SERPINE1: A Molecular Switch in the Proliferation-Migration Dichotomy in Wound-“Activated” Keratinocytes

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Significance: A highly interactive serine protease/plasmin/matrix metallo-proteinase axis regulates stromal remodeling in the wound microenvironment. Current findings highlight the importance of stringent controls on protease expression and their topographic activities in cell proliferation, migration, and tissue homeostasis. Targeting elements in this cascading network may lead to novel therapeutic approaches for fibrotic diseases and chronic wounds.

Recent Advances: Matrix-active proteases and their inhibitors orchestrate wound site tissue remodeling, cell migration, and proliferation. Indeed, the serine proteases urokinase plasminogen activator and tissue-type plasminogen activator (uPA/tPA) and their major physiological inhibitor, plasminogen activator inhibitor-1 (PAI-1; serine protease inhibitor clade E member 1 [SERPINE1]), are upregulated in several cell types during injury repair. Coordinate expression of proteolytic enzymes and their inhibitors in the wound bed provides a mechanism for fine control of focal proteolysis to facilitate matrix restructuring and cell motility in complex environments.

Critical Issues: Cosmetic and tissue functional consequences of wound repair anomalies affect the quality of life of millions of patients in the United States alone. The development of novel therapeutics to manage individuals most affected by healing anomalies will likely derive from the identification of critical, translationally accessible, control elements in the wound site microenvironment.

Future Directions: Activation of the PAI-1 gene early after wounding, its prominence in the repair transcriptome and varied functions suggest a key role in the global cutaneous injury response program. Targeting PAI-1 gene expression and/or PAI-1 function with molecular genetic constructs, neutralizing antibodies or small molecule inhibitors may provide a novel, therapeutically relevant approach, to manage the pathophysiology of wound healing disorders associated with deficient or excessive PAI-1 levels.

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SCOPE AND SIGNIFICANCE

TRANSCRIPTIONAL ACTIVATION of a global “wound repair” program accompanies the tissue response to trauma. One particular gene induced in response to injury encodes plasminogen activator inhibi-

tor type-1 (PAI-1; SERPINE1), a member of the serine protease inhibitor (SERPIN) gene family and the major physiologic regulator of the urokinase plasminogen activator (uPA)-dependent pericellular plasmin-generating cascade. Control

of PAI-1 expression/activity is critical to repair outcomes. Deficient or elevated levels of this SERPIN are causative factors in healing anomalies including excessive bleeding, thrombosis, multi-organ fibrosis, and impaired wound resolution. This paper reviews the roles of PAI-1 in stromal remodeling, cell growth, and migration.

TRANSLATIONAL RELEVANCE

PAI-1 modulates a urokinase plasminogen activator → plasmin-generating system that regulates the overall pericellular proteolytic cascade. PAI-1 titrates the extent and locale of collagen remodeling while facilitating cell migration and proliferation as part of the tissue repair program. Clarification of individual cascading pathways in this highly interdependent network of matrix proteases and protease inhibitors will provide for the rational design of focused therapies. The development of small molecule inhibitors of PAI-1 (*i.e.*, tiplaxtinin or PAI-039) that effectively attenuate pathologic remodeling suggest that targeting this SERPIN may have translational implications for treatment of chronic wounds and fibrotic disorders.

CLINICAL RELEVANCE

Chronic wounds and excessive scarring result in reduced quality of life, increased mortality/morbidity, and diminished self-esteem. The duration of the inflammatory phase, controls on proliferation, migration, apoptosis, and matrix proteolysis dictate repair outcomes. Dysregulation of PAI-1 expression in response to deficient or elevated wound site growth factors is common to repair anomalies at all tissue sites. Small molecule PAI-1 inhibitors or targeted manipulation of PAI-1 expression may have therapeutic potential in excessive scarring disorders (fibrosis) and difficult-to-heal injuries. Clarification of PAI-1 functions and identification of expression-control elements are important to the development of molecular therapeutics for PAI-1-dependent healing anomalies.

DISCUSSION

The cellular response to cutaneous injury initiates changes in gene expression required to integrate the various temporal and spatial facets of tissue repair.^{1–3} The development of elegant *in vitro* and *ex vivo* systems that recapitulate specific wound stages provide opportunities to define “trauma-activated” mechanisms that contribute to both normal and pathologic outcomes.^{2,4–7} While the healing program likely varies among the par-

ticipating elements, acquisition of a core “plastic” signature characterizes transdifferentiation between the sessile and migratory phenotypes. Prominently involved genes are those that impact control of pericellular proteolysis and matrix restructuring. These include members of the serine protease and matrix metalloproteinase (MMP) families and their respective inhibitors (*i.e.*, the serine protease inhibitors [SERPINs] and the tissue inhibitors of MMPs [TIMPs]).^{2,8–12} The SERPINs are particularly important injury-response factors as they participate in virtually all stages of repair where they regulate coagulation, fibrinolysis, inflammation, myofibroblast differentiation, and stromal remodeling. Data mining of the repertoire of plastic genes in human keratinocytes, moreover, indicated that transcripts highly expressed in the epithelial motile cohort encode urokinase plasminogen activator (uPA) and its major negative regulator plasminogen activator inhibitor type-1 (PAI-1 or SERPINE1), both critical components of the pericellular plasmin-generating cascade.^{2,6,13–15}

PAI-1 is induced in migratory cells regardless of lineage, as well as in epithelial cells undergoing epithelial-to-mesenchymal (EMT)-like transitions upon expression of the E-cadherin transcriptional repressors Snail, Slug, or E47.^{16,17} Keratinocytes and other epithelia undergo at least a partial EMT following wounding or directional migration^{18–20} with up-regulation of uPA, the uPA cell surface receptor (uPAR), and PAI-1 at the migratory front.⁶ Motile cells focalize both uPA (via interaction with uPAR) and PAI-1 (upon binding to uPA/uPAR) to the leading edge, where they function in the interrelated events of matrix restructuring and migration.^{14,21} Elements of the uPA system and the low-density lipoprotein receptor-related protein 1 (LRP1), furthermore, localize to filopodia of invasive cells and formation/internalization of the uPAR/uPA/PAI-1/LRP1 complex is required for persistent cellular locomotion.²² Fine control of focal proteolysis reorganizes extracellular matrix (ECM) architecture, affecting the dynamics of cell–ECM interactions, releases bioactive ECM fragments, and activates growth factors that stimulate migratory behavior.²² Current data are consistent with a model in which PAI-1 functions as a rheostat to regulate the spatial-temporal control of pericellular proteolysis in response to tissue injury.

PAI-1 in wound repair: *in vitro* models provide windows into *in vivo* function

PAI-1 is required for transforming growth factor beta 1 (TGF- β 1)-stimulated migration of

immortalized keratinocytes over planar substrates and through more physiological 3-D barriers.¹³ Immobilized PAI-1 functions, in fact, in a matrix-facilitating amoeboid motility with activation of the relevant signaling pathways.²³ PAI-1 is induced upon tissue trauma or during reactivation of a wound repair program *in vitro*.^{24–26} The regional restriction of uPA/PAI-1 expression, as well as the spatial/temporal distinctions among the differentiated, motile, and proliferative compartments typical of *in vivo* injury repair, are recreated during cell migration into the denuded areas of a scrape-injured monolayer.⁶ PAI-1 mRNA/protein increase rapidly in cultured epithelial cells bordering experimentally produced monolayer scrape wounds and remain elevated over the course of monolayer “healing,”^{2,14,26} closely mimicking expression events in acute epidermal injuries.^{27,28} Analysis of rather creative coupled transcriptional activation-*in situ* wound models indicated that a PAI-1 promoter-driven chimeric PAI-1 coding sequence-GFP “reporter” is expressed specifically at injury-edge cells upon trauma-induced activation and prominently “marked” keratinocyte migration trails.^{21,26} These findings led to the hypothesis that PAI-1 deposition at the cellular undersurface stimulates detachment–reattachment processes acting, thereby, as a locomoting force to propel cells away from the zone of PAI-1 accumulation. Collectively, the available data suggest that PAI-1 coordinates cycles of cell-to-substrate adhesion/disadhesion and/or maintains a repair scaffold to meet the requirements for efficient cellular migration.²² Expression alone, however, does not imply functionality, necessitating more critical assessments. Indeed, PAI-1 knock-down or antibody-mediated functional inhibition attenuated injury repair in various keratinocyte and non-epidermal cell types similar to findings in PAI-1^{−/−} cells.²⁶ Importantly, the defect in wound-associated migratory activity as a consequence of antisense-mediated PAI-1 down-regulation in cultured keratinocytes was effectively reversed (*i.e.*, “rescue” of the normal migratory response) by addition of recombinant PAI-1 immediately after scrape injury.²⁶

PAI-1 as a signaling “ligand”: consequences on cell migration

LRP1 ligand binding and/or complex formation with integrins, growth factor receptors, and proteoglycans activates intracellular signaling pathways impacting cell proliferation and migration with the motile response involving activation of Rho GTPases.²² PAI-1 can also function in a sig-

naling capacity directly affecting cell migration through engagement of LRP1 and the very low density lipoprotein receptor (VLDLR),²⁹ albeit perhaps localized on different cells within the wound bed. This would allow PAI-1 to differentially regulate the function of various cell types within the injury site microenvironment. Dissection of the involved mechanisms, however, indicates that the migration-promoting activity of PAI-1 does not require an intact protein or its protease-inhibitory property. The neutrophil elastase-cleaved form of PAI-1 is able to bind to LRP1 and impact downstream signaling, suggesting that this inactive molecule (at least with regard to its protease-inhibitory function) retains the ability to affect certain fundamental cellular processes,²⁹ but perhaps not others. Keratinocyte apoptosis, for example, is plasmin dependent;²⁶ cleaved PAI-1 may not protect keratinocytes from plasmin-initiated apoptosis in a protease-rich environment due to its inability to inhibit plasmin activation. Plasmin generation, however, is required for injury repair. Plasminogen-deficient mice exhibit a severe wound healing impairment due to retention of a significant fibrin-rich barrier to keratinocyte migration.³⁰ Thus, fine-tuning of not only expression of PAI-1, but also changes in conformational state, likely dictates its functionality with consequences on repair efficacy. The proteolytic balance, moreover, is quite different in acute as compared to chronic wounds; development of uPA/PAI-1 complexes appears to be a useful biomarker to predict opposing outcomes.^{10–12}

There are several different conformations of PAI-1 (active, latent, cleaved) and each stimulates cellular migration into 3-D collagen gels through a LRP1-dependent mechanism.²⁹ The Arg-76, Lys-80, Lys-88, Arg-118, and Lys-122 helix D residues in PAI-1 are part of the cryptic high-affinity binding site for both LRP and VLDLR, which is exposed upon formation of a complex with uPA.³¹ uPA–PAI-1 interactions with members of the LDLR family can impact signaling directly by activating their cytoplasmic domains or indirectly via uPA/uPAR.²² Although the precise mechanism is not clear, PAI-1 binding to LRP1 activates Jak/Stat signaling, resulting in actin filament polarization, nuclear translocation of activated Stat1, and increased cell migration.²⁹ All three forms of PAI-1, in fact, increase LRP1-dependent cell motility with activation of the Jak/Stat1 pathway.^{29,32} The elastase-cleaved molecule, moreover, while catalytically inactive, is as efficient as the full-length PAI-1 in promoting Jak/Stat activation and migration. While active PAI-1 is routinely cleared

from the extracellular environment in a complex with uPA/uPAR/LRP1, latent and cleaved species of PAI-1, with a preserved motile function, remain embedded in the matrix, likely serving as a reservoir to maintain cell movement. It is apparent, therefore, that key elements in the plasminogen-to-plasmin generating cascade, including PAI-1 and its functionally distinct conformations, are well poised to significantly impact various pathways in the wound repair program.

PAI-1 as a modulator of integrin function

PAI-1 also regulates integrin internalization in an LRP1-dependent manner^{33,34} resulting in cell detachment from various substrates.³³ This mechanism differs, however, from that which modulates PAI-1-stimulated migration directly via LRP1, as the requirement for uPA and uPAR may be restricted to the de-adhesion phase.^{29,32,35} Although LRP1-mediated integrin endocytosis may not be necessary for efficient cell detachment, co-endocytosis of integrins in a complex with uPAR would allow for their subcellular redistribution (*i.e.*, to the leading edge) to support cell locomotion and stromal invasion. While interactions between PAI-1 and uPA/uPAR/integrin complexes would enhance the integrin/uPAR attachment-detachment-reattachment cycle, thereby increasing cell motility, PAI-1 can utilize multiple avenues to impact LRP1-dependent movement. Complicating this process is the potential for PAI-1 to modulate syndecan-dependent keratinocyte migration. Following injury, wound margin keratinocytes synthesize and deposit unprocessed laminin-332, supporting syndecan-1 binding through the LG4/5 domain. PAI-1, which is also expressed by the wound edge epithelium, stabilizes this interaction by preventing plasmin-initiated proteolytic processing of laminin-332³⁶ and syndecan-1 shedding.³⁷ The presence of vitronectin at the wound edge can augment this process through its ability to bind PAI-1 and extend the half-life of active PAI-1 as well as engage syndecan-1. PAI-1, by titrating pericellular levels of active plasmin, promotes syndecan-1 dependent migration on unprocessed laminin-332 by preventing cleavage of the syndecan binding site LG4/5. As the proteolytic environment matures, PAI-1 and vitronectin are endocytosed and degraded.²² Syndecan-1 binding is lost due to proteolytic processing of laminin-332, as well as syndecan-1 ectodomain shedding; $\alpha_3\beta_1$ binding to processed laminin-332 begins to slow keratinocyte migration and initiate hemidesmosome formation^{36,38} (Fig. 1).

Vitronectin stabilizes PAI-1 in an active conformation, extending its half-life, potentially ampli-

fying the inhibition of focal proteolysis modulating and thereby the extent, locale, and duration of matrix remodeling. This is particularly important in the context of cutaneous injury where restoration of barrier function and tissue integrity is dependent upon keratinocyte movement. PAI-1 and vitronectin are both released from the α -granules of platelets during hemostasis, where their combined presence would presumably promote the formation of a fibrin clot and subsequently contribute to provisional matrix remodeling.³⁹ Vitronectin expression, however, is limited under normal physiological conditions but enhanced under circumstances requiring stromal remodeling (*i.e.*, wound repair⁴⁰), suggesting a continuous, albeit dynamic, molecular interaction with PAI-1 of potential physiologic significance.

While PAI-1/vitronectin complexes facilitate proteolytically based migratory processes, the interaction of these two proteins also affects cell motility through mechanisms that directly modulate cell surface receptor binding. Vitronectin promotes cellular locomotion via arginine-glycine-aspartate (RGD)-dependent interactions with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, as well as through binding to uPAR.²² The recognition site for PAI-1 on vitronectin, however, approximates those for both integrin and uPAR docking,⁴¹ and as a result, the interaction of PAI-1 with vitronectin regulates the ability of these receptors to engage vitronectin. In addition, PAI-1/vitronectin-binding blocks PAI-1 interaction with LRP1, thus preventing LRP1-dependent migration signaling.³² PAI-1, as well as being an important determinant in cell-to-substrate attachment, also affects cellular release from vitronectin by two distinct mechanisms. The affinity of PAI-1 for vitronectin is significantly higher than that of uPAR for vitronectin. Consequently, PAI-1 can competitively displace uPAR from vitronectin, initiating detachment of cells that rely mainly on uPAR for cell adhesion to vitronectin.⁴¹ However, PAI-1 is unable to promote its binding to vitronectin by competitive displacement of pre-engaged integrins. In the presence of uPA/uPAR/ α_v -integrin complexes, however, PAI-1 binding to complexed uPA will initiate integrin deactivation, promoting their detachment from vitronectin and endocytic clearance. These receptors are subsequently recycled back to the cell surface to re-engage matrix molecules and promote cell migration (Fig. 2).

PAI-1 impacts keratinocyte proliferation/migration transitions

One of the most interesting possibilities to emerge recently is the concept that certain

Figure 1. Plasminogen activator inhibitor-1 (PAI-1) regulates cell migration by modulating the pericellular proteolytic microenvironment. Interaction of active or cleaved PAI-1 with low-density lipoprotein receptor-related protein 1 (LRP1) can trigger Jak/Stat1 signaling events in a urokinase plasminogen activator (uPA)/uPA receptor (uPAR)-dependent or independent manner, leading to enhanced cell migration. PAI-1 binding to the uPAuPAR/integrin complexes will result in inhibition of the plasmin-generating proteolytic cascade, deactivation of integrins and their detachment from specific ECM ligands, and subsequent endocytic clearance of these complexes through a LRP1-dependent process. Integrins, uPAR, and LRP1 will recycle back to the cell surface. Uncontrolled uPA activity will lead to accumulation of plasmin in the cell microenvironment allowing activation of transforming growth factor beta 1 (TGF- β) and TGF- β -dependent upregulation of PAI-1. Downregulation of active plasmin will foster syndecan-1-dependent cell migration on unprocessed laminin-332 by blocking the cleavage of syndecan-1 binding site LG4/5 on laminin-332. Increased PAI-1 concentration will also block plasmin-dependent shedding of syndecan-1, promoting cell motility on laminin-332. Over time, PAI-1 levels will decrease in a progressing wound, allowing $\alpha_3\beta_1$ and $\alpha_6\beta_1$ (not shown) to engage the processed form of laminin-332 stimulating the formation of hemidesmosomes. EE, early endosome; Lyso, lysosome; ECM, extracellular matrix; Syn-1, syndecan-1; plg, plasminogen. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

motility-stimulating factors also promote, perhaps transiently as in the specific context of wound repair, growth inhibition as part of the “go or grow” proliferation/migration dichotomy.^{42,43} Senescence-associated growth arrest genes, including p16^{INK4a} and PAI-1, may actually participate in the tissue injury repair program by inhibiting proliferation while promoting migration.^{44–46} Wound leading edge keratinocytes are, in fact, less mitotically

active than cells more distant from the injury front and also express significantly greater levels of PAI-1 than the uninvolved epithelium.^{6,27,47} This has functional consequences regarding induced PAI-1 transcription/expression as a proliferation versus motile regulator of the cutaneous wound repair program, particularly when coupled with the identification of PAI-1 as a p53 target gene.⁴⁶ PAI-1 is a major contributor to the replicative

Figure 2. PAI-1 modulates cell motility on vitronectin matrices by regulating receptor-mediated adhesion. Cell attachment to vitronectin is mediated through cell surface integrins and uPA/uPAR complexes. In a PAI-1-rich cellular microenvironment, secreted PAI-1 binds to and inactivates uPA. Conformational changes in uPA are transmitted to uPAR and integrins, consequently decreasing the affinities of uPAR and integrins for vitronectin. Receptor detachment from vitronectin and their LRP1-mediated endocytic clearance allows for receptor recycling to the leading edge of the migrating cell. The unoccupied PAI-1 binding site in the SMB domain of vitronectin can now bind PAI-1, thus blocking the overlapping binding site for uPAR directly, and sterically hindering rebinding of integrins to the adjacent RGD sequence, thereby preventing cell attachment to vitronectin. In addition, should excess PAI-1 be buffered by binding to vitronectin, the PAI-1/LRP1 interaction is inhibited and LRP1-mediated stimulated Jak/Stat1 signaling suppressed. If recycled integrins are allowed to engage with unoccupied vitronectin first, excess PAI-1 is unable to displace these integrins and they become available for complex formation with uPA/uPAR. Collectively, these dynamics promote cell movement away from a PAI-1-abundant vitronectin-rich matrix onto alternative substrates that cannot be saturated by PAI-1 and where PAI-1 only regulates cell attachment through interaction with uPA/uPAR complexes. RGD, tripeptide (arginine-glycine-aspartate) integrin binding site in vitronectin; SMB, somatomedin B domain of vitronectin; VN, vitronectin. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

senescence program in various cell types and mediates TGF- β 1-induced growth restriction in human keratinocytes.⁴⁸ PAI-1 genetic deficiency or PAI-1 expression suppression by RNA interference leads to escape from both senescence-associated proliferative arrest and TGF- β 1-induced cytoskeleton in primary fibroblasts and human (HaCaT) keratinocytes.^{46,48} In support of these findings,

exogenous addition of recombinant PAI-1 to human keratinocytes effectively initiates a G₁ arrest and suppresses endothelial growth factor (EGF)- or fetal bovine serum (FBS)-stimulated growth (Fig. 3) while adenoviral-mediated PAI-1 overexpression dramatically inhibits colony expansion (Fig. 4). These data are consistent with one model suggesting that PAI-1 limits cyclin:cyclin-dependent

Figure 3. Exogenous PAI-1 inhibits endothelial growth factor (EGF)-stimulated growth of human keratinocytes. A 5-day exposure of human keratinocytes (HaCaT cells) to the stable, long half-life, PAI-1 mutant 14-1B (20 nM final concentration) effectively inhibited EGF-stimulated (10 ng/mL) proliferation (**A**). Growth suppression reflected a PAI-1-induced G₁ arrest evident as early as 24 hours after initial SERPINE1 treatment (**B**). Starting populations (1-day serum starvation prior to EGF or EGF + PAI-1 addition) were virtually devoid of DNA-synthesizing keratinocytes. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 4. Exogenous PAI-1-dependent growth arrest is mimicked by adenoviral-mediated PAI-1 overexpression. The growth of two human keratinocyte cell lines (HaCaT, HaCaT II-4), maintained in media containing fetal bovine serum for 3 days, was significantly suppressed by exposure to the stable, long half-life, PAI-1 mutant 14-1B (A). Data was obtained using a standard toluidine blue-based proliferation assay. A549 was included as a control PAI-1-responsive cell type. Growth inhibition was also evident in HaCaT cells infected with an adenovirus encoding PAI-1 and green fluorescent protein (GFP) in which PAI-1 expression was driven by a cytomegalovirus promoter, but not in keratinocytes infected with a control GFP-only adenovirus (B). While the number of initial small colonies that formed was only marginally different between the test groups, colony expansion (i.e., growth to large size) was dramatically suppressed by PAI-1 overexpression.

kinase activities, perhaps by modulating PI(3) kinase signaling or inhibiting mitogenic growth factor activation as a consequence of PAI-1-mediated suppression of uPA catalysis.⁴⁶

Clinical implications for wound healing

The plasmin-based pericellular proteolytic cascade significantly impacts wound cell function at several levels with effects not restricted to any specific cell type. Binding of uPA to PAI-1 induces myofibroblast differentiation on vitronectin matrices and uPAR cleavage regulates the fibroblast-to-myofibroblast transition.⁴⁹ In injury repair, fibroblasts are recruited to the wound site, where they differentiate into myofibroblasts that in turn perform the essential functions of matrix deposition and wound contraction. In deficient (incomplete) or exuberant (excessive scarring) repair, myofibroblasts are few (likely due to failure to recruit fibroblasts) or overly abundant and do not

effectively undergo apoptosis, respectively. uPA is also a mitogen and motogen to several cell types involved in tissue repair, by signaling through uPAR/LRP1/integrin complexes⁵⁰ and via activation of ECM-tethered growth factors.⁴⁶ PAI-1 levels in the wound microenvironment, and its stabilization by vitronectin, can modulate each of these healing-critical events. Elevated expression of PAI-1 as well as collagen, moreover, is associated with certain healing anomalies including keloid development and hypertrophic scarring while the uPA/PAI-1 ratio is increased in chronic ulcers favoring high uPA activity.⁵¹ Indeed, hypertrophic scar and keloid fibroblasts exhibit differences in ECM remodeling, likely as a result of altered TGF- β 1 signaling or response, that contribute to the basic pathophysiology of both.^{52,53} Excessive scarring consistently reflects augmented PAI-1 expression, a prominent TGF- β 1 target, particularly in the aggressive fibrotic disorders.^{52,54,55} The clinical potential in disrupting expression of this SERPIN has ramifications to keloid disease, in particular, as well as to the more general problem of tissue fibrosis and highlighted by the finding that PAI-1 small interfering RNA effectively attenuates collagen levels in keloids.^{55,56} Subcutaneous implantation of polyvinyl alcohol sponges into PAI-1^{-/-} mice resulted in a significantly reduced fibrotic response, unlike wild-type animals which developed extensive fibrin-rich deposits, consistent with the implication of PAI-1 as a key element in tissue fibrosis.⁵⁶ Importantly, PAI-1^{-/-} mice exhibit accelerated skin wound healing⁴⁴ and while the actual underlying mechanisms are unclear, effects on proliferation,⁴⁶ uPA-dependent growth factor or plasmin activation or PAI-1-vitronectin interactions^{22,57} may each play a role. In this last regard, vitronectin^{-/-} mice are, in fact, deficient in skin wound healing.⁵⁸ Furthermore, as is the case for PAI-1, the closely related SERPIN PAI-2 is similarly anti-proliferative.⁵⁹ The early transcriptional response of the PAI-1 gene to monolayer wounding and its prominence in the injury repair genetic signature are consistent with its role as regulator of the temporal cadence of cell migration and growth as part of the cutaneous injury response program for both keratinocytes and fibroblasts. This possibility is supported by the observations that PAI-1 deficiency promotes skin injury repair while plasminogen stimulated healing of acute and diabetic wounds.^{44,57} More recently, small molecule PAI-1 functional inhibitors (tiplaxtinin, TM5275) demonstrated efficacy in experimental models of TGF- β 1-induced pulmonary fibrosis and stimulated skeletal muscle repair

and regeneration in the contest of type 1 diabetes.^{60,61} Collectively, it appears that targeting PAI-1 gene expression and/or PAI-1 function with molecular genetic constructs, neutralizing antibodies or small molecule inhibitors may provide a novel, therapeutically relevant approach, to manage the pathophysiology of wound healing disorders associated with deficient or excessive PAI-1 levels.

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- Spatial and temporal regulation of the wound site pericellular proteolytic microenvironment initiates a tissue repair response coupled to a plasmin/MMP-directed stromal remodeling axis.
- Members of the serine protease and MMP families constitute a functionally complex system of interacting protease cascades and are titrated by their respective inhibitors.
- Fine regulation of serine protease/MMP expression and their topographic activity are required for proper integration of cell proliferation, migration, and stromal reconstruction as part of the wound healing program.
- PAI-1 is a multipurpose SERPIN. It is a significant contributor to the overall pericellular proteolytic balance in the wound microenvironment, regulates cell migration through its anti-proteolytic and signaling functions, and as a member of the repertoire of p53 target genes, is a likely major factor in go-versus-grow decisions in the repair response.
- Targeting individual elements in this highly interactive network of proteases and protease inhibitors may lead to novel therapeutic approaches for the treatment of fibrotic diseases and chronic wounds. PAI-1 appears to be an excellent candidate for such focused interventions.

the molecular mechanisms underlying TGF- β 1 target gene transcription and their involvement in wound healing, angiogenesis, and fibrosis. **Stacie M. Kutz, PhD**, is an associate professor in the Department of Biology at the Sage College of Albany. **Brian K. Law, PhD**, is an associate professor in the Department of Pharmacology and Therapeutics at the University of Florida in Gainesville. The research team has enjoyed a long history of productive collaborations.

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Abbreviations and Acronyms

ECM	= extracellular matrix
GFP	= green fluorescent protein
LRP1	= low-density lipoprotein receptor-related protein 1
MMP	= matrix metalloproteinase
PAI-1	= plasminogen activator inhibitor-1
RGD	= arginine-glycine-aspartate
SERPINE1	= serine protease inhibitor clade E member 1
TIMP	= tissue inhibitor of matrix metalloproteinases
tPA	= tissue-type plasminogen activator
uPA	= urokinase plasminogen activator
uPAR	= uPA receptor
VLDLR	= very-low density lipoprotein receptor