



Editorial

## Epithelial “Plasticity” in Tumor Progression and Wound Repair: Potential Therapeutic Targets in the Stromal Microenvironment

Paul J. Higgins<sup>1\*</sup>

### Focal Proteolysis: Regulation of Cell Migration and Signaling by the Serine Protease-Matrix Metalloproteinase Cascade

Epithelial transdifferentiation or cellular “plasticity” refers to a morphogenetic switch resulting in loss of normal epithelial properties, again in the expression of genes is generally restricted to the mesenchymal lineage and acquisition of a migratory phenotype. Essential during development and organogenesis (i.e., embryonic patterning), epithelial plasticity is relatively limited in the adult organism, occurring during wound healing and regenerative repair or, more typically, in tissue fibrosis and tumor metastasis [1]. The temporal and spatial regulation of the plastic phenotype is likely a collective response to specific growth factors and cues from the extracellular environment. Among the various inducers of cellular transdifferentiation, members of the transforming growth factor- $\beta$  are, perhaps, the most prominent, impacting both the emergence and persistence of the plastic restructuring.

TGF- $\beta$  promotes cellular motility and emergence of the plastic cohort through expression controls on genes that encode stromal remodeling proteins [2,3]. These include serine proteases and matrix metalloproteinases (MMP) and their respective inhibitors which support matrix disruptive as well as stabilizing processes. Stringent controls on serine protease/MMP transcription, duration of expression and topographic activity are essential for maintaining tissue homeostasis. Proteolytic networks within the pericellular microenvironment, moreover, are frequently activated by conversion of plasminogen to plasmin, a broad-spectrum protease. Plasmin targets stromal elements directly while also activating several MMPs triggering a complex cascade leading to focalized matrix degradation. The cell biologic implications of these interacting systems have been elegantly modeled *in vitro* upon addition of epidermal growth factor (EGF)+TGF- $\beta$ 1 to malignant human epithelial cells to mimic the frequently observed TGF- $\beta$ 1 elevation in the tumor microenvironment and amplified EGF receptor (EGFR) signaling typical of late-stage cancers. Combined co-stimulation resulted in the synergistic up-regulation of a defined set of pro-invasive genes, the most prominent of which encodes plasminogen activator inhibitor-type-1 (PAI-1 or

SERPINE1, the clade E member 1 of the family of serine protease inhibitors) [4]. This finding is of considerable translational relevance since increased PAI-1 levels accompany emergence of the plastic phenotype, paralleling the requirement for enhanced cell motility. Notably, PAI-1 expression is similarly increased in wound margin keratinocytes where it is deposited into cellular migration tracks, suggesting an integral role in regulating directional migration and wound closure. Coordinate up-regulation of stromal proteases with a potent upstream inhibitor of plasmin generation (i.e., PAI-1) provides a mechanism for fine control of focal proteolysis to facilitate optimal cell motility in complex environments.

PAI-1 may titrate the MMP cascade via controlled generation of pericellular plasmin modulating stromal remodeling both temporally and spatially. The established contribution of PAI-1 in various disease states (tumor progression, inflammation, scarring disorders, atherosclerosis, thrombosis, myocardial infarction, diabetes) likely reflects maintenance of a “stromal scaffold” that impacts cell survival, growth and transdifferentiation, cellular motile processes and signal transduction. Stromal PAI-1, moreover, is itself a substrate for several extracellular proteases including elastase, MMP-3 and plasmin resulting in the generation of specific PAI-1 cleavage products. “Cleaved” PAI-1 is unable to bind its targets urokinase plasminogen activator (uPA) and tissue-type PA (tPA) to inhibit plasmin-based proteolysis but retains the ability to bind to the low-density lipoprotein receptor-related protein-1 (LRP1) where it effectively augments uPA/tPA complex-independent cell migration. The mechanistic basis for this response is not clear but appears to involve LRP1 function as a key signaling mediator in several intracellular pathways via interactions with multiple adaptor and scaffolding proteins. LRP1 ligand binding and/or complex formation with additional surface receptors also activates specific mitogen-activated protein (MAP) and *src* kinases stimulating cell proliferation and migration with the motility dependent on Rho family GTPases. Alternatively, PAI-1 can also initiate signaling events that impact cell migration through engagement of LRP1 and the related very low-density lipoprotein receptor. Indeed, the different conformations of PAI-1 (active, latent as well as plasmin- or MMP-cleaved) all interact with LRP1 to enhance cell migration apparently by specific engagement of the Jak/Stat1 signaling pathway. These data are consistent with recent findings that the migratory response in transformed human epidermal keratinocytes is PAI-1-dependent and that recombinant PAI-1 alone, in the absence of added growth factors, stimulates motility comparable to that attained by growth factor supplementation. The receptor-associated protein (RAP), an LRP1 antagonist which binds LRP1 and blocks interactions with known ligands including PAI-1, inhibits the migration-promoting effects of PAI-1 and effectively inhibited EGF-stimulated motility, which is known to be dependent on LRP1/PAI-1. 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.

\*Corresponding author: Paul J. Higgins, Center for Cell Biology & Cancer Research, Albany Medical College, 47 New Scotland Avenue, Albany, New York 12208 USA, E-mail: [higginp@mail.amc.edu](mailto:higginp@mail.amc.edu)

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## Therapeutic Opportunities

Up-regulation of the serine protease inhibitor PAI-1, in tumor cells or in mesenchymal cells within the tumor microenvironment as well as by "wound-stimulated" epithelial cells, may shift the proteolytic balance to optimize creation of a migratory "scaffold". The available data clearly implicates PAI-1 as a major upstream modulator of uPA→plasmin-generating system that exerts fine control over the MMP-dependent pericellular proteolytic cascade. In this context, PAI-1 may "titrate" the extent and locale of collagen stromal remodeling to facilitate cellular invasion as part of the metastatic and tissue repair programs [5]. Further clarification of the complexity of controls and the extent of interdependency of individual cascading "arms" in this highly interactive network of matrix proteases and protease inhibitors will be necessary for the rationale design of focused therapeutic approaches for the treatment of cancer, fibrotic disorders and chronic wounds. An assessment of MMP inhibitors in clinical trials is already ongoing. The emergence of uPA and PAI-1 as significant level-of-evidence-1 prognostic markers of overall survival in breast cancer is well-established. The developments of small molecule inhibitors of PAI-1 (i.e., tiplaxtinin or PAI-039) that effectively attenuate aortic remodeling in the context of vascular injury suggest that disruption of PAI-1 function may have significant translational implications. Targeting individual elements in this highly interactive pericellular proteolytic control network may lead to novel therapeutic approaches for the treatment of cancer, fibrotic diseases and chronic wounds. It is anticipated that Cell Biology: Research & Therapy will be an effective

vehicle to communicate these translationally-important findings to the biomedical research community.

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## Author Affiliation

Top

<sup>1</sup>Center for Cell Biology & Cancer Research, Albany Medical College, USA

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