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REVIEW

PAI-1: A Multifunctional SERPIN with Complex Roles in Cell Signaling and Migration

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Abstract: Elevated levels of plasminogen activator inhibitor type-1 (PAI-1) often occur in concert with the conversion of non-motile epithelial elements into a more migratory phenotype. While essential during embryonic development, this restructuring process, referred to as epithelial-to-mesenchymal-transition (EMT) is limited in the adult organism, occurring normally during wound repair or more atypically in tumor progression. Cell motility, the focal point of EMT, requires the coordinate regulation of multiple mechanisms which ensure proper communication between cell surface receptors and the extracellular environment. PAI-1, through multifaceted interactions with both extracellular matrix (ECM) and cell surface constituents plays a critical role in modulating many of these events. This review focuses on the complex role of PAI-1 in the cellular motile program.

Keywords: PAI-1, LRP1, EMT, TGF-β1, MMP, migration, wound, vitronectin

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Introduction

During embryogenesis, the development and differentiation of functionally mature adult tissues often requires conversion of non-motile epithelial elements into a more migratory phenotype, a complex cellular restructuring process referred to as epithelial-to-mesechymal transition (EMT).[1] Temporal and spatial regulation of EMT, as well as the subsequent restitution of an epitheliod phenotype (Mesenchymal to Epithelial Transition), is regulated by specific growth factors (individually or collectively) and by cues from the extracellular environment.[1–3] In the adult organism, epithelial "plasticity" persists; it is generally limited however, occurring normally as a component of wound repair or more atypically, during tumor progression.[1,4–6] In such restricted circumstances, growth factor signaling largely dictates phenotypic outcome. Epidermal growth factor (EGF) receptor amplification and an altered cellular response to transforming growth factor-β (TGF-β), for example, are often associated with the progression of epithelial tumor cells from a relatively benign to a more aggressive phenotype with increased metastatic potential.[7–10] Model systems that employ the addition of EGF + TGF-β1 to cultured keratinocytes, to mimic the frequently observed TGF-β1 elevation in the tumor microenvironment and amplified EGFR signaling in late-stage malignancies, identified the synergistic up-regulation of a subset of pro-invasive genes the most prominent of which encodes plasminogen activator inhibitor-type-1 (PAI-1).[11,12] Importantly, elevated levels of PAI-1 often occur in concert with epithelial cell plasticity, paralleling the requirement for enhanced cell motility. PAI-1, through its inhibition of urokinase-type plasminogen activator (uPA) is critical for regulating the generation of pericellular plasmin (Fig. 1) and consequently modulating extracellular matrix proteolysis and stromal remodeling. Increased expression of PAI-1 has been associated with several pathophysiological events including tumor progression, inflammation, hypertrophic scarring, atherosclerosis, thrombosis, myocardial infarction, diabetes and obesity.[13–17] In addition to proteolytic control, the contribution of PAI-1 to promoting these pathologies is thought to occur through multiple avenues which additionally impact on cell motility. This review focuses on the most recent developments in this field and on the complex role of PAI-1 in the cellular motile program.

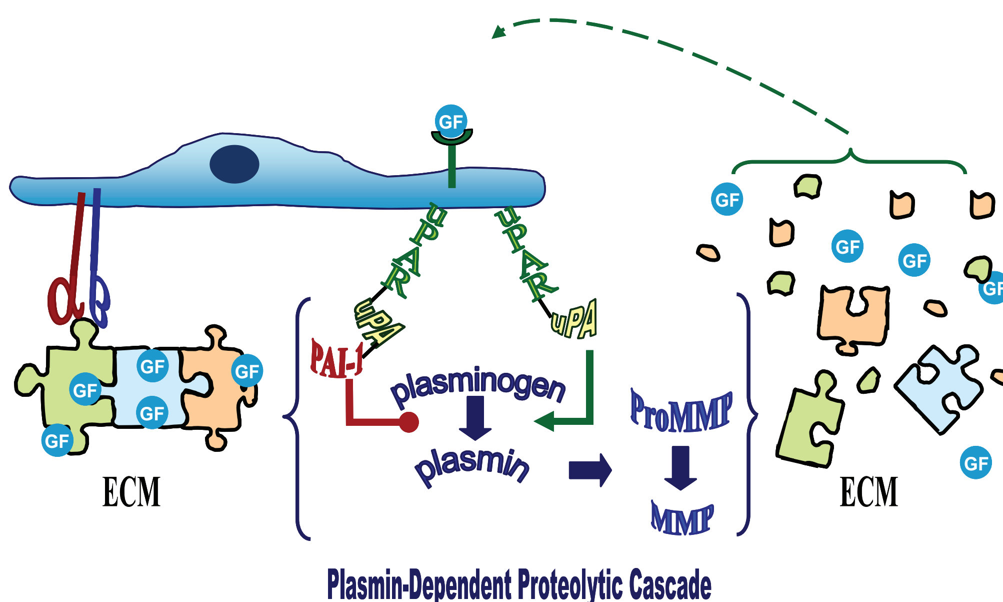

Figure 1. PAI-1 modulates cell migration by Regulating ECM proteolysis. Physiological control of pericellular proteolysis occurs primarily through the regulation of plasminogen activation at the cell surface, which, in turn contributes to downstream MMP activity. Focal proteolysis disrupts ECM architecture, breaking cell-matrix interactions with receptors such as integrins, and releasing bioactive fragments of extracellular matrix molecules, as well as growth factors that stimulate migratory behavior. PAI-1, through its ability to inhibit uPA-dependent activation of plasmin, titers this process maintaining the scaffolding necessary to facilitate cell migration.
Abbreviations: PAI-1, plasminogen activator inhibitor type-1; uPA, urokinase type plasminogen activator; uPAR, uPA receptor; MMP, matrix metalloproteinase; GF, growth factor.

PAI-1 Regulated Cell Migration: Proteolytic Events

TGF-β, an established facilitator of EMT, likely promotes invasive behavior through the transcriptional activation of genes that impact stromal remodeling and cell motility. Matrix structural elements (ie, fibronectin, collagen) and matrix-active proteases, (eg, urokinse type plasminogen activator [uPA] and matrix metalloproteinases [MMPs]) as well as the serine protease inhibitor (SERPIN), PAI-1 are up-regulated in response to TGF-β1.[18–24] EGF signaling, which is enhanced as a result of increased receptor number in various cancers,[10] also stimulates the expression of various MMPs.[22,25–28] Paradoxically, the combination of TGF-β1 and EGF synergistically up-regulates PAI-1 levels in several cell types,[11,12,18–29] despite the inability of EGF alone to increase PAI-1 levels in some systems,[12,30] and may ultimately support spatio-temporal titering of excessive plasmin-based proteolysis.

Generally, both TGF-β1 and EGF levels increase substantially following acute injury, partially due to their release from platelet α granules, but also through increased cellular expression, particularly at the wound edge.[19] These growth factors appear critical to the initial stages of cutaneous tissue regeneration through promotion of keratinocyte migration, as well as proliferation.[19,31–33] TGF-β1 and EGF also up-regulate the matrix metalloproteinase, stromelysin-2, or MMP-10 in keratinocytes.[22,28] During cutaneous wound repair, MMP-10 is specifically localized to cells in the migrating tongue where it appears to enhance migration.[22,34] Notably, PAI-1 expression also increases in keratinocytes at the wound margin and is deposited into the migration tracks of these cells, suggesting that it, as well, plays an integral role in regulating directional migration and wound closure.[35–38] Studies indicate, for example, that in non-healing wounds (also considered a model for tumor progression), failure to close may result in part from a disproportionate level of EGF and EGFR degradation,[39,40] which could arguably shift the elements balancing pericellular proteolysis. Over-expression of constitutively active MMP-10 in the epidermis has, in fact, been shown to produce deleterious effects on the coordinated migration of keratinocytes into the wound bed; an effect attributed to excessive laminin-5 (laminin-332) processing.[34] Unconstrained MMP-10 activity also leads to excessive collagenolysis[12] which impacts negatively on cell migration and ultimately, the restoration of tissue integrity. Coordinate up-regulation of proteolytic enzymes such as the MMPs, together with their upstream inhibitor PAI-1 by individual growth factors provides an exquisite mechanism for controlling focal proteolysis (Fig. 1), which is essential for cell motility. The presence of multiple growth factors contributing similar coordinate activities may conceivably augment the regulated proteolysis required to sustain or enhance motility. Indeed, the combination of TGF-β1 + EGF synergistically increases epithelial cell migration[26] and MMP expression,[26,27] as well as the expression of PAI-1.[11,12]

Focalized proteolysis also promotes the discrete release of bioactive fragments and growth factors from the stromal environment which in turn, influences cell proliferation and cell migration (Fig. 1). MMP-dependent generation of ECM fragments, for example, affects both angiogenic and anti-angiogenic activities under physiologically-relevant conditions.[41,42] MMP-2 and MMP-9, for instance, cleave collagen IV, exposing cryptic epitopes in the molecule that promote angiogenesis[43,44] while matrikines such as arrestin, canstatin, tumstatin and metastatin which are also generated from collagen IV are anti-angiogenic.[41,42] Proteolytically derived fragments from collagen XVIII (endostatin and neostatin), collagen VIII (vastatin), collagen XV (restin) and perlecan (endorepellin) also exhibit anti-angiogenic properties.[41,42] Often these ECM fragments exert their effect by competitive binding with intact ECM molecules to various cell surface receptors.[42] MMP-dependent release of laminin-332 fragments promotes epithelial cell migration. Indeed, recombinant domain III of the laminin-332 γ2 chain (which is cleaved from laminin-332 by MT1-MMP and MMP-2) binds to EGFR and initiates signaling events which culminate in enhanced cell motility.[45–47] Similarly, MMP based proteolysis of fibronectin yields fragments that affect migration (MSF),[41,48] angiogenesis, (anastellin)[49,50] cell proliferation and differentiation.[41] Proteolytic processing of the extracellular environment, therefore, impacts multiple aspects pertaining to the regulation of cell motility; PAI-1, as the major up-stream physiological inhibitor of plasmin-based proteolysis, (Fig. 1) has a critical role in modulating these events.

PAI-1 Regulated Cell Migration: Receptor Interactions

Stromal PAI-1 is a target for cleavage by extracellular proteases, including elastase, MMP-3 and plasmin.[51–53] "Cleaved" PAI-1 is unable to interact with the plasminogen activators (PA) uPA and tPA to inhibit plasmin-based proteolysis but can bind to the low-density-lipoprotein-receptor-related-protein-1 (LRP1) through a PA-complex-independent interaction (Fig. 2A) to augment migration of smooth muscle cells.[54] PAI-1 also stimulates directional migration in normal human keratinocytes (Fig. 3) and is required for TGF-β1 + EGF induced keratinocyte scattering.[11] Notably, high levels of stromal PAI-1 have been correlated with poor prognosis in several cancers, including breast, lung, ovarian and squamous cell carcinomas[13,16] suggesting that PAI-1-dependent preservation of the surrounding matrix facilitates motility of invading cells. Current observations, however, also suggest an alternative role for PAI-1 as a signaling molecule that enhances cell migration. Indeed, the different conformations of PAI-1 (active, latent, cleaved) can bind LRP1 and stimulate Jak/Stat1-dependent migration (Fig. 2A).[54–56] Consequently, even though 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 migratory function, remain embedded in the matrix to sustain cell migration. This paradigm supports the correlation of high PAI-1 levels with poor prognosis.

LRP1, in addition to its function as a major endocytic receptor, is also a key signaling mediator in several pathways due, in part, to its ability

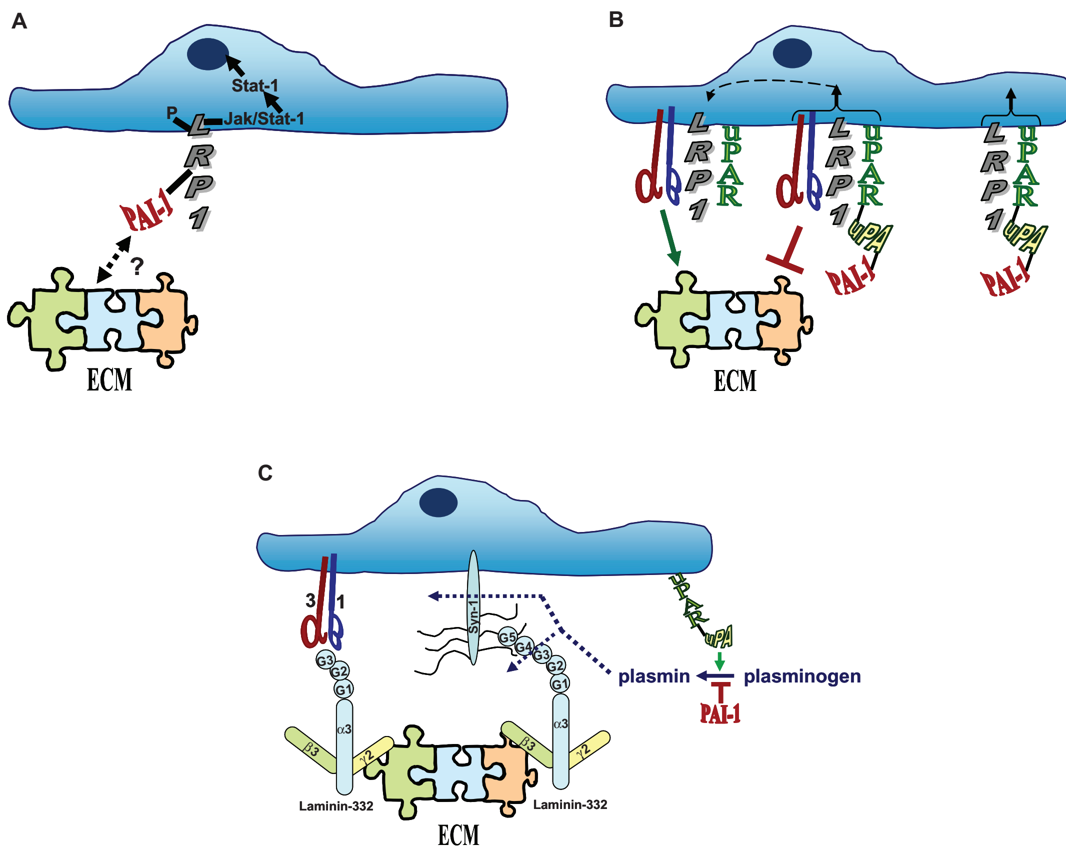

Figure 2. PAI-1 modulates cell migration through cell surface receptors. **A**) PAI-1 binding to the LRP1 in a non-uPA/uPAR dependent manner, triggers Jak/Stat1 signaling events that culminate in enhanced cell migration. It is unclear whether this process necessitates PAI-1 interaction with the ECM. **B**) PAI-1 binding to uPA/uPAR results in the internalization of the PAI-1/uPA/uPAR complexes in an LRP1 dependent manner. PAI-1 binding to uPA/uPAR can also trigger detachment of cell surface integrins from their ECM ligands and subsequent internalization in an LRP1-uPA/uPAR-dependent manner. In each case, receptors (integrin, uPAR, LRP1) recycled back to the cell surface, while uPA and PAI-1 are degraded. **C**) In this hypothetical model, PAI-1, through its ability to titer active plasmin, promotes syndecan-1 dependent migration on unprocessed laminin-332 by preventing cleavage of the syndecan binding site LG4/5. Additionally, PAI-1 inhibition of plasmin activation facilitates migration on unprocessed laminin-332 by reducing the shedding of syndecan-1 from the cell surface. As the proteolytic environment matures and PAI-1 levels decrease, integrins α3β1 and α6β4 (not shown) engage the proteolytically cleaved, or processed form of laminin-332 and begin to establish hemidesmosomes.

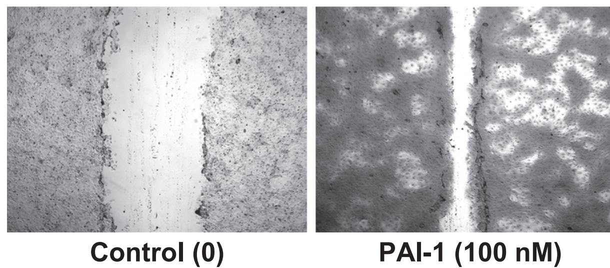

Figure 3. PAI-1 enhances keratinocyte migration. Primary human keratinocytes isolated from neonatal foreskin, were seeded onto tissue culture plastic coated with collagen type-1 (30 ug/ml), in complete (growth factor supplemented) keratinocyte medium (Cascade Biologics, Invitrogen; Carlsbad, CA) and grown to confluence. The medium was then changed to Defined Keratinocyte SFM (Invitrogen) without growth factors for 24 hours, after which monolayers were scrape-wounded with a pipet tip, washed and incubated in Defined Keratinocyte SFM ± PAI-1 (100 nM) for 24 hours. Monolayers were then fixed with paraformaldehyde and briefly stained with crystal violet to delineate the wound edge, then measured for change in wound site closure. Images were collected at 5x magnification on a Nikon OptiPhot-2.

to support interactions with multiple adaptor and scaffolding proteins.[57] The intracellular domain of LRP1 (following Regulated Intramembrane Proteolysis) also translocates to the nucleus, where it appears to negatively regulate amyloid precursor protein (APP) intracellular domain-dependent gene transcription through an interaction with Tip60.[58,59] LRP1 ligand binding and/or its complex formation with cell surface moieties such as integrins,[60–62] growth factor receptors[63,64] and proteoglycans[65] appears to activate MAP and Src family kinases,[64,66–69] regulate cell proliferation[63,64,70–72] and stimulate cell migration.[66,73]

PAI-1 activity has been associated with activation of ERK signaling events,[74] regulation of cell proliferation through Akt,[75,76] modulation of TGF-β signaling through αvβ3,[77] recruitment of cellular effectors during renal fibrosis[78] and control of fibronectin matrix assembly through αvβ5 and α5β1 integrins.[79] PAI-1 also regulates levels of cell surface integrins by triggering their internalization in an LRP-dependent manner,[61,77,80] resulting in cell detachment from a variety of substrates[61,80] (Fig. 2B). The mechanism supporting this function appears to differ however, from that which modulates PAI-1stimulated migration directly via LRP1, as uPA is required for detachment, but not for the migratory response.[54,55,61,80] Nevertheless, it is apparent from these studies that PAI-1 can utilize multiple avenues to impact on cell migration through LRP1 (Fig. 2A and 2B). Studies suggest that ligand binding to LRP1 affects Schwann cell motility through activation of the Rho family GTPases.[73] Notably, Rho family GTPase activity has been connected with enhanced expression/induction of Jak/Stat signaling.[81,82] The potential contribution of these interactions in PAI-1-stimulated cell locomotion via the LRP1 however, remains to be determined.

Syndecan-1 binding to the LG 4/5 domain of unprocessed laminin-332 appears necessary for keratinocyte migration[83,84] and may, therefore, contribute to the rate at which wound healing proceeds.[85–87] Notably, cleavage of the α3 subunit of laminin-332 by plasmin, which occurs between the integrin and syndecan binding sites within the LG domain (Fig. 2C), converts laminin-332 from a pro-migratory factor to one that impedes cell motility and supports hemidesmosome formation.[88] A potential role exists, therefore, for PAI-1 in modulating syndecan-dependent keratinocyte migration during wound healing. In this model (Fig. 2C), keratinocytes at the wound margin begin to synthesize and deposit unprocessed laminin-332, supporting syndecan-1 binding through the LG 4/5 domain. PAI-1, which is also up-regulated in cells at the wound edge, stabilizes this interaction by preventing plasmin-based proteolytic processing of laminin-332[88] and syndecan-1 shedding.[89,90] The presence of vitronectin (VN) at the wound edge can augment this event through its ability to extend the half-life of active PAI-1 (discussed below), as well as engage syndecan-1[91] and focalize PAI-1. As the proteolytic environment matures, PAI-1 and VN are endocytosed and degraded.[92,93] Syndecan-1 binding is lost due to proteolytic processing of laminin-332, as well as syndecan-1 ectodomain shedding and α3β1 binding to processed laminin-332 begins to slow keratinocyte migration and initiate hemidesmosome formation.[88]

PAI-1 Regulated Cell Migration: Interactions with Vitronectin

PAI-1-VN interactions impact on several mechanisms associated with cell migration. VN stabilizes and extends the half-life of active, PA-binding PAI-1, amplifying the inhibition of focal proteolysis and thereby preserving the stromal architecture necessary for cell migration.[94,95] This is particularly important following cutaneous injury where restoration of barrier function and tissue

integrity is dependent upon keratinocyte migration. PAI-1 and vitronectin are both released from the alpha granules of platelets during hemostasis, where their joint presence would presumably facilitate the formation of a fibrin clot and subsequently contribute to provisional matrix remodeling.[96,97] PAI-1 expression is additionally up-regulated in keratinocytes at the wound margin[36,37] highlighting the involvement of this SERPIN in initiating tissue repair. Vitronectin, which exhibits limited expression under normal physiological conditions,[98–101] is also enhanced under circumstances requiring stromal remodeling, such as wound repair[102–104] and tumor progression[105–110] suggesting a continuing, albeit dynamic, molecular interaction of physiologic significance.

While PAI-1-VN complexes facilitate migratory processes by preserving stromal architecture, the interaction of these two proteins also affects cell migration through mechanisms that directly modulate cell surface receptor binding (Fig. 4). VN promotes cell migration via RGD-dependent interactions with αvβ3 and αvβ5 integrins,[111–114] as well as through binding to the urokinase-type-plasminogen-activator-receptor, uPAR.[115,116] The binding site for PAI-1 on VN, however, approximates those for both integrin and uPAR binding[117] and, as a result, the interaction of PAI-1 with VN interferes with the ability of VN to engage these receptors[115–119] (Fig. 4). Similarly, the PAI-1 and LRP1 interaction-dependent migration is blocked

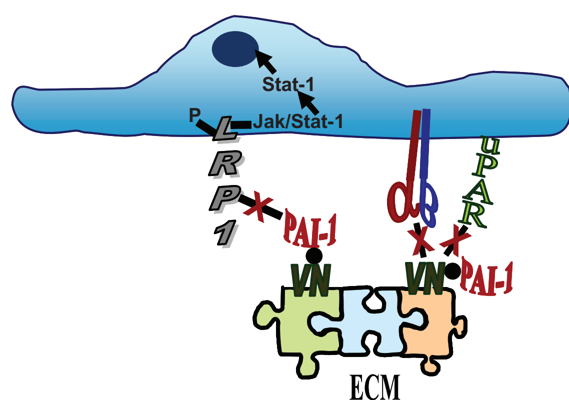

Figure 4. PAI-1-VN interactions disrupt receptor binding events. PAI-1 binds to the VN molecule in a region that overlaps the uPAR and integrin binding sites. Consequently, the higher affinity PAI-1-VN interaction disrupts the capacity for VN to engage these receptors. In a similar manner, VN binding to PAI-1 inhibits PAI-1 binding to LRP1 and Jak/Stat1 mediated migration.

by VN binding to PAI-1[55] (Fig. 4). Collectively, it is clear that the interaction of these two molecules has the potential to affect cell motility on multiple levels.

PAI-1, in addition to regulating cell-to-substrate attachment, also regulates cellular detachment from VN by two distinct mechanisms. The affinity of PAI-1 for VN is sufficient to trigger the release of uPAR from vitronectin.[115,116,118] In addition, PAI-1 in the presence of uPA/uPAR complexes, can initiate detachment of integrins from their ECM ligands and promote their endocytic clearance.[61,80] Subsequently, these receptors are recycled back to the cell surface to re-engage matrix molecules and promote cell migration[57] (Fig. 2B).

Summary

Clearly, cell motility, which involves attachment and detachment of cell surface receptors from the ECM, requires focused regulation of a series of complex events and the coordination of multiple mechanisms which ultimately ensure appropriate communication between cell surface receptors and the extracellular environment. PAI-1, through its multifaceted interactions with both ECM and cell surface constituents plays a central role in modulating many of the temporally-regulated and spatially-controlled events that contribute to managing this intricate process in both physiologic and pathophysiologic contexts. Understanding the factors and stimuli that influence PAI-1 expression levels and activity therefore offers us an attractive avenue for the future of drug development.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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