture of the overlying epithelium is apparent. Inflammatory cells and excessive fibrin deposits are evident in the underlying stroma. Magnification bar is 50 µm for both panels.

samples from these mice. Surprisingly, a homozygous Pg deficiency ($PG^{-/-}$) was not embryonic lethal with mice surviving well into adulthood. A diminished growth rate in deficient mice shortly after weaning; runtiness and apathic nature in a few mice; and diminished fertility were reported (56). No significant impact on hematological parameters were observed, which was unlike that reported for mice deficient for both u-PA and t-PA ($UPA^{-/-}$: $TPA^{-/-}$) where it was demonstrated that these mice suffered severe anemia, beyond the age of 20 weeks, which was associated with the development of cachexia (58).

3.2. Spontaneous Phenotypes

3.2.1. Thrombosis

Fibrin deposition was found to occur in a number of organs with some associated pathological consequences. Spontaneous fibrin deposition was observed but not to the same extent in all organs. Organs targeted in this deficiency were liver (Figure 3A), lung, pancreas, thymus, adrenal tissue, ovary and uteri and stomach with associated gastric and colonic ulcerations (Figure 3B). The occurrence of gastric and colonic ulcerations was most likely due to fibrin occlusion of small blood vessels. Rectal prolapse, most likely due to fibrin-mediated vasocclusion, with associated rectal ulceration, necrosis, inflammation and bacterial contamination, was a common phenotype in these mice. It was reported that a small number of deficient mice demonstrated a significant enlargement of one or both kidneys which was associated with distension of the pelvis and urine accumulation. In this case, no obvious sign of fibrous adhesions were noted in the peritoneum (56). Additionally, studies to determine fibrinolytic capacity in these mice, through analyzing their ability to degrade a pulmonary clot, indicated that clot lysis was significantly delayed in these mice (56). Reconstitution with murine plasminogen normalized the thrombolytic potential indicating that plasminogen plays a critical role in *in vivo* fibrin clot dissolution (59).

3.2.2. Growth, Development and Fertility

Little differences in growth and behavioral development were observed between $PG^{-/-}$ and *WT* mice (60). Between 2 and 21 days of age, weight gain and physical development milestones, *i.e.*, ear detachment, eye opening, and teeth eruption, were similar. Differences in physical development were observed only after 4 weeks of age with less weight gain and delayed vaginal patency in $PG^{-/-}$ mice. Behavioral development during 2-21 days of age was similar. These studies consisted of analyses of the development of reflexes, reaction to gravitational positioning, neuromotor ability, motor coordination, locomotor activity, integration of motor and vestibular systems, olfactory development, and incidence of audiogenic seizure susceptibility. Additionally, this study indicated that there was some alteration in reactivity in response to stress and in hormone processing and neuroendocrine regulation.

Ovulation efficiency in young, age-matched *WT*, $PG^{+/-}$, and $PG^{-/-}$ female mice was studied after stimulation with gonadotropin and indicated a slight, but not statistically significant, reduction in ovulation efficiency (61). The temporal onset of follicular wall rupture after

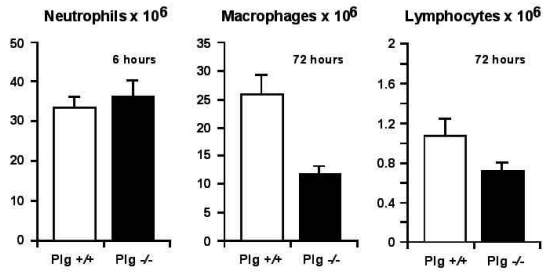

Figure 4. Leukocyte migration in response to thioglycollate in wild-type, $PG^{+/+}$ and $PG^{-/-}$ mice. Cell counts are documented at the time of peak response for both genotypes. Reprinted from Plow, E., Ploplis, V.A., Carmeliet, P., and Collen, D. (1999) Plasminogen and cell migration *in vivo* in Fibrinolysis & Proteolysis, Vol. 13 pp. 49-53, with permission from Churchill Livingstone.

stimulation with gonadotropin was also studied and indicated that ovulation was not delayed in $PG^{-/-}$ mice. Histological analyses of ovaries indicated that there were no abnormalities. Additionally, physiological ovulation, which is controlled by endogenous gonadotropin, also supported a lack of effect on ovulation efficiency in $PG^{-/-}$ animals.

Although ovulation efficiency in $PG^{-/-}$ mice was not significantly different than that observed in *WT* mice, a significant percentage of $PG^{-/-}$ female mice failed to become pregnant with the severity dependent on the extent of the C57BL6 strain background (62). Lactational competence has also been shown to be significantly diminished with 75% of $PG^{-/-}$, backcrossed 10 generations into the C57BL6 strain, unable to sustain lactation for 10 days. Those that were able to sustain lactation over this time period, as well as those that failed to lactate, demonstrated histological abnormalities of their mammary glands.

Mammary gland remodeling during involution was also different in $PG^{-/-}$ mice, relative to that in $PG^{+/-}$ and *WT* mice, with a proportionate reduction in median gland mass, after 5 days of involution which was significantly less severe in $PG^{-/-}$ mice. Additionally, the histology of involuting mammary glands was also markedly different in these mice with no evidence of fibrin accumulation in $PG^{-/-}$ mammary glands compared to *WT* mice.

3.3. Induced Phenotypes

3.3.1. Inflammation and Infection

A direct assessment of the inflammatory response was made in $PG^{-/-}$ mice utilizing the inflammatory stimulant, thioglycollate (63). While the kinetics and extent of early neutrophil recruitment into the stimulated peritoneal cavity was similar to that observed in *WT* mice, macrophage and lymphocyte recruitment were severely compromised (Figure 4).

Many studies have indicated that pathogens utilize host Pg to invade and degrade tissue barriers. Other studies have shown that some gram-positive *streptococci* and *Yersinia* activate Pg with their endogenous activators (64,65). In order to delineate the role of Pg in various stages of the infection process, a number of infectivity studies have been performed in $PG^{-/-}$ mice.

Analyses of the effect of host Pg deficiency on the infectivity of relapsing *Borrelia* fever indicated a decreased spirochetal load in the brains of these mice relative to *WT* mice (66). However, this deficiency did not appear to affect cutaneous tissue to blood penetration indicating a selective use of this protein during the infection process.

Studies of erythrocytes infected with human (*Plasmodium falciparum*) and murine (*Plasmodium chabaudi*) malaria parasites have identified Pg activator activity that is not observed in uninfected cells (67). Additional studies have shown that serine protease inhibitors and antibodies to uPA inhibit the rupture of these cells by malaria parasites. Surprisingly, studies in mice deficient for components of the fibrinolytic system (uPA, tPA, Pg, uPAR) have shown that these proteins are not required for the erythrocytic life cycle of this pathogen (Table 1) (68).

It has been demonstrated that Group A *streptococci* can acquire surface-associated Pm-like activity when incubated with human plasma (69). Further studies have shown that the a1a2 repeats of PAM, a M-like surface protein present in Group A *streptococci*, contains a strong binding site for HPg which is mediated by the kringle 2 domain of HPg. As a result of this interaction, and coupled with the presence of the bacterial-derived HPg activator, streptokinase, HPm is generated on the cell surface (70) which can provide the proteolytic activity required for invasion into host tissue.

Additional studies have been performed on the role of Pg in the virulence of the gram-negative bacteria *Yersinia pestis*, which is the causative agent of plague. A number of strains of this pathogen produce a cell surface-associated Pg activator that appears to play an important role during the systemic infection stage (65). Studies in $PG^{-/-}$ mice demonstrated an increased resistance to this organism, which corresponded to an increase in LD_{50} of about 100-fold (71). This would indicate that Pg plays a major role in the pathogenicity of *Yersinia pestis.*

3.3.2. Vascular Remodeling

3.3.2.1. Arterial Remodeling after Electrical Injury

A number of studies have indicated that components of the fibrinolytic system play a role in smooth muscle cell migration (72-74). Arterial intimal thickening during vascular remodeling after injury involves smooth muscle cell proliferation in the media and migration across the internal elastic lamina into the intimal compartment, where deposition of extracellular matrix protein occurs and contributes to luminal stenosis (75,76). In order to determine whether Pg plays a role in this repair process, neointima formation was evaluated in $PG^{-/-}$ mice following electrical injury of the femoral artery (77). In this model, destruction of medial smooth muscle cells and denudation of the intimal endothelial cells occurs at the site of injury.

Table 1. Responses to Murine Malaria *P. vinkei* in Mice Deficient for Components of the Fibrinolytic System

Parasite	Mouse Genotype	Number	Time to >50% parasitemia (days)	Survival
P. vinkei	*WT*	12	7.6 ± 0.6	0/12
	PG$^{-/-}$	12	8.0 ± 1.1	0/12
	UPA$^{-/-}$	12	7.5 ± 0.5	0/12
	TPA$^{-/-}$	6	7.5 ± 0.5	0/6
	UPAR$^{-/-}$	6	7.2 ± 0.4	0/6

A transient platelet-rich thrombus is also formed. In *PG*$^{-/-}$ mice, the wound healing process is significantly impaired with delayed necrotic debris removal, reduced inflammatory cell infiltration and smooth muscle cell accumulation, and a decreased neointima. However, smooth muscle cell proliferation and reendothelialization were similar for both genotypes. Results from this study support a role for Pg in vascular wound healing following electrical injury, most likely by affecting cellular migration.

3.3.2.2. Transplant Atherosclerosis

Since a Pg deficiency results in reduced inflammatory recruitment and diminished smooth muscle cell migration, as demonstrated in various models, the effect of this deficiency on other vascular injury/repair models has been investigated. A number of models that mimic some of the clinical phenotypes of atherosclerosis have been employed.

The molecular mechanisms associated with transplant arteriosclerosis (arterial graft disease) are poorly understood but this disease is believed to be initiated by an immune reaction to donor graft antigens (78,79). It has been observed that host inflammatory cells invade the donor blood vessel producing cytokines and chemotactic agents that result in vascular smooth muscle cell proliferation and migration (78-80). In order for these inflammatory and smooth muscle cells to migrate through tissue barriers, cell surface-associated proteases are required. Potential candidates are proteins associated with the fibrinolytic and metalloprotease systems. However, their role in transplant arteriosclerosis had not been conclusively delineated.

A mouse model of transplant arteriosclerosis has been developed and involves the grafting of a carotid artery from a donor mouse onto a recipient mouse with histoincompatibility in the H-2 region (81). Studies performed in *PG*$^{-/-}$ mice demonstrated diminished adventitial and medial inflammation, retarded elastic laminae degradation, reduced media necrosis, and suppressed smooth muscle cell proliferation and migration into the intimal compartment (82) relative to *WT* mice.

Another study utilized a model of vein graft stenosis. This study involved the grafting of a segment of the left external jugular vein to the left (ipsilateral) carotid artery in *PG*$^{-/-}$ and *WT* mice (83). In contrast to the arterial transplant model, results from this study indicated that there were no significant differences in neointima formation in patches 20 days after surgery between *PG*$^{-/-}$ and *WT* mice. However, there was a higher proportion of CD45 cells in the neointima of the venous patch from *PG*$^{-/-}$ mice relative to *WT* mice. This may be due to an inability of these leukocytes to penetrate the elastin- and collagen-rich neointima that is formed rapidly resulting in an entrapment of these cells.

3.3.2.3. Apo E$^{-/-}$: PG$^{-/-}$

A number of mice models have evolved that attempt to differentiate specific mechanisms associated with the development and progression of atherosclerosis (84,85). Transgenic and gene knock out mice have been generated in which genes that regulate the atherosclerotic process have been altered i.e., apo E and LDL receptor (LDLR) (86,87). These mice, when placed on high fat diets, develop hypercholesterolemia and spontaneous atherosclerotic lesions.

A study investigating the effect of Pg deficiency on the development of atherosclerosis in *apo E*$^{-/-}$ mice (*PG*$^{-/-}$: *apo E*$^{-/-}$) demonstrated that the absence of Pg accelerated lesion development in the proximal and distal aorta relative to *apo E*$^{-/-}$ mice (88). Results from this study suggest that a Pg deficient state amplifies the atherosclerotic process in the context of other underlying atherogenic conditions.

3.3.2.4.Vascular Cuffs

Vascular remodeling, which has been described for pathologies such as atherosclerosis and restenosis, involves extracellular proteolysis. A model of vascular remodeling utilized polyethylene cuff placement around arteries. In this model inflammatory cell recruitment into the vessel wall occurs shortly after cuff placement (89). Inhibition of inflammation in this model with dexamethasone resulted in significantly reduced neointima formation in rabbit carotid arteries (90). These results suggest that in this model the inflammatory response plays a significant role in neointima formation associated with vascular remodeling.

Since studies have implicated Pg in extracellular matrix proteolysis and the inflammatory response, an investigation of the role of plasmin(ogen) in vascular remodeling after placement of polyethylene cuffs was performed in *PG*$^{-/-}$ mice (91). Histological and morphometric analyses were performed 2, 5, 14, and 28 days after cuff placement. The results indicated that inflammatory response, elastic lamina degradation, and neointima formation were similar between *WT* and *PG*$^{-/-}$ mice. However, focal medial atrophy was apparent in *PG*$^{-/-}$ mice which was also evident in *PG*$^{-/-}$/*FG*$^{-/-}$ mice implicating a fibrin-independent function of Pg that mediates in preventing medial atrophy following injury. Medial compartment enlargement did not occur in *PG*$^{-/-}$ mice but did occur in *PG*$^{-/-}$/*FG*$^{-/-}$ mice implicating fibrin as an

impediment for enlargement. The presence of unresolved fibrin in the adventitia of *PG*$^{-/-}$ mice may act as a physical barrier to expansion/remodeling following injury. These studies indicate that plasmin(ogen) plays a role in vascular remodeling events and that fibrinolysis may be an important contributory event during the repair process.

3.3.2.5. Endoluminal Arterial Injury

Another vascular injury model utilized a flexible guidewire to initiate injury to the luminal surface of carotid arteries in *PG*$^{-/-}$ and *WT* mice (92). Shortly after injury, denudation of the endothelial intimal layer, exposure of the internal elastica lamina, disruption of the medial layer of smooth muscle cells, and attachment of platelets and leukocytes were observed. Three weeks post-injury, increases in the adventitial, medial, and intimal layers were observed in *WT* mice arteries but only a slight increase in medial and intimal compartments was observed in *PG*$^{-/-}$ arteries. No increase in adventitial compartment was observed in *PG*$^{-/-}$ arteries, consistent with a diminished inflammatory response. Compensatory dilation of the lumen was observed in response to injury in *WT* arteries but not *PG*$^{-/-}$ arteries. Dilation may be the result of infiltrating leukocytes or Pm-mediated activation of TGF-β. Unresolved medial thrombus was evident in *PG*$^{-/-}$ arteries and not in *WT* arteries consistent with the fibrinolytic function of Pg.

The conflicting observations in these vascular injury models may be the result of different methods used to induce injury and resultant diverse mechanisms initiated after injury during the repair process. This would suggest that vascular remodeling events are complex and multifactoral.

3.3.3. Wound Healing

3.3.3.1. Skin Injury

Pg has been implicated in playing a role in the degradation of extracellular matrices, a physiological event essential for tissue remodeling such as wound healing of the skin. This process involves the formation of a fibrin-rich provisional matrix at the site of the wound, infiltration of inflammatory cells, proliferation and migration of keratinocytes at the wound edges, and formation of granulation tissue. Since fibrin is a major component of the skin wound healing process and keratinocytes express components of the fibrinolytic system (93-97), Pm is also a likely player during this process. In order to directly determine if Pg plays an active role during skin wound healing, studies were performed in *PG*$^{-/-}$ mice (98). For this study, incisional wounds were made in *PG*$^{-/-}$, *PG*$^{+/-}$, and *WT* control mice and examined at 2 to 7 day intervals. While cell migration and tissue remodeling, in general, were not compromised as evident by the infiltration of inflammatory cells, formation of granulation tissue, and neovascularization within the wound field, keratinocyte migration from the wound edges was impaired in *PG*$^{-/-}$ mice. This was most probably due to an inability to degrade the fibrin provisional matrix since healing times were corrected in *PG*$^{-/-}$/*FG*$^{-/-}$ mice (99).

3.3.3.2. Corneal Injury

Corneal wound healing was studied in *PG*$^{-/-}$ mice utilizing a excimer laser photorefractive keratectomy injury model (100). This study assessed fibrin resolution and restoration of clarity following injury. It is thought that corneal repair is dependent on a balance between provisional matrix deposition immediately following injury, its resolution, and ultimate re-epithelialization. Eyes were examined over a 21 day period following injury. Re-epithelialization was rapid and complete within 3 days for both *WT* and *PG*$^{-/-}$ mice. Corneal fibrin(ogen) was extensive in *PG*$^{-/-}$ mice on days 1 and 3 following injury but sparse in *WT* mice. Fibrin ultimately resolved in *WT* mice but persisted in *PG*$^{-/-}$ mice over the 21 day period contributing towards corneal opacity and scarring.

Another study of corneal injury/repair in *PG*$^{-/-}$ mice utilized a blade to create corneal epithelial defects (3 mm) (101). Corneal epithelial defects healed quickly in *WT* mice with the generation of a transparent cornea. In *PG*$^{-/-}$ mice the healing process was impaired and complicated by a severe inflammatory response, corneal opacity, due to unresolved scar tissue and fibrin deposition, and stromal neovascularization. Similar studies in *PG*$^{-/-}$/*FG*$^{-/-}$ mice restored a normal healing process.

Results from both of these studies indicate that Pm plays a role in corneal tissue repair processes through its fibrinolytic capacity in removing injury-induced provisional fibrin matrices.

3.3.3.3. Liver Injury/Repair

Key events in liver regeneration are cellular proliferation and tissue remodeling which would involve matrix protein degradation. An earlier study, utilizing partial hepatectomy of *UPA*$^{-/-}$ mice, indicated that hepatocyte proliferation was diminished relative to *WT* mice resulting in transiently impaired liver regeneration (102). Since matrix degradation is an important event in remodeling after acute injury and Pg has been implicated in matrix protein degradation, either directly or through activation of other proteolytic pathways, a study of liver regeneration after acute injury was performed utilizing *PG*$^{-/-}$ mice (103). For this study, carbon tetrachloride was used to induce an acute liver injury in *PG*$^{-/-}$ and *WT* control mice. Two days after injury, *WT* and *PG*$^{-/-}$ livers looked diseased and similar with a pale lacey appearance. At day 7 only the *WT* liver was restored to normal. *PG*$^{-/-}$ livers remained diseased as long as 2.5 months after injury and demonstrated a persistent damage to centrilobular hepatocytes. Unlike *UPA*$^{-/-}$ mice, these unresolved lesions were not due to alterations in cellular proliferation. While fibrin deposits persisted for at least a month in the centrilobular areas of *PG*$^{-/-}$ mice, *PG*$^{-/-}$ mice in a *FG*$^{-/-}$ background did not correct the extended disease state. This study indicates that Pg plays an important role in remodeling events following acute liver injury possibly through direct or indirect mechanisms of degradation of necrotic tissue.

3.3.3.4. Cardiac Injury

Early cardiac wound healing events involve recruitment of inflammatory cells to the site of injury and degradation of extracellular matrix protein and necrotic cardiomyocytes (104). Studies have indicated that likely candidate proteases that facilitate inflammatory cell migration into the wound are Pm, MMPs, and cathepsin (105). Additional studies have shown that mice lacking MMP-9 or

uPA have reduced inflammatory cell recruitment to the site of cardiac injury and are protected against cardiac rupture (106).

Since Pg is a substrate for uPA and Pm can activate MMP-9, a study to determine if Pg plays a direct role in cardiac wound healing following induction of myocardial infarction (MI) was performed (107). For this study myocardial infarction was induced in *PG*$^{-/-}$ and *WT* mice by permanent ligation of the main left coronary artery (108). Structural analyses were performed 1, 2, and 5 weeks after infarction. Left ventricle (LV) function, measured by cardiac output and LV pressure development, was assessed 2 weeks after infarction. Infarct healing, the result of infiltration of macrophages, (myo)fibroblasts, and endothelial cells, was abolished in *PG*$^{-/-}$ mice for at least 5 weeks after MI. As a result, necrotic cardiomyocytes were still evident and the formation of granulation tissue and fibrous tissue did not occur. Both MMP-2 and MMP-9 activity, MMPs that can be activated by Pm, were diminished in the infarcted heart implicating a role for these proteases in cardiac healing responses. Additionally, architectural changes in the left ventricle were similar in *PG*$^{-/-}$ and *WT* mice except for a less pronounced thinning of the infarcted wall in *PG*$^{-/-}$ mice, most likely due to a persistent presence of necrotic myocardiocytes. Surprisingly, LV function was attenuated only slightly in *PG*$^{-/-}$ mice. Results from these studies indicate that Pg plays a role in cardiac wound healing events and support its involvement in other wound healing processes.

3.3.4. Excitotoxin-Induced Neurodegeneration

Components of the fibrinolytic system, i.e., tPA and Pg, have been implicated in neuronal death in the hippocampus after exposure to excitatory amino acids. Studies in *TPA*$^{-/-}$ mice demonstrated a resistance to neuronal death induced by excitotoxins acting through all 3 glutamate receptors (kainate, AMPA, and NMDA) (109), directly implicating this protein in excitoxin-mediated neuronal death.

While the liver is the primary source of Pg, it is also produced in the hippocampus, a region in the brain that is particularly sensitive to neuronal death (110). Since both tPA and Pg co-localize in the hippocampus these two proteins have been implicated in mediating degeneration. In order to determine if Pg is a substrate for tPA during neuronal degeneration, studies were performed in *PG*$^{-/-}$ mice to assess resistant to excitoxin-induced neuronal death (111). For this study, *PG*$^{-/-}$ mice were injected with the glutamate analog kainate into the hippocampus and neuronal survival determined 5 days later. Resistance to excitotoxic injury in *PG*$^{-/-}$ mice was found to be equivalent to that observed for *TPA*$^{-/-}$ mice. Additionally, studies infusing α_2-antiplasmin (AP) into kainate-challenged *WT* mice resulted in resistance to neuronal death, therefore, directly implicating Pm in promoting hippocampal excitotoxic neuronal death. To determine if the protective mechanism is fibrin-dependent, similar studies were performed in *PG*$^{-/-}$/*FG*$^{-/-}$ mice (112). These mice were equally resistant to excitoxin-induced neuronal degeneration implicating other Pm substrates in this process. The candidate substrate that appears to be involved in this process has been identified as laminin. This protein, which is expressed in the hippocampus, disappears after excitoxin injection just before neuronal death (113). Laminin degradation was shown to be blocked in *TPA*$^{-/-}$ mice, after excitoxin challenge, or by infusion of a Pm inhibitor in *WT* mice. These studies suggest that sensitization to excitoxin-mediated hippocampal neuronal death is mediated through tPA/Pm catalyzed disruption of neuron-laminin interactions.

3.3.5. Axonal Degeneration and Demyelination

Since studies have indicated that tPA/Pm contributes to excitotoxin-induced neuronal death, most likely through degradation of laminin and resultant disruption of neuron/extracellular matrix interactions, analysis of a role for Pg in inflammatory neuronal degeneration was made. In this study the sciatic nerve was injured in *PG*$^{-/-}$ mice and *WT* control mice (114). Axonal demyelination was found to be exacerbated in *PG*$^{-/-}$ mice relative to *WT* mice. Axonal damage correlated positively with an increase in fibrin(ogen) deposition. Elimination of fibrinogen in *PG*$^{-/-}$ mice ameliorated axonal degeneration and suggests that fibrin is the Pm substrate during inflammatory axonal damage.

3.3.6. Cerebral Ischemic Infarction

It has been demonstrated that focal cerebral ischemia induced by middle cerebral artery occlusion is associated with excitoxin-mediated neuronal degeneration. Additionally, administration of tPA has been shown to exacerbate ischemic infarct size whereas a deficiency of tPA resulted in a reduced ischemic infarct size (115). These observations would suggest a role for tPA/Pm in events associated with cortical ischemic neuronal degeneration. In order to determine if Pg plays a role in focal cerebral ischemic infarction, the left middle cerebral artery in *PG*$^{-/-}$ mice was ligated and the infarct size quantitated 24 hrs later (116). Relative to *WT* controls, focal cerebral infarct size in *PG*$^{-/-}$ mice was significantly larger. Similar studies in alpha 2–antiplasmin deficient (*AP*$^{-/-}$) mice resulted in smaller infarcts. Since the total fibrin burden was much larger in *PG*$^{-/-}$ mice than in *AP*$^{-/-}$ mice, it has been suggested that an alteration in hemostatic balance in *PG*$^{-/-}$ mice results in a transient increase in intravascular fibrin deposition in the penumbra of the infarct.

3.3.7. Cancer Growth and Metastasis

3.3.7.1. Lewis Lung Carcinoma

The proteolytic degradation of extracellular matrix barriers by tumor cells has been identified as the first event in processes of invasion and angiogenesis of solid tumors (117). Localization of uPA activity on the cell surface, through its' interaction with uPAR, has been shown to be vital to the invasive capacity of several tumor cells and inhibition of this interaction can cause tumor cells to become dormant (118). For example, in experiments performed on nude mice, down-regulation of uPAR expression, using antisense vectors in a transplanted glioblastoma model, successfully inhibited tumor formation (119). Increased uPA activity has been reported in the more malignant phenotypes of astrocytoma (120). Clinically, it has been demonstrated that uPA, uPAR, and PAI-1 are elevated in patients with kidney cancer and these levels correlate with its aggressive phenotype (121). Additionally, elevated levels of uPAR appear to be a marker of poor prognosis in colorectal, breast, and squamous cell carcinomas (122-124). Studies in *UPA*$^{-/-}$

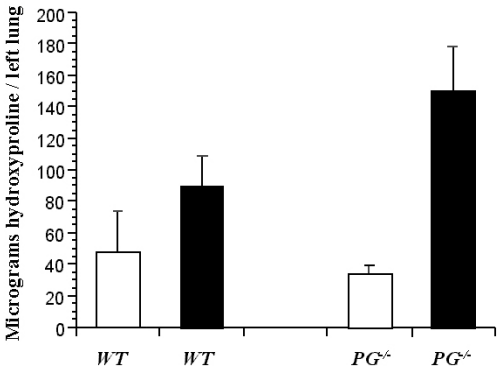

Figure 5. Hydroxyproline content in lungs 14 days after saline (white boxes, n=3) or bleomycin treatment (black boxes) of *WT* (n=4) and *PG*$^{-/-}$ (n=5) mice. Values are means ± SE performed in duplicate.

mice, utilizing dimethybenz(a)anthracene and croton oil, indicated a delay in the induction of primary cutaneous melanocytic neoplasia (125). Other studies in *UPA*$^{-/-}$ and *PAI-1*$^{-/-}$ mice, utilizing T241 fibrosarcoma tumor model, indicated that host expression of these proteins are essential for primary tumor growth (126). In order to investigate a direct role for Pg in the growth and dissemination of tumors, studies utilizing Lewis Lung Carcinoma in *PG*$^{-/-}$ mice were performed (127). While primary tumors were smaller, less hemorrhagic, and demonstrated reduced skin ulceration in *PG*$^{-/-}$ mice, primary tumors developed in both *PG*$^{-/-}$ and *WT* control mice with no difference in the rate of appearance. Additionally, dissemination to regional lymph nodes were delayed in *PG*$^{-/-}$ mice relative to *WT* control mice but there were no quantitative differences in lung metastasis.

3.3.7.2. Polyoma Virus Middle T Antigen-Induced Mammary Carcinoma

In order to determine if host Pg plays a role in mammary tumor growth and metastasis, *PG*$^{-/-}$ mice were crossed with transgenic mice expressing Polyoma middle T antigen under the control of the mouse mammary tumor virus long terminal repeat (128). Well-differentiated adenocarcinomas developed equivalently in *PG*$^{-/-}$ and transgenic mice and no obvious histological differences were observed. However, lung metastasis was significantly reduced in *PG*$^{-/-}$ mice.

Results from both of these studies would indicate that tumor type, location of growth and dissemination, and involvement of other proteolytic pathways could potentially dictate whether or not Pg is a contributing factor.

3.3.8. Glomerulonephritis

Fibrin deposits are observed in the glomerulus in severe proliferative and crescentic forms of human glomerulonephritis (GN). Since the glomerulus is particularly susceptible to injury induced by fibrin deposits, renal function is severely compromised (129). Also associated with these forms of GN are the accumulation of T cells and monocytes and cross-linking of fibrin within the Bowman's space and in the glomerular tuft. As a result of the chemotactic effects of fibrin, macrophages accumulate in the Bowman's space and this accumulation of macrophages and fibrin form the hallmark crescent appearance of this disease (130-132). Dysregulation of the fibrinolytic system has been demonstrated in experimental crescentic GN and in human GN (133,134). Little is known of the protective effect of Pm in preventing glomerular fibrin deposition in crescentic GN. In order to directly investigate the role of Pg in immune initiated glomerular injury, proliferative GN was induced in *PG*$^{-/-}$ mice (135). This model consisted of planting sheep anti-mouse GBM globulin in the glomeruli of mice previously sensitized to sheep globulin. Animals are then studied 10 days after the anti-GBM globulin injection. In *WT* mice, this results in a rapidly, progressive GN with renal impairment. A deficiency of Pg resulted in a severe functional and histological exacerbation of glomerular injury and supports the role of this protein in protecting the glomerulus from acute inflammatory injury.

3.3.9. Pulmonary Fibrosis

Studies have shown that acute and chronic pulmonary diseases are associated with impaired fibrinolytic activity within the lung. However, the specific role of the fibrinolytic system during lung injury and repair is unknown.

The chemotherapeutic drug, bleomycin, has been used as an agent for inducing acute lung injury in a number of animal models (136). Intratracheal administration of bleomycin in rodents results in inflammatory and fibrotic responses similar to what has been observed in patients with pulmonary fibrosis (137). Studies in PAI-1 overexpressing mice, challenged with bleomycin, demonstrated enhanced collagen deposition in the lungs relative to *WT* mice while a deficiency of PAI-1 resulted in collagen levels equivalent to PBS-treated *WT* controls (138). Additional studies have shown that treatment with uPA in bleomycin-treated rats and mice results in diminished collagen deposition (139,140). These studies implicate a role for the fibrinolytic system in regulating the pathologies associated with the repair process following acute lung injury. Studies in bleomycin-treated *PG*$^{-/-}$ mice demonstrated enhanced collagen deposition relative to *WT* controls (Figure 5) (141). While fibrin co-localized with collagen in fibrotic lesions, studies in *FG*$^{-/-}$ mice indicated that its presence was not essential for the development of fibrotic lesions (Figure 6) (142,143). Additionally, while a pulmonary hemorrhagic phenotype developed in *WT* mice this was not observed in *PG*$^{-/-}$ mice. This may be the result of diminished macrophage recruitment and activation of MMP-12 in the lungs of *PG*$^{-/-}$ mice relative to *WT* mice.

These studies support a role of the fibrinolytic system in events associated with the repair process following acute lung injury.

4. PERSPECTIVES

Indirect studies have implicated a critical role for Pg in hemostatic as well as nonhemostatic events, the latter of which involves cell migratory and tissue remodeling processes including early developmental processes such as spermatocyte migration, ovulation, and trophoblast

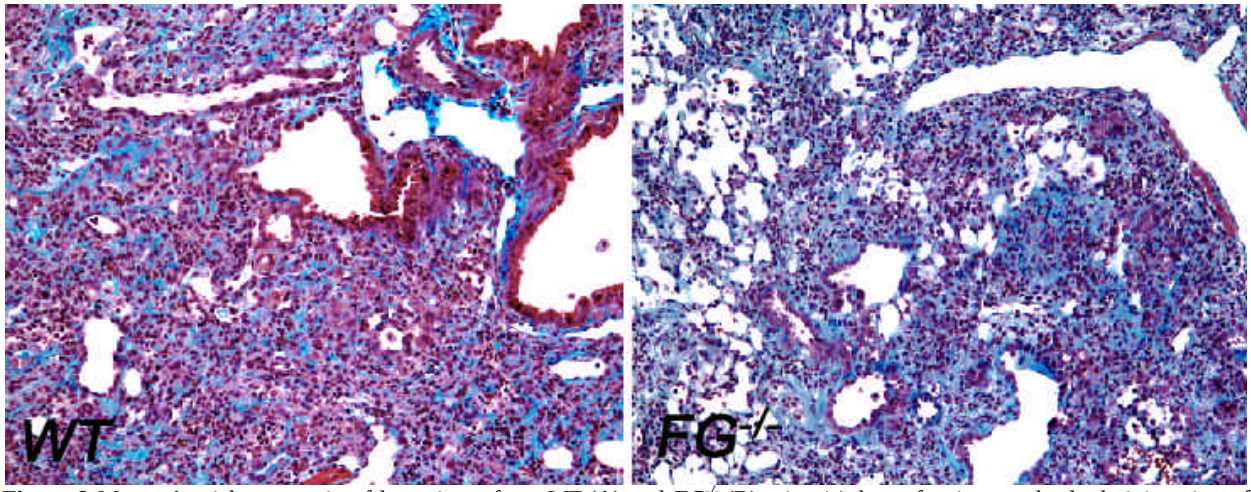

Figure 6. Masson's trichrome stain of lung tissue from *WT* (A) and *FG*$^{-/-}$ (B) mice 14 days after intratracheal administration of bleomycin. Collagen deposits (blue) are evident within the fibrotic lesions (original magnification 200X).

invasion during embryogenesis. Therefore, it was surprising that *PG*$^{-/-}$ mice survive to adulthood, are fertile, and carry a litter to full term. As a result, a number of direct studies in *PG*$^{-/-}$ mice have served to confirm or to question the relative importance of this protein in a number of physiological and pathophysiological events. While it has been demonstrated that Pg plays an important role in maintaining vascular patency by resolving fibrin clots, a deficiency does not necessarily lead to an immediate and life-threatening event. This has also been demonstrated clinically where *PG*$^{-/-}$ humans have been identified by presenting with other overt phenotypes such a ligneous conjunctivitis. Nevertheless, studies in *PG*$^{-/-}$ mice have identified a strong relationship between Pg and fibrin(ogen) in not only hemostasis but also in cell migratory and tissue remodeling processes. Additionally, other fibrin(ogen)-independent mechanisms have also been revealed from studies with these mice and indicate that the substrate specificity of Pm *in vivo* is more diverse. As a result of these studies with mouse models of Pg deficiency, the pathophysiology of this defect in humans can be more fully appreciated.

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6. REFERENCES

1. D. Collen: On the regulation and control of fibrinolysis. *Thromb Haemost* 43, 77-89 (1980)
2. Hoylaerts M, D. C. Rijken & H. R. Lijnen: Kinetics of the activation of plasminogen by human tissue plasminogen activator. *J Biol Chem* 257, 3912-3919 (1982)
3. Vassalli J. D., D. Baccino & D. Belin: A cellular binding site for the Mr 55,000 form of the human plasminogen activator, urokinase. *J Cell Biol* 100, 86-92 (1985)
4. Plow E. F., F. Freany, J. Plescia & L. A. Miles: The plasminogen system and cell surfaces: Evidence for plasminogen and urokinase receptors on the same cell type. *J Cell Biol* 103, 2411-2430 (1986)
5. Hajjar K. A., & N. M. Hamel: Identification and characterization of human endothelial cell membrane binding sites for tissue plasminogen activator and urokinase. *J Biol Chem* 265, 2908-2916 (1990)
6. Valinsky, J. E., E. Reich & N. M. Douarin: Plasminogen activator in the bursa fabricus: correlations with morphogenetic remodeling and cell migration. *Cell* 25, 471-476 (1980)
7. Schafer B. M., K. Maier, U. Eickhoff R. F. Todd & M. D. Kramer: Plasminogen activation in healing human wounds. *Am J Pathol* 144, 1269-1280 (1994)
8. Strickland S. E. Reich & M. I. Sherman: Plasminogen activator in early embryogenesis: Enzyme production by trophoblast and parietal endoderm. *Cell* 9, 231-240 (1976)
9. Gross J. L., D. Moscatelli & D. B. Rifkin: Increased capillary endothelial cell protease activity in response to angiogenic stimuli in vitro. *Proc Natl Acad Sci USA* 80, 2623-2627 (1983)
10. Dano K, P. A. Andreasen, J. Grondahl-Hansen P. Kristensen, L.S. Nielsen & L. Skriver: Plasminogen activators, tissue degradation and cancer. *Adv Cancer Res* 44, 139-266 (1985)
11. Nielsen L. S., G. M. Kallerman, N. Behrendt, R. Picone, K. Dano & F. Blasi: A 55,000-65,000 Mr receptor for urokinase-type plasminogen activator. Identification in human tumor cell lines and partial purification. *J Biol Chem* 263, 2358-2363 (1988)
12. Mak T. W., G. Rutledge & D. J. Sutherland: Androgen-dependent fibrinolytic activity in murine mammary carcinoma (Shionogi SC 115) cells in vitro. *Cell* 7, 223-226 (1976)
13. Ossowski L. & E. Reich: Antibodies to plasminogen activator inhibit human tumor metastasis. *Cell* 35, 611-619 (1983)
14. H. C. Kwaan: Plasminogen-plasmin system in tumor invasion. *Jap J Thromb Haemost* 5, 79-97 (1994)

15. O'Reilly M. S., L. Holmgren, Y. Shing, C. Chen, R. A. Rosenthal, M. Moses, W. S. Lane, Y. Cao, E. H. Sage & J. Folkman: Angiostatin: a novel inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 79, 315-328 (1994)
16. Weitkamp L. K., S. A. Guttormsen & J. S. Schultz: Linkage between the loci for the Lp(a) lipoprotein and plasminogen. *Human Genetics* 79, 80-82 (1988)
17. Armstrong V. W., P. Cremer, E. Eberle, A. Manke, F. Shulze, H. Wieland, H. Kreager, & D. Seidel: The association between serum Lp(a) concentration and angiographically assessed coronary arteriosclerosis. Dependence on serum LDL levels. *Atherosclerosis* 62, 249-257 (1986)
18. Falkenberg M., J. Tjarnstrom, P. Ortenwall, M. Olausson & B. Risberg: Localization of fibrinolytic activators and inhibitors in normal and atherosclerotic vessels. *Thromb. Haemost.* 75, 933-938 (1996)
19. Robbie L. A., N. A. Booth & A. J. Brown: Inhibitors of fibrinolysis are elevated in atherosclerotic plaque. *Arterioscler. Thromb. Vasc. Biol.* 16, 539-545 (1996)
20. Forsgren M., B. Raden, M. Israelsson, K. Larsson & L_O. Heden: Molecular cloning and characterization of a full-length cDNA clone for human plasminogen. *FEBS Lett* 213, 254-260 (1987)
21. Degen S. J. F., S. M. Bell, L. A. Schaefer & R. W. Elliot: Characterization of the cDNA for mouse plasminogen and localization of the gene to mouse chromosome 17. *Genomics* 8, 49-61 (1990)
22. Petersen T. E., M. R. Martzen, A. Ichinose & E. W. Davie: Characterization of the gene for human plasminogen, a key proenzyme in the fibrinolytic system. *J Biol Chem* 265, 6104-6111 (1990)
23. P. A. Sharp: Speculations on RNA splicing. *Cell* 23, 643-646 (1981)
24. Hayes M.L. & F.J. Castellino: Carbohydrate of human plasminogen variants. II. Structure of the asparagine-linked oligosaccharide unit. *J Biol Chem* 254, 8772-8776 (1979a)
25. Hayes, M. L. & F. J. Castellino: Carbohydrate of human plasminogen variants. II. Structure of the O-glycosidically-linked oligosaccharide unit. *J Biol Chem* 254, 8777-8776 (1979b)
26. Wang H., M. Prorok, R. K. Bretthauer & F. J. Castellino: Serine-578 is a major phosphorylation locus in human plasma plasminogen. *Biochemistry* 36, 8100-8106 (1997)
27. Pirie-Shepherd S. R., R. D. Stevens, N. L. Andol, J. J. Enghild & S. V. Pizzo: Evidence for a novel O-linked sialylated trisaccharide on Ser248 of human plasminogen 2. *J Biol Chem* 272, 7408-7411 (1997)
28. G. L. Horton: Isolation of glycopeptides containing O-linked oligosaccharides by lectin affinity chromatography on jacalin-agarose. *Anal Biochem* 191, 262-267 (1990)
29. Robbins K. C., L. Summaria, B. Hsieh & R. J. Shah: The peptide chains of human plasmin. Mechanism of activation of human plasminogen to plasmin. *J Biol Chem* 242, 2333-2342 (1967)
30. Violand B. N. & F. J. Castellino: Mechanism of urokinase-catalyzed activation of human plasminogen. *J Biol Chem* 251, 3906-3912 (1976)
31. Horrevoets A. J. G., A. E. Smilde, J. C. Fredenburgh, H. Pannekoek & M. E. Nesheim: The activation-resistant conformation of recombinant human plasminogen is stabilized by basic residues in the amino-terminal hinge region. *J Biol Chem* 270, 15770-15776 (1995)
32. Sottrup-Jensen L., H. Claeys, M. Zajdel, T. E. Petersen & S. Magnusson: The primary structure of human plasminogen: isolation of two lysine-binding fragments and one "mini" plasminogen (MW, 38,000) by elastase-catalyzed-specific limited proteolysis. *Prog Chem Fibrinolysis and Thrombolysis* 3, 191-209 (1978)
33. Magnusson S., T. E. Petersen, L. Sottrup-Jensen & H. Claeys: Complete primary structure of prothrombin: Isolation and reactivity of ten carboxylated glutamic residues and regulation of prothrombin activation by thrombin. In: *Proteases and biological control.* Eds: Reich E, Rifkin, DB, Shaw, E, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 123-149 (1975)
34. McMullen B. A. & K. Fujikawa: Amino acid sequence of the heavy chain of human a-factor XIIa (activated Hageman factor). *J Biol Chem* 260, 5328-5341 (1985)
35. Pennica D., W. E. Holmes, W. J. Kohr, R. N. Harkins, G. A. Vehar, C. A. Ward, W. F. Bennett, E. Yelverton, P. H. Seeburg, H. L. Heyneker, D. V. Goeddel & D. Collen: Cloning and expression of human tissue-type plasminogen activator cDNA in E. Coli. *Nature* 301, 214-221 (1983)
36. Steffens G. J., W. A. Gunzler, F. Otting, E. Frankus & L. Flohe: The complete amino acid sequence of low molecular mass urokinase from human urine. *Hoppe-Seyler's Z Physiol Chem* 363, 1043-1058 (1982)
37. McLean J. W., J. E. Tomlinson, W-J. Kuang, D. L. Eaton, E. Y. Chen, G. M. Gless, Scanu A. M. & R. M. Lawn: cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. *Nature* 330, 132-137 (1987)
38. Berge A. & U. Sjorbring: PAM, a novel plasminogen-binding protein from Streptococcus pyogenes. *J Biol Chem* 268, 25417-25424 (1993)
39. DiCosta S. S. & M. D. P. Boyle: Interaction of a group A Streptococcus within human plasma results in assembly of a surface plasminogen activator that contributes to occupancy of surface plasmin-binding structures. *Microb Pathog* 24, 341-349 (1998)
40. Wistedt A. C., H. Kotarsky, D. Marti, U. Ringdahl, F. J. Castellino, J. Schaller & U. Sjobring: Kringle 2 mediates high affinity binding to an internal sequence in streptococcal surface protein PAM. *J Biol Chem* 273, 24420-24424 (1998)
41. Urano T., B. A. K. Chibber & F. J. Castellino: The reciprocal effects of epsilon-aminohexanoic acid and chloride ion on the activation of human [Glu1] plasminogen by human urokinase. *Proc Natl Acad Sci USA* 84, 4031-4034 (1987a)
42. Urano T., V. S. de Serrano, P. J. Gaffney & F. J. Castellino: The control of the urokinase-catalyzed activation of human glutamic acid 1-plasminogen by positive and negative effectors. *J Biol Chem* 262, 15959-15964, (1987b)
43. Menhart N., L. Sehl, R. F. Kelley & F. J. Castellino: Construction, expression and purification of recombinant kringle 1 of human plasminogen and analysis of its interaction with omega-amino acids. *Biochemistry* 30, 1948-1957 (1991)
44. Menhart N., S. G. McCance, L. C. Sehl & F. J. Castellino: Functional independence of the kringle 4 and

kringle 5 regions of human plasminogen. *Biochemistry* 32, 8799-8806 (1993)
45. Sehl L. C., & F. J. Castellino: Thermodynamic properties of the binding of alpha, omega-amino acids to the isolated kringle 4 region of human plasminogen as determined by high sensitivity titration calorimetry. *J Biol Chem* 265, 5482-5486 (1990)
46. Hoover G. J., N. Menhart, A. Martin, S. Warder & F. J. Castellino: Amino acids of the recombinant kringle 1 domain of human plasminogen that stabilizes its interaction with ? -amino acids. *Biochemistry* 32, 10936-10943 (1993)
47. McCance S. G., N. Menhart & F. J. Castellino: Amino acid residues of the kringle-4 and kringle-5 domains of human plasminogen that stabilizes their interactions with omega-amino acid ligands. *J Biol Chem* 269, 32405-32410 (1994)
48. Chang Y., I. Mochalkin, S. G. McCance, B. Cheng, A. Tulinsky & F. J. Castellino: Structure and ligand binding determinants of the recombinant kringle 5 domain of human plasminogen. *Biochemistry* 10, 3258-3271 (1998) 1998.
49. Aoki N., M. Moroi, Y. Sakata, N. Yoshida & M. Matsuda: Abnormal plasminogen – a hereditary molecular abnormality found in a patient with recurrent thrombosis. *J Clin Invest* 61, 1186-1195 (1978)
50. Miyata T., S. Iwanaga, Y. Sakata & N. Aoki: Plasminogen Tochigi: Inactive plasmin resulting from replacement of alanine 600 by threonine in the active site. *Proc Natl Acad Sci USA* 79, 6132-6136 (1982)
51. Ichinose A., E. S. Espling, J. Takamatsu, H. Saito, K. Shinmyozu, I. Maruyama, T. E. Petersen, & E. W. Davie: Two types of abnormal genes for plasminogen in families with a predisposition for thrombosis. *Proc Natl Acad Sci USA* 88, 115-119 (1991)
52. Mingers A. M., A. Philapitsch, P. Zeitler, V. Schuster, H. P. Schwarz & H. W. Kreth: Human homozygous type I plasminogen deficiency and ligneous conjunctivitis. *APMIS* 107, 62-72 (1999)
53. Kraft J., W. Lieb, P. Zeitler & V. Schuster: Ligneous conjunctivitis in a girl with severe type I plasminogen deficiency. *Graefe's Archive for Clinical and Experimental Ophthalmology* 238, 797-800 (2000)
54. Schuster V., A-M. Mingers, S. Seidenspinner, Z. Nüssgens, T. Pukrop & H. W. Kreth: Homozygous mutations in the plasminogen gene of two unrelated girls with ligneous conjunctivitis. *Blood* 90, 958-966 (1997)
55. Schuster V., S. Seidenspinner, P. Zeitler, C. Escher, U. Pleyer, W. Bernauer, E. R. Stiehm, S. Isenberg, S. Seregard, T. Olsson, A-M. Mingers, C. Schambeck & H. W. Kreth: Compound-heterozygous mutations in the plasminogen gene predispose to the development of ligneous conjunctivitis. *Blood* 93, 3457-3466 (1999)
56. Ploplis V. A., P. Carmeliet, S. Vazirzadeh, I. Van Vlaenderen, L. Moons, E. F. Plow & D. Collen: Effects of disruption of the plasminogen gene on thrombosis, growth, and health in mice. *Circulation* 92, 2585-2593 (1995)
57. Bugge T. H., M. J. Flick, C. C. Daugherty & J. L. Degen: Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. *Genes & Development* 9, 794-807 (1995)
58. Carmeliet P., L. Schoonjans, L. Kieckens, B. Ream, J. Degen, R. Bronson, R. De Vos, J. J. van den Oord, D. Collen & R. C. Mulligans: Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* 368, 419-424 (1994)
59. Lijnen H. R., P. Carmeliet, A. Bouché, L. Moons, V. A. Ploplis, E. F. Plow & D. Collen: Restoration of thrombolytic potential in plasminogen-deficient mice by bolus administration of plasminogen. *Blood* 88, 870-876 (1996)
60. Hoover-Plow J., N. Wang, & V. Ploplis: Growth and behavioral development in plasminogen gene-targeted mice. *Growth, Development & Aging* 63, 13-32 (1999)
61. Ny A., G. Leonardsson, A-C. Hägglund, P. Hägglöf, V. A. Ploplis, P. Carmeliet & T. Ny: Ovulation in plasminogen-deficient mice. *Endocrinology* 140, 5030-5035 (1999)
62. Lund L. R., S. F. Bjorn, M. D. Sternlicht, B. S. Nielsen, H. Solberg, P. A. Usher, R. Osterby, I. J. Christensen, R. W. Stephens, T. H. Bugge, K. Dano & Z. Werb: Lactational competence and involution of the mouse mammary gland require plasminogen. *Development* 127, 4481-4492 (2000)
63. Ploplis V. A., E. L. French, P. Carmeliet, D. Collen & E. F. Plow: Plasminogen deficiency differentially affects recruitment of inflammatory cell populations in mice. *Blood* 91, 2005-2009 (1998)
64. Lottenberg R., L. E. des Jardin, H. Wang & M. D. P. Boyle: Streptokinase-producing streptococci grown in human plasma acquire unregulated cell-associated plasmin activity. *J Infect Dis* 166, 436-440 (1992)
65. Sodeinde O. A., Y. V. Subrahmanyam, K. Stark, T. Quan, Y. Bao & J. D. Goguen: A surface protease and the invasive character of plague. *Science* 258, 1004-1007 (1992)
66. Gebbia J. A., J. C. G. Monco, J. L. Degen, T. H. Bugge & J. L. Benach: The plasminogen activation system enhances brain and heart invasion in murine relapsing fever borreliosis. *J Clin Invest* 103, 81-87 (1999)
67. Roggwiller E., A-C. Fricaud, T. Blisnick & B. Braun-Breton: Host urokinase-type plasminogen activator participates in the release of malaria merozoites from infected erythrocytes. *Mol Biochem Parasitol* 86, 49-59 (1997)
68. Rosenthal P. J., A. Semenov, V. A. Ploplis & E. F. Plow: Plasminogen activators are not required in the erythrocytic life cycle of malaria parasites. *Mol Biochem Parasitol* 97, 253-257 (1998)
69. D'Costa S. S. & M. D. Boyle: Interaction of a group A Streptococcus within human plasma results in assembly of a surface plasminogen activator that contributes to occupancy of surface plasmin binding structures. *Microbial Pathogen* 24, 341-349 (1998)
70. Ringdahl U., M. Svensson, A. C. Wistedt, T. Renn, R. Kellner, W. Muller-Esterl & U. Sjobring: Molecular co-operation between protein PAM and streptokinase for plasmin acquisition by Streptococcus pyogenes. *J Biol Chem* 273, 6424-6430 (1998)
71. Goguen J. D., T. Bugge & J. L. Degen: Role of the pleiotropic effects of plasminogen deficiency in infection experiments with plasminogen-deficient mice. *Methods* 21, 179-183 (2000)
72. Clowes A. W., M. M. Clowes, Y. P. Au, M. A. Reidy & D. Belin: Smooth muscle cells express urokinase during

mitogenesis and tissue-type plasminogen activator during migration in injured rat carotid artery. *Circ Res* 67, 61-67 (1990)
73. Jackson C. L. & M. A. Reidy: The role of plasminogen activation in smooth muscle cell migration after injury. *Ann NY Acad Sci* 667, 141-150 (1992)
74. Reidy M. A., C. Irvin & V. Lindner: Migration of arterial wall cells. Expression of plasminogen activators and inhibitors in injured rat arteries. *Circ Res* 78, 405-414 (1996)
75. Libby P. D., D. Schwartz, E. Brogi, H. Tanaka & S. K. Clinton: A cascade model for restenosis. A special case of atherosclerotic progression. *Circulation* 86 (Suppl. III), 47-52 (1992)
76. Reidy M. A., D. Jackson & V. Lindner: Neointimal proliferation: control of vascular smooth muscle cell growth. *Vasc Med Rev* 3, 156-167 (1992)
77. Carmeliet P., L. Moons, V. Ploplis, E. Plow & D. Collen: Impaired arterial neointima formation in mice with disruption of the plasminogen gene. *J Clin Invest* 99, 200-208 (1997)
78. Russell M. E., A. F. Wallace, W. W. Hancock, M. H. Sayegh, D. H. Adams, N. E. Sibinga, L. R. Wyner & M. J. Karnovsky: Upregulation of cytokines associated with macrophage activation in the Lewis-to-F344 rat transplantation model of chronic cardiac rejection. *Transplantation* 59, 572-578 (1995)
79. P. Libby: Molecular bases of the acute coronary syndromes. *Circulation* 91, 2844-2850 (1995)
80. Nagano H., R. N. Mitchell, M. K. Taylor, S. Hasegawa, N. L. Tilney, & P. Libby: Interferon-gamma deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. *J Clin Invest* 100, 550-557 (1997)
81. Shi C., M. E. Russell, C. Bianchi, J. B. Newell & E. Haber: Murine model of accelerated transplant arteriosclerosis. *Circ Res* 75, 199-207 (1994)
82. Moons L., C. Shi, V. Ploplis, E. Plow, E. Haber D. Collen & P. Carmeliet: Reduced transplant arteriosclerosis in plasminogen-deficient mice. *J Clin Invest* 102, 1788-1797 (1998)
83. Shi C., A. Patel, D. Zhang, H. Wang, P. Carmeliet, G. L. Reed, M-E. Lee, E. Haber, & N. E. S. Sibinga: Plasminogen is not required for neointima formation in a mouse model of vein graft stenosis. *Circ Res* 84, 883-890 (1999)
84. Jokinen M. P., T. B. Clarkson & R. W. Prichard: Recent advances in molecular pathology: animal models in atherosclerotic research. *Exp Mol Pathol* 42, 1-28 (1985)
85. Knowles J. W. & N. Maeda: Genetic modifiers of atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 20, 2336-2345 (2000)
86. Zhang S. H., R. L. Reddick, J. A. Piedrahita & N. Maeda: Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258, 468-471 (1992)
87. Ishibashi S., M. S. Brown, J. L. Goldstein, R. D. Gerard, R. E. Hammer & J. Herz: Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J Clin Invest* 92, 883-893 (1993)
88. Xiao Q., M. J. S. Danton, D. P. Witte, M. C. Kowala, M. T. Valentine, T. H. Bugge, & J. L. Degen: Plasminogen deficiency accelerates vessel wall disease in mice predisposed to atherosclerosis. *Proc Natl Acad Sci USA* 94, 10335-10340 (1997)
89. Kockx M. M., G. R. DeMeyer, W. A. Jacob, H. Bult & A. G. Herman: Triphasic sequence of neointimal formation in the cuffed carotid artery of the rabbit. *Arterioscler Thromb* 12, 1447-1457 (1992)
90. Hirosumi J., A. Nomoto, Y. Ohkubo, C. Sekiguchi, S. Mutoh, I. Yamaguchi & H. Aoki: Inflammatory responses in cuff-induced atherosclerosis in rabbits. *Atherosclerosis* 64, 243-254 (1987)
91. Drew A. F., H. L. Tucker, K. W. Kombrinck, D. I. Simon, T. H. Bugge & J. L. Degen: Plasminogen is a critical determinant of vascular remodeling in mice. *Circ Res* 87, 133-139 (2000)
92. Busuttil S. J., C. Drumm, V. A. Ploplis & E. F. Plow: Endoluminal arterial injury in plasminogen deficient mice. *J Surg Res* 91, 159-164 (2000)
93. Morioka S., G. S. Lazarus, J. L. Baird & P. L. Jensen: Migrating keratinocytes express urokinase-type plasminogen activator. *J Invest Dermatol* 88, 418-423 (1987)
94. Grondahl-Hansen J., L. R. Lund, E. Ralfkiaer, V. Ottevanger & K. Dano: Urokinase- and tissue-type plasminogen activators in keratinocytes during wound reepithelialization in vivo. *J Invest Dermatol* 90, 790-795 (1988)
95. Romer J., L. R. Lund, J. Eriksen, E. Ralfkiaer, R. Zeheb, T. D. Gelehrter, K. Dano & P. Kristensen: Differential expression of urokinase-type plasminogen activator and its type-1 inhibitor during healing of mouse skin wounds. *J Invest Dermatol* 97, 803-811 (1991)
96. Romer J., L. R. Lund, J. Eriksen, C. Pyke, P. Kristensen & K. Dano: The receptor for urokinase-type plasminogen activator is expressed by keratinocytes at the leading edge during re-epithelialization of mouse skin wounds. *J Invest Dermatol* 102, 519-522 (1994)
97. Schaefer B. M., K. Maier, U. Eickhoff, R. F. Todd & M. D. Kramer: Plasminogen activation in healing human wounds. *Am J Pathol* 144, 1269-1280 (1994)
98. Romer J., T. H. Bugge, C. Pyke, L. R. Lund, M. J. Flick, J. L. Degen & K. Dano: Impaired wound healing in mice with a disrupted plasminogen gene. *Nat Med* 2, 287-291 (1996)
99. Bugge T. H., K. W. Kombrinck, M. J. Flick, C. C. Daugherty, M. J. Danton & J. L. Degen: Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell* 87, 709-719 (1996)
100. Drew A. F., H. L. Schiman, K. W. Kombrinck, T. H. Bugge, J. L. Degen & A. H. Kaufman: Persistent corneal haze after excimer laser photokeratectomy in plasminogen-deficient mice. *Invest Ophthal Vis Sci* 41, 67-72 (2000)
101. Kao W. W., C. W. Kao, A. H. Kaufman, K. W. Kombrinck, R. L. Converse, W. V. Good, T. H. Bugge & J. L. Degen: Healing of corneal epithelial defects in plasminogen- and fibrinogen-deficient mice. *Invest Ophthalmol Vis Sci* 39, 502-508 (1998).
102. Roselli H. T., M. Su, K. Washington, D. M. Kerins, D. E. Vaughan & W. E. Russell: Liver regeneration is transiently impaired in urokinase-deficient mice. *Am J Physiol* 275, G1472-G1479 (1998)
103. Bezerra J. A., T. H. Bugge, H. Melin-Aldana, G. Sabla, K. W. Kombrinck, D. P. Witte & J. L. Degen:

Plasminogen deficiency leads to impaired remodeling after toxic injury to the liver. *Proc Natl Acad Sci US*A 96, 15143-15148 (1999)
104. Cleutjens J. P. M., J. C. Kandala, E. Guarda, R. V. Guntaka & K. T. Weber: Regulation of collagen degradation in the rat myocardium after infarction. *J Mol Cell Cardiol* 27, 1281-1292 (1994)
105. Dollery C. M., J. R. McEwan & A. M. Henney: Matrix metalloproteinases and cardiovascular disease. *Circ Res* 77, 863-868 (1995)
106. Heymans S., A. Luttun, D. Nuyens, G. Theilmeier, E. Creemers, L. Moons, G. D. Dyspersin, J. P. M. Cleutjens, M. Shipley, A. Angellilo, M. Levi, O. Nube, A. Baker, E. Keshet, F. Lupu, J. M. Herbert, J. F. Smits, S. D. Shapiro, M. Baes, M. Borgers, D. Collen, M. J. Daemen & P. Carmeliet: Inhibition of plasminogen activators or matrix metalloproteinases prevent cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. *Nat Med* 10, 1135-1142 (1999)
107. Creemers E., J. Cleutjens, J. Smits, S. Heymans, L. Moons, D. Collen, M. Daemen & P. Carmeliet: Disruption of plasminogen gene in mice abolishes wound healing after myocardial infarction. *Am J Pathol* 156, 1865-1873 (2000)
108. Lutgens E., M. J. A. P. Daemen, E. D. de Muinck & J. F. M. Smits: Chronic myocardial infarction in mice: structural and functional consequences. *Cardiovasc Res* 41, 586-593 (1999)
109. Tsirka S. E., A. Gualandris, D. G. Amaral & S. Strickland: Excitoxin induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. *Nature* 377, 340-344 (1995)
110. Sappino A., R. Madani, J. Huarte, D. Belin, J. Kiss, A. Wohlwend & J-D. Vassali: Extracellular proteolysis in the adult murine brain. *J Clin Invest* 92, 679-685 (1993)
111. Tsirka S. E., A. D. Rogove, T. H. Bugge, J. Degen & S. Strickland: An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. *J Neurosci* 17, 543-552 (1997)
112. Tsirka S. E., T. H. Bugge, J. L. Degen & S. Strickland: Neuronal death in the central nervous system demonstrates a non-fibrin substrate for plasmin. *Proc Natl Acad Sci USA* 94, 9779-9781 (1997)
113. Z. L. Chen & S. Strickland: Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. *Cell* 26, 917-925 (1997)
114. Akassoglou K., K. W. Kombrinck, J. L. Degen & S. Strickland: Tissue plasminogen activator-mediated fibrinolysis protects against axonal degeneration and demyelination after sciatic nerve injury. *J Cell Biol* 149, 1157-1166 (2000)
115. Wang Y. F., S. E. Tsirka, S. Strickland, P. E. Stieg, S. G. Soriano & S. A. Lipton: Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. *Nat Med* 4, 228-231 (1998)
116. Nagai N., M. De Mol, H. R. Lijnen, P. Carmeliet & D. Collen: Role of plasminogen system components in focal cerebral ischemic infarction. A gene targeting and gene transfer study in mice. Circulation 99, 2440-2444 (1999)
117. D. Hanahan & J. Folkman: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86, 353-364 (1996)
118. Yu W., J. Kim & L. Ossowski: reduction in surface urokinase receptor forces malignant cells into a protracted state of dormancy. *J Cell Biol* 137, 767-777 (1997)
119. S. Monaham: Biological significance of the expression of uPARs in brain tumor. *Bioscience* 4, d178-187 (1999)
120. Zhang X., Z. Fei, X. Bu, H. Zhen, Z. Zhang, J. Gu & Y. Chen: Expression and significance of urokinase type plasminogen activator gene in human brain gliomas. *J Surg Oncol* 74, 90-94 (2000)
121. Swiercz R., J. D. Wolfe, A. Zaher & J. Jankun: Expression of the plasminogen activation system in kidney cancer correlates with its aggressive phenotype. *Clin Cancer Res* 4, 869-877 (1998)
122. Abe J., T. Urano, H. Konno, Y. Erhan, T. Tanaka, N. Nishino, A. Takada & S. Nakamura: 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, 2602-2611 (1999)
123. Bianchi E., R. L. Cohen, A. T. Thor, R. F. Todd, I. F. Mizukami, D. A. Lawrence, B. M. Ljung, M. A. Shuman & H. S. Smith: The urokinase receptor is expressed in invasive breast cancer but not in normal breast tissue. *Cancer Res* 54, 861-866 (1994)
124. Pedersen H., N. Brunner, D. Francis, K. Osterlind, E. Ronne, H. H. Hansen, K. Dano, & J. Grondahl-Hansen: Prognostic impact of urokinase, urokinase receptor, and type 1 plasminogen activator inhibitor in squamous and large cell lung cancer tissue. *Cancer Res* 54, 4671-4675 (1994)
125. Shapiro R. L., J. G. Duquette, D. F. Roses, I. Nunes, M. N. Harris, H. Kamino, E. L. Wilson & D. B. Rifkin: Induction of primary cutaneous melanocytic neoplasms in urokinase-type plasminogen activator (uPA)-deficient and wild-type mice: cellular blue nevi invade but do not progress to malignant melanoma in uPA-deficient animals. *Cancer Res* 56, 3597-3604 (1996)
126. Gutierrez L. S., A. Schulman, T. Brito-Robinson, F. Noria, V. A. Ploplis & F. J. Castellino: Tumor development is retarded in mice lacking the gene for urokinase-type plasminogen activator or its inhibitor, plasminogen activator inhibitor-1. *Cancer Res* 60, 5839-5847 (2000)
127. Bugge T. H., K. W. Kombrinck, Q. Xiao, K. Holmbäck, C. C. Daugherty. D. P. Witte & J. L. Degen: Growth and dissemination of Lewis Lung Carcinoma in plasminogen-deficient mice. *Blood* 90, 4522-4531 (1997)
128. Bugge T. H., L. R. Lund, K. K. Kombrinck, B. S. Nielsen, K. Holmbäck, A. F. Drew, M. J. Flick, M. D. P. Witte, K. Dano, & J. L. Degen: Reduced metastasis of Polyoma virus middle T antigen-induced mammary cancer in plasminogen-deficient mice. *Oncogene* 16, 3097-3104 (1998)
129. P. Kincaid-Smith: Coagulation and renal disease. *Kidney Int* 2, 183-190 (1972)
130. Holdsworth S. R., N. M. Thomson, E. F. Glasgow & R. C. Atkins: The effect of defibrination on macrophage participation in rabbit nephrotoxic nephritis: studies using glomerular culture and electron microscopy. *Clin Exp Immunol* 37, 38-43 (1979)
131. Thomson, N. M., J. Moran, I. J. Simpson & D. K. Peters: Defibrination with ancrod in experimental immune complex nephritis. *Clin Exp Immunol* 20, 527-557 (1975)
132. Naish P., G. B. Penn, D. J. Evans & D. K. Peters: The effect of defibrination on nephrotoxic serum nephritis in rabbits. *Clin Sci* 42, 643-646 (1972)

133. Malliaros J., S. R. Holdsworth, J. Wojta, J. Erlich & P. G. Tipping: Glomerular fibrinolytic activity in antiGBM glomerulonephritis in rabbits. *Kidney Int* 44, 557-564 (1993)
134. Rondeau E., B. Mougenot, R. Lacave, M. N. Peraldi, E. K. O. Kruithof & J. D. Sraer: Plasminogen activator inhibitor 1 in renal deposits of human nephropathies. *Clin Nephrol* 33, 55-60 (1990)
135. Kitching R., S. R. Holdsworth, V. A. Ploplis, E. F. Plow, D. Collen, P. Carmeliet & P. G. Tipping: Plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis. *J Exp Med* 185, 963-968 (1997)
136. Phan S. H., R. S. Thrall & C. Williams: Bleomycin-induced pulmonary fibrosis: effects of steroid on lung collagen metabolism. *Am Rev Respir Dis* 124, 428-434 (1981)
137. I. Y. Adamson & D.H. Bowden: The pathogenesis of bleomycin-induced pulmonary fibrosis. *Am J Pathol* 77, 185-197 (1974)
138. Eitzman D. T., R. D. McCoy, X. Zheng, W. P. Fay, T. Shen, D. Ginsburg & R. H. Simon: Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest* 97, 232-237 (1996)
139. Hart D. A., P. Whidden, F. Green, J. Henkin & D. E. Woods: Partial reversal of established bleomycin-induced pulmonary fibrosis by rh-urokinase in a rat model. *Clin Invest Med* 17, 69-76 (1994)
140. Sisson T. H., N. Hattori, Y. Xu & R. H. Simon: Treatment of bleomycin-induced pulmonary fibrosis by transfer of urokinase-type plasminogen activator genes. *Hum Gene Ther* 10, 2315-2323 (1999)
141. Swaisgood C. M., E. L. French, C. Noga, R. H. Simon & V. A. Ploplis: The development of bleomycin-induced pulmonary fibrosis in mice deficient for components of the fibrinolytic system. *Am J Pathol* 157, 177-187 (2000)
142. Ploplis V. A., J. Wilberding, L. McLennan, Z. Liang, I. Cornelissen, M. DeFord, E. D. Rosen & F. J. Castellino: A total fibrinogen deficiency is compatible with the development of pulmonary fibrosis in mice. *Am J Pathol* 157, 703-708 (2000)
143. Hattori N., J. L. Degen, T. H. Sisson, H. Liu, B. B. Moore, R. G. Pandrangi, R. H. Simon & A. F. Drew: Bleomycin-induced pulmonary fibrosis in Fg-null mice. *J Clin Invest* 106, 1341-1350 (2000)

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